Spontaneous Discharge Patterns in Cochlear Spiral Ganglion Cells Before the Onset of Hearing in Cats

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Submitted 25 April 2007; accepted in final form 5 August 2007


INTRODUCTION

Many mammals, including the kitten, are altricial, and they spend the first several days to weeks as neonates before appreciable hearing sensitivity develops. During this prehearing period, projections of spiral ganglion cells target zones in the cochlear nucleus undergo refinement to form precisely segregated cochleotopic terminal fields (for review, see Cant 1998). A similar cochleotopic organization of auditory projections is formed and preserved at essentially all levels of the neuraxis including the auditory cortex. These projections ultimately relay information about sound frequency, which is encoded spatially in the cochlea and thus provide the basis for tonotopic mapping through out the central auditory system. In auditory nuclei receiving binaural input, the projections of each ear are segregated and preserved during ontogeny, and these connections provide a means to locate sounds in the environment through neural computations. Understanding the mechanisms involved in the refinement, segregation, and preservation of these maps is of great interest.

The initial topographical adjustments and final refinement of the cochlear nerve terminal fields in cochlear nuclei of the cat are essentially completed before hearing begins (Cant 1998; Leake et al. 1992, 2002; Snyder and Leake 1997; Snyder et al. 1997). However, the mechanisms underlying these prehearing developmental processes are not yet clear. In the visual system, segregation and refinement of central retinotopic projections occurs in neonatal mammals before onset of vision (McLaughlin and O’Leary 2005). The detailed refinements of retinotopic projections are thought to depend critically on both molecular guidance and patterned neural activity in retinal ganglion cells (Cang et al. 2005; Demas et al. 2006; Hindges et al. 2002; McLaughlin et al. 2003a-c; Mrsic-Flogel et al. 2005; O’Leary and McLaughlin 2005; Ruthazer and Cline 2004; Ruthazer et al. 2003; Stellwagen and Shatz 2002; Yates et al. 2004).

Of particular interest is the observation that spontaneous waves of correlated rhythmic neural activity in retinal ganglion cells provide a signal that is critical for the establishment of fully refined retinotopic maps at all levels of the neuraxis (Cang et al. 2005; Demas et al. 2006; McLaughlin et al. 2003c; Mrsic-Flogel et al. 2005; O’Leary and McLaughlin 2005; Ruthazer et al. 2003; Stellwagen and Shatz 2002). Spontaneous rhythmic discharge has been reported in brain stem auditory pathways of prehearing birds and mammals (Gummer and Mark 1994; Kotak and Sanes 1995; Lippe 1994; Rubsamen and Schafer 1990). The presence of rhythmic bursting among cochlear ganglion cells in the embryonic chicken has also been established recently and is reminiscent of rhythmic discharge patterns in the retina (Jones et al. 2001). The idea that rhythmic discharge patterns play a role in guiding refinements in central cochleotopic fields has been suggested by a number of investigators (Gummer and Mark 1994; Jones et al. 2001, 2006; Lippe 1994, 1995). Not firmly established in the mammal is the question of whether spiral ganglion cells themselves exhibit such spontaneous activity patterns. Neurons of the auditory nerve in cats are known to be spontaneously active as early as 2 days postnatal (P2) (Carlier et al. 1975; Romand 1984; Walsh and McGee 1987). However, it is not clear whether spiral ganglion cells (SGCs) of neonatal kittens exhibit the necessary repetitive spontaneous bursting discharge patterns. We hypothesize that spontaneous rhythmic bursting discharge patterns are present in mammalian SGCs before hearing begins and that such discharge patterns serve to guide activity-dependent cen-
tral refinements in mammals. To critically address the first of these assertions, we recorded spontaneous activity of SGCs in neonatal kittens ranging in age from P3 to P9.

METHODS

Animals and surgical approach

The care and use of animals in this study were approved by the Institutional Animal Care and Use Committee at the University of California at San Francisco (UCSF) and conformed to all National Institutes of Health guidelines. All animals included in this study were bred in a closed colony maintained at UCSF. Queens were bred for periods of ≤24 h, so the gestation period for each litter was known within ±12 h. Total number of days postconception (dpc, number of days of gestation plus number of days postnatal) is used to define age, because this value correlates best with development of the organ of Corti (Sato et al. 1999) and development of electrophysiological response properties in the auditory nerve (Fitzakerley et al. 1998). This report includes recordings made in eight young kittens ranging in age from 69 to 75 dpc (corresponding to postnatal ages P3–P9) and in one older cat studied at 102 dpc (P36) as summarized in Table 1.

The animals were anesthetized with inhaled isoflurane (3% for induction; 1–2% for maintenance). A tracheotomy tube was inserted, and animals were maintained on a small animal pressure-controlled ventilator (Kent Scientific). The respiratory rate, heart rate, and body temperature were monitored throughout the experimental procedures, and body temperature was maintained using a warm water recirculating blanket with a thermostatic coupler. The head was stabilized using a nylon screw mounted on the skull with dental acrylic and three small bone screws. The auditory bulla on the left side was exposed and opened to permit access to the cochlea. In the youngest animals, mesenchyme was dissected from the middle ear to visualize the round window. The round window membrane was excised and excised to allow direct visualization of Rosenthal’s canal in the hook region and lower basal turn of the cochlea. In younger kittens (before ossification of the modiolus), the recording micropipettes could be inserted directly into the spiral ganglion. In older kittens, a small opening into the modiolus, was made using a tip of a 30-gauge needle to lift a small flap of bone over Rosenthal’s canal. Glass micropipettes (Flaming/Brown micropipette puller, model P-97, Sutter Instrument Co.) filled with 0.5 M KCL in 0.05 M Tris buffer (pH 7.6) were lowered into the scala tympani using a micromanipulator and advanced into the ganglion using a hydraulic microdrive. A silver wire was plated with silver chloride, placed in the muscle posterior to the bulla, and used as a reference electrode. Microelectrode impedance ranged from 5 to 70 MΩ and was measured in situ using an electrometer (model WPI 767-B, World Precision Instruments).

TABLE 1. Summary of ages for animals studied

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Days Gest.</th>
<th>Postnatal Age</th>
<th>Total Age</th>
<th>Isolated</th>
<th>Used</th>
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<td>66</td>
<td>10</td>
<td>102</td>
<td>3</td>
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</table>

Days Gest., days of gestation; Total Age, total days post-conception (dpc); Isolated, number of cells isolated; Used, number of cells used to form metrics for spontaneous discharge and bursting.

Electrophysiological recording

The discharge activity of SGCs, ongoing ECG, and ventilation were recorded digitally (12-bit conversion, 44,100-Hz sampling). Electrophysiological activity was recorded for up to ~14 min. Figure 1A shows a typical spiral ganglion neuron recording and signal-to-noise ratio generally achieved in this study. Analysis of discharge patterns was accomplished off-line using the digital records. Throughout this report, mean values will be expressed as the mean ± SD (n), where n = sample size. Useful data were obtained from cellular recordings having 1) a signal-to-noise ratio (spike amplitude/ background noise) that permitted unambiguous spike detection and 2) a recording duration that was sufficient to glean useful information about cell discharge. We used the product of the number of spikes multiplied by the time between the first and last spike of a record as an objective metric (spikes-seconds) to exclude cells. Cells not providing ≥50 spikes-seconds of well resolved spike activity were excluded from analysis.

Metrics documented for each neuron included total recording time (time between first and last spike), total number of spikes, spike rate (total number of spikes/total recording time), mean spike time interval, SD of the spike interval (SDi), and interval coefficient of variation (CVi). The CVi for spontaneously discharging auditory ganglion cells ranges between 0.6 and 1.0, with a mean of ~0.8 in the mature mammal (cat; Walsh et al. 1972) and ~1.0 for the mature bird (Jones and Jones 2000). CVi’s ranging between 0.7 and 1.0 reflect the near stochastic discharge of mature auditory neurons, which is often described as a quasi-Poisson excitation or renewal process. CVi was reported for all neurons in which recording times were sufficient to determine whether bursting was present and adequate numbers of spikes occurred to permit the calculation of the burst index (below). Burst rate was calculated as a simple count of the total number of bursts divided by the total recording time. Bursts were defined in the...
context of a preceding period of low activity, and this was signaled by
long spike intervals. For the purpose of counting bursts, we defined a
minimum preburst period (PBp, ms). This was used as a threshold to
define the onset spike of bursts. Here PBp was set equal to three times
the mean spike interval for the entire discharge record. This is the
same criterion used by Gummer and Mark, (1994). The onset of a
prospective burst was signaled by a spike having a spike interval equal
to or greater than the PBp. A burst was counted only if the onset spike
was followed by a group of two or more spikes having spike intervals
less than PBp. The word “spontaneous” in this study means “in the
absence of sound stimulation.”

**Determination of bursting versus entrainment**

Entrainment to cardiac or ventilation rhythms, which could be
misinterpreted as bursting, was determined by 1) visual inspection of
the time records; 2) cross-correlation; and 3) probability density
functions. Entrained cells were excluded from summary spontaneous
discharge data. Bursting was evaluated using two computational
methods: 1) probability density functions and 2) burst index (BI), a
dimensionless metric useful for identifying and ranking bursting
patterns [originally referred to as burst factor (BF)] (Jones and Jones

**VISUAL INSPECTION OF TIME RECORDS AND CROSS-CORRELATION.** The
original spike records were displayed and recording quality con-
firmed. Spike onset times were scored and evaluated for obvious signs
of entrainment (e.g., inspected for regular bursting at rates equal to
ECG or ventilation rates). Spike rate and onset time were plotted.
Plots of cardiac and ventilation cycle onset times were added to spike
time plots. These combined plots were examined visually in detail for
evidence of SGC entrainment. Neurons found to discharge in a
manner that was linked in time to either cycle were designated as
“entrained.” An example of such a plot and a detailed description of
the graphical method used for inspection is provided in Supplemental
Fig. 1.1

Cross-correlation of spike times with cardiac or ventilation cycle
onset times involved constructing two separate period histograms for
the recorded spike onset times (Perkel et al. 1967). One period
histogram was based on cardiac cycle onsets and the other on
ventilation cycle onsets. The term “period histogram” will be used
herein to indicate the final result of a cross-correlation. The duration
of the time window for a cross-correlation and resultant period
histogram was equal to the mean cardiac or ventilator period, and we
used bins that were 10 ms long. The time window was centered on
the onset of each cardiac or ventilation cycle. Theoretically, for a neuron
exhibiting discharge activity that is randomly generated and independ-
ent of the cardiac and/or ventilation cycle, the distribution of the
period histogram amplitudes should be relatively flat, reflecting a
uniform probability distribution across the time window (Perkel et al.
1967).

**PROBABILITY DENSITY FUNCTIONS.** A simple spike time histogram
was created to represent discharge probability as a function of time.
The number of spikes occurring in the space of each time bin was
taken as the discharge amplitude (spike count), and this was plotted as
a function of time, where each time bin corresponded to one cycle
period of ECG or ventilation. The time represented by the total
number of bins was equal to the total time of the recording. If spike
discharge was strictly linked to the ECG or ventilation cycle, each
cycle (bin) would have a comparable number of counts, and the spike
discharge amplitudes would distribute evenly across all bins through-
out the time histogram. Similarly, if spike discharge was randomly
generated and independent of ECG and ventilation events, spike
discharge amplitude would also be randomly distributed throughout
the time histogram. However, if spike discharges were nonstochastic

and occurred in recurrent bursts interspersed with silent periods, the
distribution of spike counts would not be uniform and would not
follow a Poisson distribution as described below.

**USING THE VC TO IDENTIFY NONUNIFORM PROBABILITY DISTRIBUTIONS.** To construct frequency distribution functions for period
histograms and spike time histograms, the amplitude of a given time
bin \(i\) (measured in spikes) was denoted \(g(i)\), \(i = 1, 2, \ldots, m\), where \(m\)
was the total number of bins in the period or time histogram
window. Values of the frequency distribution array, \(p(k)\), were calcu-
lated by counting the number of times that \(g(i)\) was exactly equal to
\(k\) \((k = 0, 1, 2, \ldots)\). Thus \(p(2)\) was the number of bins of the period or
time histogram that contained exactly two spikes. The weighted mean
\((\bar{M}_w\), also widely referred to as expected value) and variance \((\sigma^2_w)\)
of the frequency distribution function \(p(k)\) were calculated as follows

\[
\bar{M}_w = \sum_{k=0}^{q} \frac{p(k)}{m} k
\]

and

\[
\sigma^2_w = \sum_{k=0}^{q} \frac{p(k)}{m} (k - \bar{M}_w)^2
\]

where \(k\) is the number of counts (spike counts), \(q\) is the maximum
number of spike counts in any bin, \(p(k)\) is the number of bins with a
spike count of \(k\), and \(m\) is the total number of bins. The VC, a single
value describing the amplitude (spike count) distribution of the period
histogram \((V_{CP})\) or spike time histogram \((V_{CTH})\), was calculated using the following equation.

\[
VC = \sigma^2_w / \bar{M}_w
\]

The VCs of sampled SGC spike discharge data were contrasted
with the VCs of Monte Carlo simulations having an equal number of
spikes. Spike onset times in simulations were randomly assigned over
the same recording period as sampled data, and these “simulated spike
trains” were cross-correlated with the mechanical event cycles (car-
diac and ventilation) present in the sampled data records. This process
produced a period histogram for simulated data. In a similar fashion,
the simulated spike trains were accumulated in appropriate time bins
of the spike time histogram and the results were compared with those
for sampled spike data. Frequency distribution functions were con-
structed for the simulations, and VC values were calculated from the
weighted mean and variance as described above.

**INTERPRETING VCS.** Theoretically, if SGC spike discharges are
independent of cardiac and ventilation cycles, the distribution of period
histogram amplitudes, \([g(i)]\), should approach that produced by a
stochastic Poisson excitation process and approximate those produced
by Monte Carlo simulations. The same is true for amplitudes (spike
counts) of the spike time histograms. The resulting frequency distri-
bution \([p(k)]\) would in those cases follow or approach the theoretical
Poisson probability distribution \(P(k)\), which is given by \(P(k) =
!n \frac{e^{-k} k^k}{k!}\), where \(e\) is a constant equal to the mean counts per bin, \(k\)
is the spike count, and \(k!\) is \(k\) factorial (Batschelet 1979). The value
\(P(k)\) for any given \(k\) is the fraction of the total number of bins that will
contain \(k\) spike counts if each spike event is equally likely to enter any
of the bins and must enter one. For a true Poisson frequency distri-
bution, the resulting VC is 1.0 (\(VC = 1.0\)), because in that case, the
weighted mean and variance are equal \((\sigma^2_w = \bar{M}_w)\). Values of VC
substantially larger or smaller than 1.0 were taken to indicate sub-
stantial deviation from a Poisson distribution and thus were indicative
of spike event times that were linked to the ventilation or cardiac cycle
in period histograms or that were produced by nonstochastic bursting
activity represented in spike time histograms. Values of VCs obtained
from simulated and sampled data were compared and statistical
contrasts made using the Wilcoxon signed-rank test.
NOMENCLATURE FOR VCS. As noted above, general reference to the VC will be made using the acronym VC. VCs derived from period histograms (PHs) will be referred to as VCPH and those derived from time histograms (THs) as VCTH. To distinguish VCs based on cardiac (ECG) and ventilation (vent) data, the corresponding subscripts will be added (i.e., VCPH ECG, VCPH vent, VCTH ECG, VCTH vent). Finally, there are occasions where VCs are pooled for ECG and ventilation data, and these are referred to as VCPH ECG and VCPH vent.

BI. BI was calculated only for those records with 11 or more spikes. The intervals in each spike record were ranked from longest to shortest. The longest intervals were used to calculate BI. The four longest intervals were used for records containing 11–80 spikes. If >80 spikes were recorded, the number of longest intervals used was calculated as 5% of the total number of spikes (truncated to an integer). The BI was calculated as follows

\[ BI = \frac{A \times B}{(\text{total sample time})} \]

or

\[ BI = \frac{(\text{total time of the longest intervals})}{(\text{total sample time/total number of intervals})} \times \frac{(\text{mean interval length of the longest intervals})}{(\text{total sample time})} \]

The first component \( A \) of the BI equation reflects the proportion of time that a cell spent in long silent periods. The second component \( B \) provides an adjustment of the BI for the relative amount of activity present during discharge bursts such that the greater the contrast between silent and active periods, the greater was the BI. BI < 1.0 signals the presence of bursting, and the greater the BI, the more pronounced is the bursting (Jones and Jones 2000; Jones et al. 2001).

RESULTS

Action potential discharges (APs) of SGCs were recorded from the cochleas of eight prehearing neonates (age, 69–75 dpc; P3–P9) and one 3-wk-old kitten. The typical developmental status of the organ of Corti and spiral ganglion in the prehearing neonates is shown in the histological sections shown in Fig. 1, B and C. At the ages examined, the SGCs have not yet developed somatic myelin. All recordings were made in the absence of auditory stimulation. A total of 112 SGCs were isolated for study. Sixty-six of these cells provided useful data with recordings of sufficient quality and adequate numbers of APs to carry out data analysis. Responses to cardiac or ventilation cycles, when present, appeared to be epiphenomena superposed on the background of ongoing spontaneous activity. Such phenomena have been reported in neonatal mice and birds (Jones and Jones 2000; Sanes and Walsh 1998). The discharge patterns of 17 of these 66 useful cells (−25%) were clearly seen graphically to be in part synchronized with the mechanical action of the heart beat or with the respiratory ventilation cycle. We have designated all 17 of these cells as entrained cells. Data from these entrained cells were not used to generate metrics for spontaneous discharge activity, although data from six of the cells were used to show the effects of entrainment on period histograms and VCs. The discharge activity of the remaining well-characterized 49 SGCs (48 neurons in neonates) was designated as “spontaneous” (non-entrained), and these data served as the basis for the quantitative description of SGC spontaneous discharge activity.

Cross-correlation: period histograms and entrainment

An example of a typical period histogram of an entrained neuron is shown in Supplemental Fig. 2. Spike counts in the PHs of entrained cells showed considerable bias in spike distribution toward some phase of the ECG or ventilation cycle. VCs for such PHs (VCPH ECG and VCPH vent) were considerably >1.0 and ranged ≤27. The mean value for the pooled VCs was ~8.6 (see VCPH ECG and VCPH vent; Table 2), and this served as an illustration of the values expected from spike discharge driven in part by cardiac or ventilation cycles.

In contrast, the spike distributions in PHs from cells of the spontaneous group were relatively uniform across ECG and ventilation cycles. A typical example (VCPH vent = 1.41) is shown in Supplemental Fig. 3. Individual values of VCs for PHs from cells of the spontaneous group are shown in Fig. 2 (A: ECG, VCPH ECG; B: ventilation, VCPH vent). The distribution of VCPH for sampled spike data (●) and simulated data (△) showed considerable overlap, indicating that spike discharge in sampled neurons generally approximated the distribution for random spike discharge for both ECG and ventilation cycles. The range of VCs for both ECG and ventilation cycles was slightly larger for sampled data. Only a few cells (5) had VCs >2.0, which was the upper limit of VCs obtained from simulated data.

Summary values of the data in Fig. 2 are listed in Table 2. The mean values of VCPH ECG and VCPH vent for all sampled and simulated groups were <2.0 (Table 2) and were consistent with relatively independent discharge patterns. There were no significant differences in mean values of VCPH ECG for simulated and sampled ECG data. There was a very small difference (0.18) in the means for simulated and sampled ventilation data (VCPH vent; Table 2; P < 0.001). This difference (i.e., 0.18) was less than the SD of all other simulation groups, and in our view, was too small to be of practical importance. The means for

![Image](http://jn.physiology.org/ by 10.220.33.5 on October 29, 2017)
Acronyms are combined to form column label designations as follows: VCPH ECG is the variation coefficient from a cardiac cycle period histogram. For period results from sampled (Sampled) and simulated discharge data (Simulated). “Difference” is the difference in mean VC values for sampled and simulated data. Time histograms (THs). There were 7 VCs for the entrained’ group because 1 SGC was entrained to both ECG and ventilation thus contributing 2 VCs. Compare at various ages postconception. Spike rates ranged from 0.06 to 8.6; Table 2). Together, these results showed that entrainment by ECG and ventilation played a negligible role in the pooled VCs (ECG and ventilation) for sampled data were VCPH ECGandvent = 1.3 ± 0.5 (51) and for simulated data were VCPH ECGandvent = 1.1 ± 0.2 (51), and these may be contrasted with that for the entrained cells (i.e., VCPH ECGandvent = −8.6; Table 2). Together, these results showed that entrainment by ECG and ventilation played a negligible role in forming the activity patterns of the spontaneous group.

Spike rates

As reported by many investigators, the spontaneous discharge rate of SGCs in the neonatal kitten was considerably lower than rates observed for most cells in older kittens and adults (Walsh and Romand 1992). Figure 3 summarizes the distribution of spontaneous spike discharge rates for neonates at various ages postconception. Spike rates ranged from 0.06 to 56 spikes/s. Only two ganglion cells had spike rates >10 spikes/s. The mean spontaneous discharge rate across all 48 SGCs in neonates was 3.09 ± 8.2 (48) spikes/s. Neonatal spike rates reported here are similar to those reported by Romand (1984) for 6-day-old kittens (i.e., −4 spikes/s, figure line labeled C). This contrasts markedly with the rate of 88.6 spikes/s for an SGC recorded in the 1-mo-old kitten; a spontaneous discharge rate that falls within the upper ranges reported for mature cats (60–100 spikes/s) (Kiang 1965; Liberman 1978; Walsh and McGee 1987; Walsh et al. 1972). Romand (1984) reported the mean discharge rates for the adult as −45 spikes/s and for a 1-mo-old kitten as −27 spikes/s, and these values are represented on Fig. 3 (lines labeled A and B, respectively).

Bursting patterns

The pattern of spike discharge over time was also remarkably different for neonates in comparison to mature animals as shown in Figs. 4 and 5. Figure 4 shows examples of the original recordings from SGCs of a mature animal (top trace)

FIG. 3. Summary of discharge rates obtained from neonates in this study (mean rate = 3.08 ± 8.2 spikes/s, n = 48). Graph reflects spike rate (spikes/s) distribution as a function of neonatal age in days postconception (dpc). Days 69 to 75 dpc correspond approximately to P3–P9 postnatal days in this study. Forty-eight neurons are represented. One outlier at 56 spikes/s was obtained from a very short record of 6 s and thus may not well represent the general spike rate of the cell. Mean recording duration for the group of neurons was ~151 s. Horizontal lines labeled A, B, and C reflect mean spike rates reported by Romand (1984) for adult cat (A > −45 spikes/s) and 1-mo-old (B > −27 spikes/s) and 6-day kitten (C ∼ 4 spikes/s).

The pattern of spike discharge over time was also remarkably different for neonates in comparison to mature animals as shown in Figs. 4 and 5. Figure 4 shows examples of the original recordings from SGCs of a mature animal (top trace)
and a neonate (bottom trace). The discharge in the mature animal reflected relatively continuous, irregular spike discharges that contained no appreciable long periods of inactivity, whereas the discharge pattern in the neonate occurred in a series of bursts interrupted by long silent periods. The variation in discharge rate over much longer periods of time is better appreciated in discharge rate plots as shown in Fig. 5. The panels of Fig. 5 show results from the same two cells shown in Fig. 4. Each point in the plots of Fig. 5 represents the onset time (x-axis, s) of a neural spike discharge and equivalent spike rate (y-axis, spikes/s), where spike rate was calculated as reciprocal of time interval between current and previous spike. Neuron represented in A (g001D) was recorded from a 1-mo-old kitten (102 dpc) and displays a rather continuous stochastic discharge pattern. In contrast, neuron in B was recorded from a 71-dpc (P5) neonate, sg15014D. B: striking repetitive periods of high activity separated by periods of low activity or silence. Scales for time axes are considerably different for A and B. Overall spike discharge rates were also remarkably different. Numerical summary: A, age = 102 dpc (P36), spike rate = 88 spikes/s, CVi = 0.89; BI = 0.9; B, age = 71 dpc (P5); spike rate = 1.8 spikes/s; CVi = 4.1; BI = 8.8; burst rate = 4.1 bursts/min. ECG and ventilation (Vent) timing marks are indicated.

Bursting patterns represent a substantial deviation from the normal quasi-Poisson discharge pattern of a mature SGC, and for this reason, the CV of spike intervals (CVi) in the neonate was considerably >1.0 (mean CVi = 2.9 across neurons). In addition, the BI provided a more specific metric that emphasized the contrasts between multiple long silent and active periods. A BI value of 8.8 for cell sg15014D of Fig. 5B is consistent with its prominent bursting pattern. Bursting patterns were apparent in graphic records when recordings were of sufficient length to capture several sequential active and quiet periods of discharge. This required relatively long continuous recordings. The average record duration was ~151 s. Bursting discharge patterns were characteristic of all but one (k001bD) neuron in neonates. Only three neurons had BI values <2.0, and only the aforementioned single cell showed the absence of bursting based on the graphical discharge record and on the values for CVi and BI (k001bD: CVi = 1.08; BI = 0.78).

CVi ranged from 1.08 (1 nonbursting cell) up to 10.4, and the mean CVi was 2.9 for all nonentrained neonatal neurons. The mean BI for the group was 5.2 and BI ranged from 0.78 (1 nonbursting cell) to 16.5. In all but one cell, CVi and BI values were >1.1, reflecting dominant nonstochastic discharge patterns associated with the basic spontaneous spike activity and the presence of prominent periods of both silence and high activity (bursting). The overall distributions of values for both CVi and BI were independent of the duration of the recorded activity and CVi was independent of the mean discharge rate. These distributions of CVi and BI are shown in Figs. 6 and 7. Salient quantitative features of spontaneous activity in the neonatal and 1-mo-old kittens are summarized in Table 3.

Bursting patterns were also evident from the THs of spike discharge records. Figure 8 shows the VC values for the spike THs (VCTH values) of nonentrained SGC neurons. One can gain an appreciation for how bursting varied across SGC cells from Fig. 8. VCTH values for sampled (●) and simulated (△) data showed striking differences in distributions. More pronounced bursting is associated with larger VCTH values. Those SGC neurons with the lowest VC values for sampled data were cells with very low discharge rates and relatively few spikes. The mean values for sampled and simulated data were substan-
tially different whether the analysis time bins were based on the ECG period or ventilation period (see VCTH ECG and VCTH vent, \( P < 0.001 \); Table 2). The means for the pooled VCTHs (ECG and ventilation) for sampled data were VCTH ECG and vent = 8.2 ± 8.9 (49) and for simulated data were VCTH ECG and vent = 1.2 ± 0.4 (49). These remarkable differences further showed the nonstochastic nature of spontaneous SGC bursting discharge patterns for the group of neurons as a whole.

**Burst rates**

Generally, the period of time between recurring bursts was not constant for a given cell, but rather, it varied considerably throughout long records. The ganglion cell sg15014D shown in Fig. 5B provides a good example of such variation. Burst rate ranged from 0.3 to 23.4 bursts/min for the cells of the spontaneous group. The mean rate was 4.3 ± 5.0 (36). The distribution of burst rates across cells is shown in Fig. 9. For most cells (92%), burst rates were <11 bursts/min, and the majority of these were <5 bursts /min. There was no evidence of a systematic increase in burst rate with age in the kitten.

### TABLE 3. Summary data for spontaneous discharge activity

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<th>Age</th>
<th>Duration</th>
<th>Spikes</th>
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<th>Interval</th>
<th>CVi</th>
<th>BI</th>
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Mean, SD, and number of neurons \((n)\) are presented for several discharge metrics including recording duration \( (\text{Duration}, \text{s}) \), total number of spikes \( (\text{Spikes}) \), discharge rate \( (\text{Rate}, \text{sp/s}) \), interspike interval \( (\text{Interval}, \text{ms}) \), interval coefficient of variation \( (\text{CVi}) \), burst index \( (\text{BI}) \), burst rate, \( (\text{bursts/minute}) \). Means do not include cells evidencing modulation (i.e., entrainment) by cardiac or ventilation cycles. NA, not applicable.

### DISCUSSION

The results of this study show that spontaneous discharge activity in SGCs of prehearing kittens is dominated by repeating bursts of neural discharge interspersed with periods of low activity. This rhythmic bursting discharge activity occurs despite overall low discharge rates. The pattern of activity is fundamentally different from the tonic quasi-Poisson spontaneous activity found in mature SGCs. Bursting patterns occurred in the absence of sound stimulation and were independent of potential stimuli associated with cardiac and ventilation cycles. With the possible exception of being slightly less
regular in burst-to-burst period, the recurrent bursting was similar to that reported for ganglion cells in the prehearing bird (Jones et al. 2001). Burst rates for prehearing chicken embryos ranged from 1 to 54 bursts/min, with only one cell producing a rate >30 (means: through stage 39 = >9.8 burst/min, through stage 43 = >35 bursts/min) Based on the average interburst interval reported, Gummer and Mark (1994) apparently observed burst rates on the order of 7.5–95 bursts/min. The range of burst rates in the kitten (i.e., ~0.3–23) overlapped the lower end of those reported for the chicken and wallaby. We did not see a systematic increase in burst rate with age in the kitten as seen in the bird (Jones et al. 2001).

The spontaneous discharge rates of SGCs of early neonates reported here and elsewhere (P2; Carlier et al. 1975; Romand 1984; Walsh and McGee 1987) were very low, and it is reasonable to question whether such low discharge rates would be effective in influencing synaptic refinements in higher-order auditory projections. There is evidence in neonates, however, that central postsynaptic neurons of auditory projections exhibit unusual substantial and prolonged responses to single presynaptic spike discharges, and respond even more dramatically to bursts of such activity (Kotak and Sanes 1995). Thus neonatal central circuits may be predisposed to respond to isolated spikes and bursting patterns present in neonatal SGC neurons.

Distinguishing the roles of spontaneous and driven rhythmic activity in neonatal kittens

Stimulus-driven rhythmic responses of auditory nerve fibers have been reported during the first postnatal week in kittens (Carlier et al. 1975; Pujol 1972; Walsh and Mcgee 1986, 1988). This sound evoked activity should be distinguished from the spontaneous rhythmic patterns shown here for two reasons. First, given the high thresholds of auditory fibers to sound (>90 dB SPL), it is unlikely that external acoustic stimuli play any significant role in driving activity patterns before P10. Second, even if sound levels were sufficient to activate cells during this period, one might expect external stimuli to interfere with the normal endogenous spontaneous rhythms. Hypothetically this could degrade rather than contribute to activity-dependent processes underlying refinements and segregation centrally.

Implications of these findings for auditory development

There are at least two important questions to address. First, what mechanisms give rise to the bursting rhythms in SGCs, and second, what possible role does such activity play in development?

BURSTING MECHANISM. We have argued previously that, in the absence of sound stimuli, the discharge patterns of SGCs in prehearing animals as well as in the mature animal likely arise endogenously from within the cochlear neuroepithelium (Jones and Jones 2000; Jones et al. 2001, 2006). Our hypothesis is that SGC discharge patterns depend on the release of excitatory neurotransmitter from hair cells at afferent synapses. Therefore the hair cell is seen as the primary source of excitation for spontaneous SGC bursting activity. This hypothesis is consistent with long held ideas about spontaneous ganglion cell discharge in mature animals and is supported by considerable evidence (Adrian 1943; Annoni et al. 1984; Flock and Russel 1976; Furukawa and Ishii 1967; Furukawa et al. 1972; Glowatzki and Fuchs 2002; Harris and Flock 1967; Hudspeth 1986; Ishii et al. 1971; Katz 1969; Rossi et al. 1977; Schessler et al. 1991; Siegel 1992; Siegel and Dallos 1986). However in the mature animal, excitation is a relatively steady stochastic process. Given the nonstochastic bursting characteristics of SGC activity in the prehearing animal, we presume that a corresponding nonstochastic excitatory process must exist to generate such activity. Indeed, there is evidence suggesting that the inner hair cell (IHC) itself could provide the mechanism. During prehearing stages, IHCs are capable of generating spontaneous recurrent action potentials [mammals: (Kros et al. 1998; Marcotti et al. 2003a,b, 2004); birds: (Fuchs and Evans 1990; Fuchs and Sokolowski 1990; Sokolowski and Cunningham 1999)]. Here we propose that spike discharge in the IHC leads to the discharge of SGCs and recurrent IHC spikes form the basis for the bursting patterns observed in this study. The cochlear neuroepithelium is likely competent to mediate such activity at these ages in that the synaptic apparatus is present, postsynaptic glutamate receptors are expressed, and they are functional (Glowatzki and Fuchs 2002; Knipper et al. 1997; Luo et al. 1995; Sobkowicz et al. 1982). Moreover, IHC discharge has been shown to trigger exocytosis (Beutner and Moser 2001) and excite SGC terminal boutons (Glowatzki and Fuchs 2002). The ability of IHCs to generate spikes is transient, but it exists precisely during neonatal prehearing periods and is lost with the onset of hearing.

ACTIVITY-DEPENDENT MECHANISMS. COCHLEOTOPIC REFINEMENT, AND SPONTANEOUS BURSTING DISCHARGE PATTERNS. As outlined in the Introduction, waves of spontaneous rhythmic discharge appear in the retina before the onset of vision. These patterns of activity are thought to serve Hebbian processes (Hebb 1949) operating centrally during the refinement of retinotopic projections (Katz and Shatz 1996; Ruthazer and Cline 2004). Retinal waves serve to synchronize the discharge of ganglion cells of small focal retinal regions, and this process depends critically on local retinal networks (see Feller 2002). The spatiotemporal pattern of activity is thought to be more important than the mere presence of ganglion cell activity. Indeed, the evidence suggests that central refinement and segregation requires correlated activity arising from spatially localized receptive areas on the retina (McLaughlin et al. 2003c; Mrsic-Flogel et al. 2005; Ruthazer and Cline 2004).

The rhythmic bursting patterns in SGCs of the kitten are reminiscent of retinal discharges. If this SGC activity serves Hebbian cochleotopic refinement processes centrally, there must be a mechanism that can coordinate the activation of neighboring SGC to produce a correlated discharge. To our knowledge, circuitry like the cellular networks generating retinal waves does not exist in the cochlea. However, there are other unique features of cochlear afferent innervation that can lead to a correlated pattern of discharge in small groups of adjacent SGCs.

Generally, in the mature cochlea, each radial SGC innervates only one IHC, although each IHC is innervated by a group of 20–40 SGCs (Liberman 1980, 1982; Spoendlin 1969). This anatomical fact ensures that the activity of all SGCs innervating one IHC is strictly dependent on and thus correlated with activity in the common presynaptic receptor cell. A somewhat
less precise but similar configuration is already present at birth in the mammal (Perkins and Morest 1975; Simmons et al. 1991). According to these reports, during the first 2 neonatal days, the vast majority of SGC dendrites branch to innervate two or three adjacent IHCs, although occasionally more contacts are seen (≥8). Thereafter, dendritic branching and multiple IHC contacts decrease abruptly, and by P3, >60% of SGCs innervate a single IHC. By the end of the first neonatal week, the adult configuration is in place.

Given the common termination of scores of ganglion cells on a single IHC, each IHC must coordinate the activity of a group of SGCs. At the earliest ages, some ganglion cells might be activated by two or more IHCs, but this likely resolves quickly to an exclusive functional relationship with one IHC. Because IHCs are capable of regenerative spike discharge and excitation of SGC terminal boutons at these stages (as discussed above), it follows that such discharge would lead to the correlated activity of scores of SGCs. We hypothesize that for each individual SGC recorded in this study, there were scores of companion SGCs that discharged in a linked coordinated manner. In the presence of repetitive IHC spike discharge, SGCs would exhibit correlated recurrent bursting discharge activity. Ultimately, such correlated activity would be linked to the position occupied by a single IHC in the organ of Corti. This hypothesis simultaneously accounts for the required correlated activity and the strict local cochleotopic organization necessary to drive central Hebbian refinement processes.

An additional mechanism capable of orchestrating correlated activity among IHCs within a small region of the organ of Corti may be required to further refine the spatial organization of higher-order cochleotopic groups. Cochlear efferent terminals are known to form transiently on IHCs in the neonate and one possible mechanism is that activity in these efferent terminations could serve to modulate and thus correlate activity among IHCs locally (Glowatzki and Fuchs 2000; Goutman et al. 2005; Walsh and Mcgee 1997). A complete picture of factors contributing to the patterns of activity in IHCs and SGCs has yet to emerge. It is conceivable that correlated activity of IHCs and in turn SGCs also contributes to ear-specific segregation and refinement at levels of the auditory pathway where binaural projections converge. The working hypotheses outlined above remain to be critically tested.

In addition to guiding central refinements, the recurrent IHC spike discharge may be responsible in part for refinement of SGC terminal contacts in the organ of Corti itself. Although, IHCs are clearly capable of sustaining recurrent spike discharges in the neonate, the regulation of such discharge in vivo remains an exciting open question.

The spontaneous bursting patterns reported here for the kitten were similar to patterns found in cochlear ganglion cells of the early prehearing chicken embryo (Jones et al. 2001) and both patterns remind us of spontaneous retinal discharges. Despite many differences in the cochleas of birds and mammals, the discharge similarities during prehearing periods may reflect shared developmental processes. Whether the working hypothesis proposed here for the mammal applies to the bird is an interesting question and remains to be explored.

These results are consistent with the hypothesis that spontaneous rhythmic bursting activity in SGC of prehearing mammals plays a critical role in the refinement of central cochleotopic projections. Clear from the present study is the fact that these spontaneous discharge patterns are present during the period of final refinement of cochleotopic projections from the cochlea to the cochlear nucleus (Leake et al. 2002). Furthermore, the rhythmic bursting discharge of ganglion cells occurs in vivo precisely during the transient prehearing period following the onset of repetitive IHC spike discharge in vitro (Kros et al. 1998; Marcotti et al. 2003a,b, 2004), the appearance of synaptic ribbons and onset of IHC exocytosis (Beutner and Moser 2001; Glowatzki and Fuchs 2002; Sobkowicz et al. 1982), and during the stabilization of terminal contacts of individual SGCs onto single IHCs and reorganization of efferent terminals (Glowatzki and Fuchs 2000; Goutman et al. 2005; Perkins and Morest 1975; Simmons et al. 1991). Finally, given the putative critical role for the IHC in the hypothesis above, it is worth noting that cochleotopic refinements in the cochlear nucleus fail to occur in neonates when IHCs are destroyed at birth by ototoxic drugs (Leake et al. 2006). Such a lesion would, among other things, eliminate normal spontaneous activity in SGCs. Together, these observations suggest both a mechanism for stimulus-independent rhythmic bursting of ganglion cells and a possible role for such discharge patterns in guiding the prehearing activity-dependent refinement in central auditory relays.

Acknowledgments
We thank M. Jensen and F. Foley for excellent assistance.

Grants
This study was supported by National Institute of Deafness and Other Communication Disorders Grants 5R01-DC-00160 to P. A. Leake and R01-DC-02753 and R01-DC-003776 to T. A. Jones.

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J Neurophysiol • VOL 98 • OCTOBER 2007 • www.jn.org


