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Original Paper

Temporal population code of concurrent vocal signals in the auditory midbrain

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Abstract. Unique patterns of spike activity across neuron populations have been implicated in the coding of complex sensory stimuli. Delineating the patterns of neural activity in response to varying stimulus parameters and their relationships to the tuning characteristics of individual neurons is essential to ascertaining the nature of population coding within the brain. Here, we address these points in the midbrain coding of concurrent vocal signals of a sound-producing fish, the plainfin midshipman. Midshipman produce multiharmonic vocalizations which frequently overlap to produce beats. We used multivariate statistical analysis from single-unit recordings across multiple animals to assess the presence of a temporal population code. Our results show that distinct patterns of temporal activity emerge among midbrain neurons in response to concurrent signals that vary in their difference frequency. These patterns can serve to code beat difference frequencies. The patterns directly result from the differential temporal coding of difference frequency by individual neurons. Difference frequency encoding, based on temporal patterns of activity, could permit the segregation of concurrent vocal signals on time scales shorter than codes requiring averaging. Given the ubiquity across vertebrates of auditory midbrain tuning to the temporal structure of acoustic signals, a similar temporal population code is likely present in other species.

Keywords. Hearing - Signal segregation - Ensemble coding - Correlated firing - Temporal patterns

Abbreviations. *ACF*: autocorrelation function *dF*: difference frequency *ISI*: interspike interval *SDF*: spectral density function *VSdF*: vector strength of synchronization to difference frequency

Introduction

Specific patterns of activity among neuronal ensembles have been proposed as a mechanism of coding complex signals and scenes (Meister *1996*; Laurent *1999*; Castelo-Branco et al. *2000*). Comparisons with single-unit data indicate that combining the temporal firing patterns of small ensembles of neurons can substantially improve sensory information coding (Warland et al. *1997*; also see Laubach et al. *2000*). Yet, how patterns of neural firing correlate with complex signals across a large population of neurons, how they change with variation in signal parameters, and how they relate to individual neuron tuning, still remain largely unanswered questions in many systems. Here, we use multivariate statistical analysis to assess the response patterns of a large population of midbrain neurons to concurrent vocal signals and establish their relationship to the tuning of individual neurons.

Nesting male plainfin midshipman (*Porichthys notatus*) produce long-duration multi-harmonic signals known as hums to attract females to their nest (Brantley and Bass *1994*). The concurrent hums of neighboring males produce periodic amplitude and phase modulations at the difference frequencies (dFs) between their spectral components with dFs <10 Hz (Fig. 1a; review: Bass et al. *1999*). Behavioral experiments show individuals can segregate concurrent signals with small dFs (McKibben and Bass *1998*; D.A. Bodnar and A.H. Bass, unpublished data). Previous studies have demonstrated that midbrain neurons synchronize spike bursts and exhibit differential tuning to beat dFs (Bodnar and Bass *1997*). Approximately 35% of midbrain neurons show moderate synchronization to all dFs between ±10 Hz (broad dF selective; Fig. 1b). The remaining units show distinct peaks at a particular dF (narrow dF selective units). The majority of narrow dF selective units (75%) show symmetrical peaks at both positive and negative dFs (4 Hz, 8 Hz; Fig. 1b). The remaining narrow dF selective neurons have a single asymmetric peak at one dF (narrow dF sign-selective units; not shown). These data suggest that temporal patterns of activity will vary across different dF tuned cell groups in response to beats with different dFs. Hence, there is a potential temporal population code for dF within the auditory midbrain.

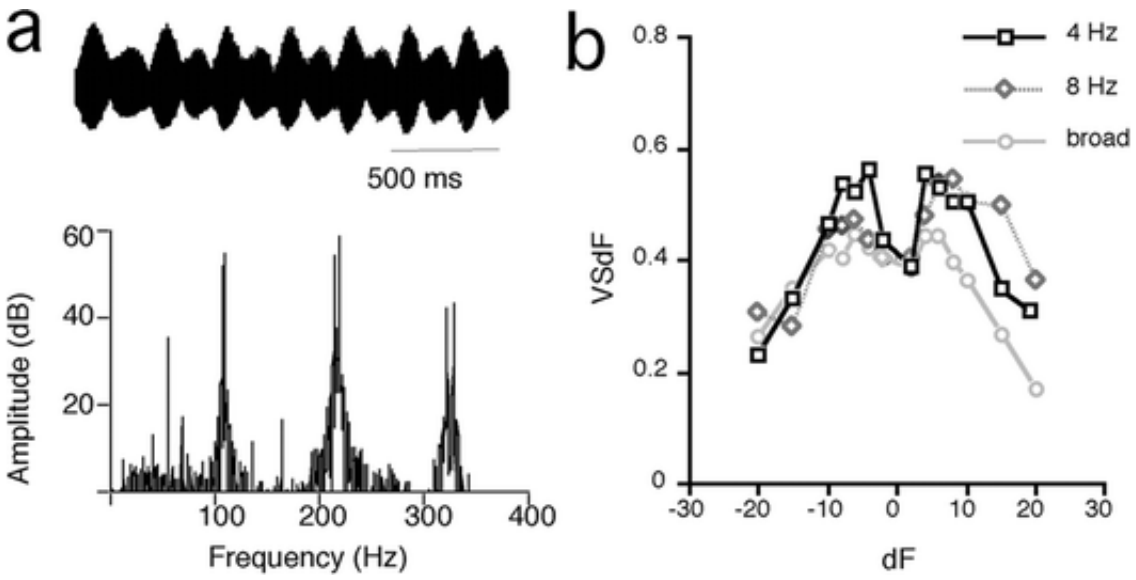

Fig. 1a,b. Midshipman concurrent vocal signals and their encoding by individual midbrain neurons. **a** An example of a beat waveform produced by concurrent hums of individual males and the amplitude spectrum of the waveform. The amplitude modulations in the signal occur at the difference frequency (dF) between the fundamentals and upper harmonics which can be seen by the double peaks in the amplitude spectrum. **b** Examples of the vector strength of synchronization to dF (VS_{dF}) versus dF of individual midbrain neurons (adapted from Bodnar and Bass *1997*). VS_{dF} measures the degree of phase locking to dF. The plots show a neuron with broad dF tuning (no significant changes in dF synchronization for dFs between ±10 Hz), and units with symmetrical tuning (peak synchronization to a particular dF, both positive and negative) to dF=4 Hz or 8 Hz

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Here, we test the hypothesis that a population code of patterned spike activity emerges within the midbrain and is at least in part the result of dF tuning of individual neurons. Using multivariate statistical methods, we test for changes in patterns in overall firing rates as well as the relative magnitude and phase of dF-dependent spike activity among different dF-tuned cell groups. Our results show that across a large population sample of neurons from multiple animals, there are no significant differences in firing rates of neurons in response to beats of different dFs. However, for beats with different dFs, the relative magnitude and phase of dF-dependent activity varied between units that differ in their dF tuning. These data establish the presence of a temporal population code for dF within the auditory midbrain that is derived from the tuning of individual neurons.

Materials and methods

Surgical procedures, recording methods, and stimulus system have been described previously (Bodnar and Bass *1997, 1999*). Single-unit responses were measured for a standard stimulus set of beats in which one frequency component was held constant at 90 Hz and the other varied from ±10 Hz in 2-Hz increments and at 12 dB above threshold for most units. We compiled the responses of 83 single units from 36 animals (1-6 units/animal) into a composite population, thus providing an assessment of population coding across a large number of neurons. These neurons formed the basis of analysis in previous studies (Bodnar and Bass *1997, 1999*).

In our population, we included neurons with broad and symmetrical dF tuning, but excluded narrow dF sign-selective neurons (see above). This was done because one of our interests was to determine whether the sign of dF (positive or negative) is encoded within the population output of symmetric narrow dF-selective and broad-selective units. Given that the spike outputs of sign-selective units already carry this information, their activity will enhance whatever patterns are revealed in the analysis of symmetrical and broad units.

To quantify patterned activity, autocorrelation functions (ACFs) and their spectral density functions (SDFs) were computed for the spike trains of each neuron. The ACF of a spike train measures the degree of similarity in the occurrence of spikes for a given time delay (see Gabbiani and Koch *1998* for an overview of the method). Peaks in the ACF indicate repeated patterns of activity at specific time intervals throughout the spike train. The SDF is the Fourier transform of the ACF and measures the magnitude of the dF-dependent spike activity detected within the ACF. This method has been previously utilized in a number of studies assessing auditory responses to multiharmonic signals (Simmons and Ferragamo *1993*; Simmons et al. *1993*; Cariani and Delgutte *1996a*, *1996b*).

For data presented here, spike trains were binned at 5 ms. In an initial preliminary analysis of some datasets, spikes were also tested at bin sizes of 1 ms, 2 ms, 8 ms, and 10 ms (Fig. 2). The peaks of the autocorrelation function were augmented with larger bin size while the magnitude of the dF component of the SDF of each cell group increased linearly. This indicates that the relative contribution of the cell groups to patterned activity was maintained with changes in bin size. The average of spike rate of midbrain neurons across dFs is approximately 20 spikes s^{-1} which has a corresponding interval size of 50 ms. A bin size of 5 ms was chosen in order to maximize the magnitude of the dF component of the SDF for statistical analysis while maintaining a relatively small bin size compared to the interval size predicted by the spike rate (10%).

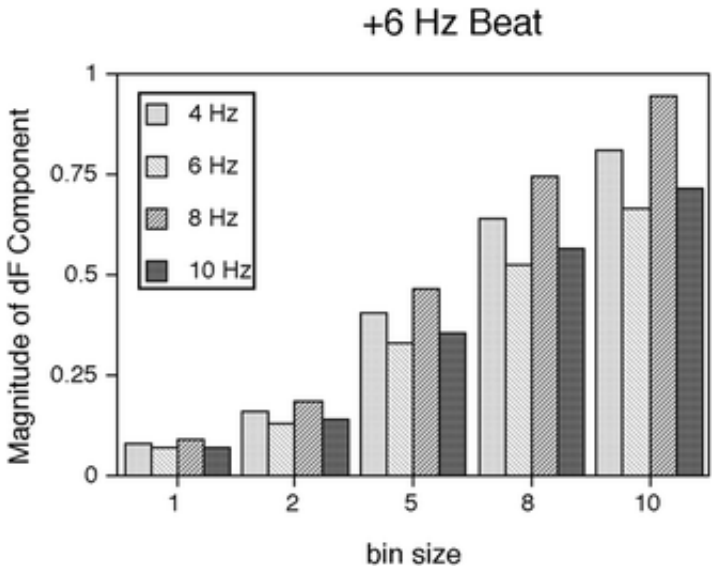

Fig. 2. Effect of bin size on the dF component of the SDF. A histogram of the dF component of the spectral density function (SDF) of the narrow dF selective cell groups for autocorrelation functions (ACFs) computed for a +6-Hz beat with different bin sizes (1 ms, 2 ms, 5 ms, 8 ms, and 10 ms). The height of each bar represents the magnitude of the dF component of the SDF (*y*-axis) for each cell group at a given bin size (*x*-axis)

Examples of the ACFs and SDFs for four individual neurons in response to a +6 Hz beat are shown in Fig. 3a, b. The units are from the same animal, but have different dF tuning. All four units show periodic changes in their ACFs at time delays of approximately 160 ms, corresponding to 6 Hz dF. This periodicity and its magnitude is shown in the corresponding SDFs for each unit. Here, the magnitude of the 6 Hz unit actually has the lowest SDF value.

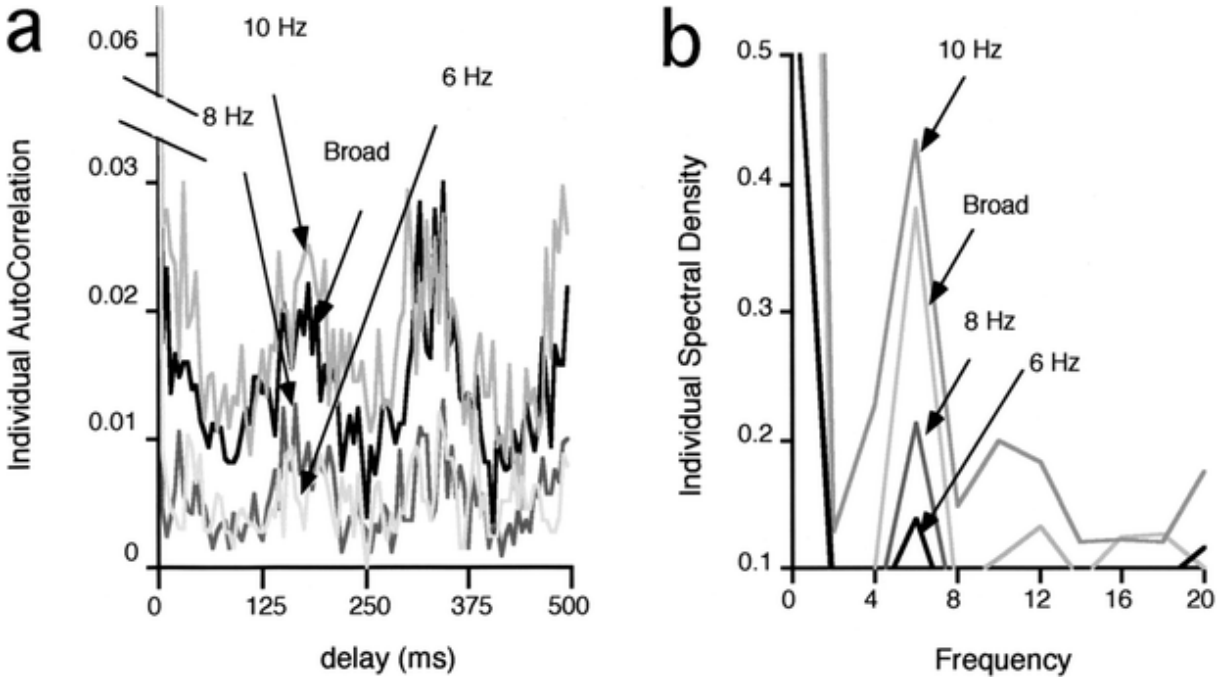

Fig. 3a,b. Examples of ACFs and SDFs of individual neurons. **a** The ACFs of four midbrain neurons from the same animal with different dF tuning (6 Hz, 8 Hz, 10 Hz, Broad) in response to a +6-Hz beat. All four units show an apparent periodicity in their ACF at 6 Hz. **b** The SDFs of the ACFs of the neurons in **a**. All four units show a distinct peak in their SDF at 6 Hz

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To compare dF-dependent spike activity, the d.c. (0 Hz) and dF components of the SDFs of individual units were measured in response to each beat stimulus. The magnitude of the d.c. component reflects the mean firing rate of a unit, while the dF magnitude reflects the variability in the spike activity due to dF periodicity in the signal. The magnitude of the dF component can also be approximated by the product of the vector strength of synchronization to dF and spike rate (after Rees and Palmer *1989*; also see Bodnar and Bass *2001a*). The computation of ACFs and SDFs was used here because analysis over a large number of neurons and dFs was more easily executed. In addition, the mean phase angle of firing during the dF period was computed separately for each unit. The mean phase of firing indicates the time point within the beat cycle that periodic spike activity is centered around.

Graphical displays of average values of variables for different dFs can show some differences both in terms of general trends as well as more complex patterning. However, the variance of values largely determines whether these changes are significant. For example, small differences that are only barely discernable in a graphical display, may be significant if the variability in the parameter is small across dFs. To ascertain whether these visually identified differences had any validity, we used a repeated measures ANOVA with two "within factors" and two "between factors" to test the data sets (SuperANOVA software). The "within factors" were stimulus dF and the sign of dF. The "between factors" were dF-tuned cell type and individual animals. This type of multivariate analysis allows one to assess the general effects of different factors as well as interactions between them. A significant general effect of a factor indicates that across all other factors there was an overall change in the variable with respect to that factor. For example, a significant general effect of dF indicates that all cell types and individuals show the same general changes in response with changes in dF. A significant interaction between two factors indicates that there are different patterns of change within one factor due to the other factor. For example, a significant interaction between dF and cell type indicates that within each cell type group, changes in dF produce different patterns of change.

The design of our stimulus system imposed certain limitations on our interpretation of the effects of different factors on the phase of firing. Specifically, the possible presence of frequency dependent phase shifts in the loudspeaker response could not be ruled out. Because the speaker enclosure is completely sealed to prevent water leakage, the phase of movement of the diaphragm could not be measured. Therefore, we could not determine whether any general effects of dF or sign were due to phase shifts in the stimuli rather than neuron response; hence, these parameters were not considered in our analysis. However, relative changes in phase of firing between groups as detected by an interaction between dF and cell group were still valid, as these could not result from phase shifts in the speaker output alone.

In our analysis, we included individual animals as a factor in order to minimize possible effects of biases created by multiple samples from the same individual in the analysis of the effects of other factors. In addition, we also performed a two within (dF and sign) and one between (cell type) repeated measures ANOVA on a modified data set in which we averaged the values for neurons from the same animal within the same dF cell type (individual could no longer be a factor as there was never more than one individual within cell group). This removes sampling biases within a cell group. Any correlations between neurons from the same animal but in different cell groups would tend to obscure detection of patterns between cell groups. Hence, the presence of significant interactions between cell type and dF or sign indicates an effect robust enough to outweigh any within animal correlations.

Results

The stochastic nature of neural activity most often makes it difficult to assess the temporal relationships of activity across a large population of neurons with varying response characteristics from raw spike train data. Specifically, the responses of neurons vary widely both in terms of spike rate and synchronization characteristics. In addition, neurons also show some variance in their spike times from repetition to repetition. Thus, it is difficult to discern visually to what degree there is, on average, a consistent temporal relationship in the activity of neurons of a given cell type and between neurons of different types. Pooled ACFs provide a useful means of visualizing and quantifying the patterns of activity of neurons across a large population of neurons. The ACF of an individual neuron detects the time intervals at which spike activity regularly occurs. Strong peaks in pooled ACFs thus indicate time intervals at which correlated activity across a group of neurons repeats throughout the signal.

The mean ACF of each dF-tuned group in response to a beat (+6 Hz) is shown in Fig. 4a. The ACF of each cell group shows a peak at approximately 167 ms and 333 ms indicating that all the groups have some degree of correlated firing at these time intervals. Furthermore, the activity patterns appear to repeat throughout the population with a periodicity equivalent to dF. This is verified by the peak in the SDF function at the 6 Hz dF for each cell group (Fig. 4b). However, the magnitude of this peak varies across the cell groups, which suggests that the composition of the pattern ensembles in the ACF is determined by the dF tuning of individual units.

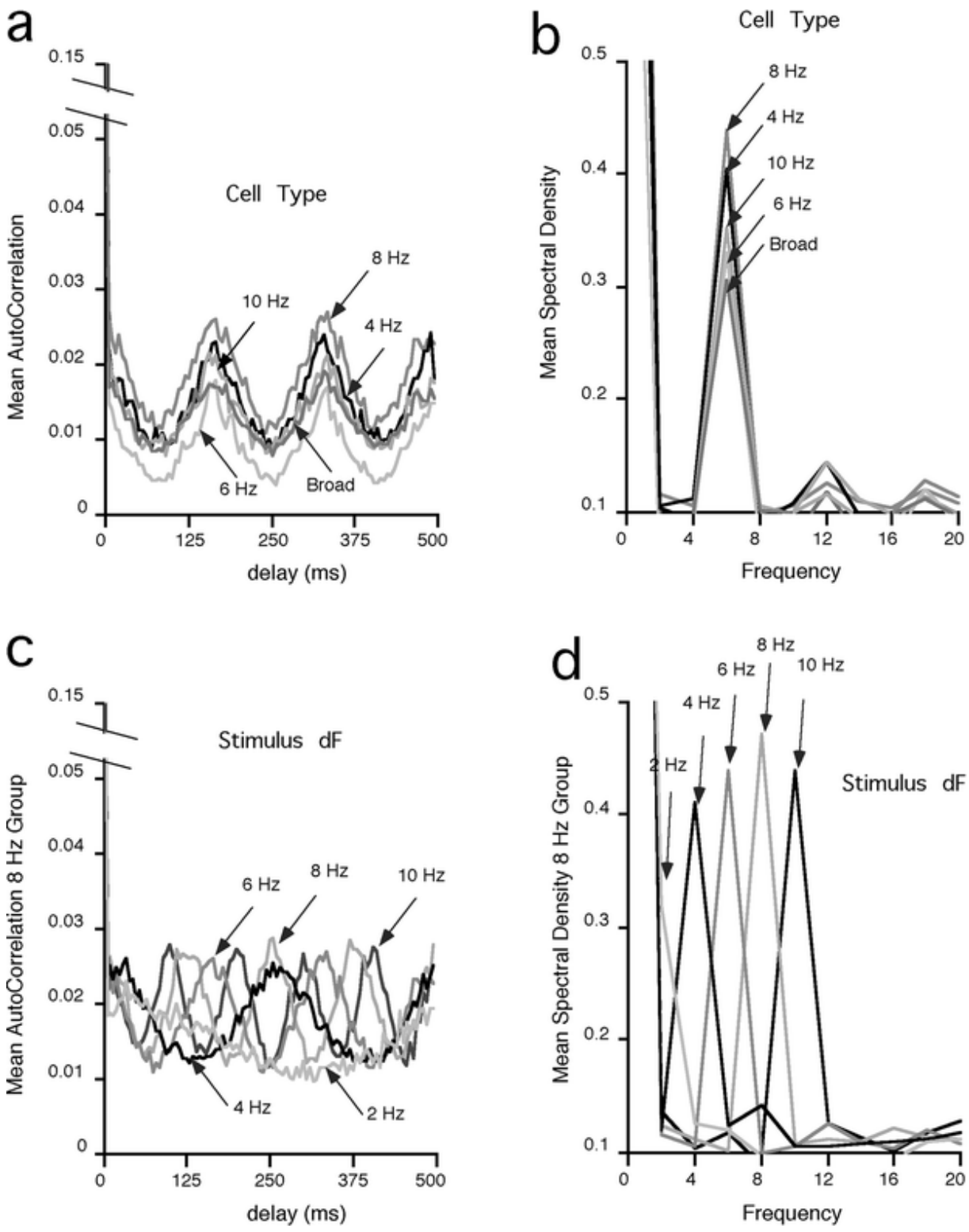

Fig. 4a-d. Midbrain neuron dF cell group responses to beat signals. **a** The mean ACF of each dF cell group in response to a +6-Hz beat. **b** The mean SDF of each dF cell group in response to a +6-Hz beat. **c** The mean ACFs of the 8-Hz cell group in response to beats with dF=+2 Hz, +4 Hz, +6 Hz, +8 Hz, and +10 Hz. **d** The mean SDFs of the 8-Hz cell group in response to beats with dF=+2 Hz, +4 Hz, +6 Hz, +8 Hz, and +10 Hz

The ACFs of one dF cell group (8 Hz), in response to beats with varying dFs, are shown in Fig. 4c. In response to each dF, this cell group shows periodicity in its ACF corresponding to dF. However, the magnitude of the dF components of the SDFs of the ACFs of each beat response varies as dF changes (Fig. 4d). This indicates that temporally patterned activity within a cell group depends on stimulus dF.

In periodic signals, the phase angle of firing reflects the relative timing of spike activity during a stimulus cycle. Examples of the phase distributions for two different dFs are shown in Fig. 5a, b. Each cell group had a different distribution of mean phases in response to the same stimulus. As noted in the methods, the overall phase of firing in response to changes in dF may result from frequency dependent phase shifts in the speaker. However, such phase shifts in the speaker cannot account for differences in the relative phase of firing between cell groups in response to the same signal and, hence, must result from mechanisms within the auditory system.

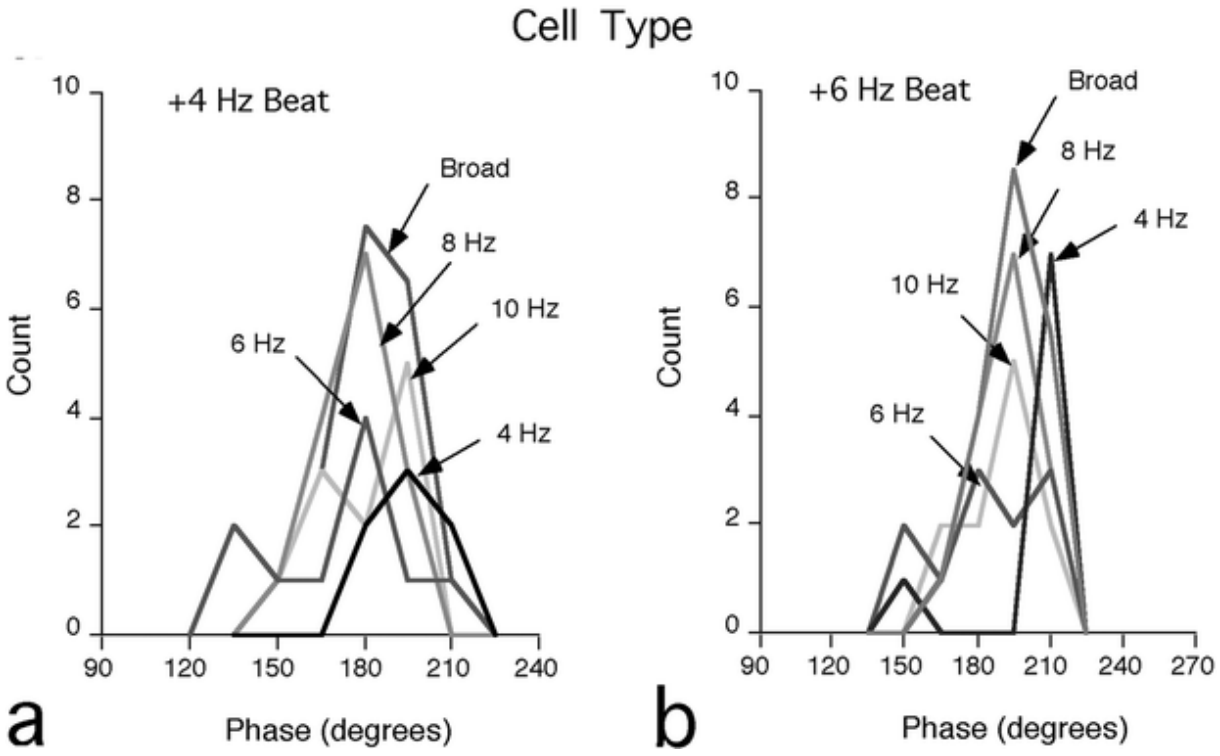

Fig. 5a,b. The relative mean phase of firing for population responses of midbrain neurons beat signals. Each plot shows the distribution of mean phase of firing of each dF cell group in response to a +4-Hz beat (**a**) and a +6-Hz beat (**b**). In both cases the broad cell group is scaled by one-half

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Together, the ACF and phase data suggest that different patterns of activity may arise with variation in the dF of beat stimuli, thereby providing a population code of dF. To directly test this hypothesis, we compared the magnitude of the d.c., dF components of the SDF, and mean phase of firing across dF cell types for each beat dF. The plots in Fig. 6 show these values for each cell group in response to each dF; the left column shows responses to -dF beats while the right column shows responses to +dF beats. These plots show the mean values of the designated parameter for each cell type across each dF. Each stimulus dF is represented by a gray scale while the magnitude of a value is represented by the height of each bar. A particular pattern of activity across the population is related to the relative parameter values of each cell type along a given dF. Thus, variation in these relative values with changes in dF suggest that there are differences in the patterns of spike rate, dF-dependent activity and phase of firing.

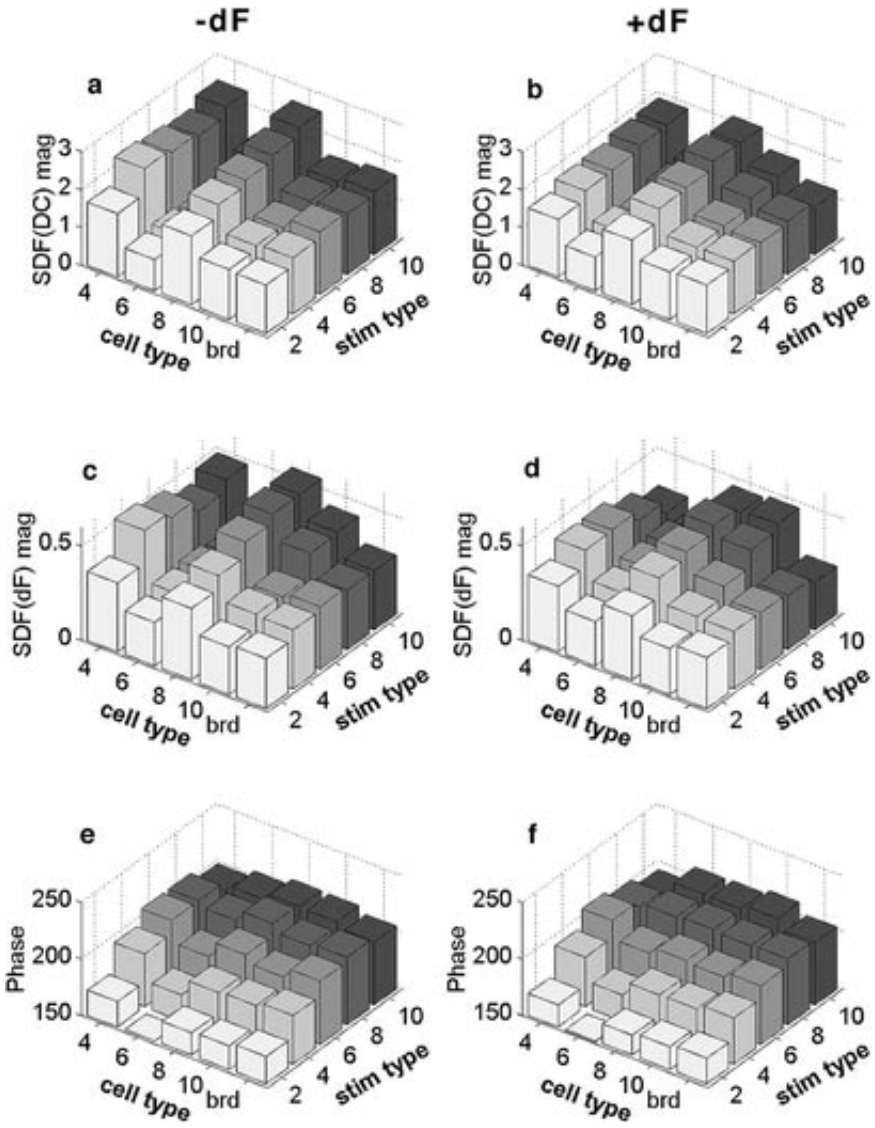

Fig. 6a-f. Patterned responses of midbrain neurons to beats with different dFs. The height (*z*-axis) of the histogram bars in each plot represents the mean value of the designated parameter with dF cell type on the *x*-axis and stimulus ±dF on the *y*-axis. Each stimulus dF is represented by a *gray scale*. **a**, **b** The d.c. component of the SDF. **c**, **d** The dF component of the SDF. **e**, **f** Mean phase of firing

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The changes in the d.c. component are shown in Fig. 6a, b. Some small differences in the relative values of d.c. values can be seen at some dFs. However, statistical tests showed no significant interaction between stimulus dF and dF cell type. Thus, spike rate does not vary among different dF cell types in response to beats with different dFs. There was also no significant effect of the sign of dF nor any interactions of the sign of dF with cell type. Thus, across the population, spike rate does not provide reliable information regarding either beat dF or the spectral composition.

The values of the magnitude of the dF component are shown in Fig. 6c, d. Here, the relative values of each cell group do appear to change across different dFs. For example, compare the relative values of +10 and -10 Hz beats. In the case of -10 Hz the magnitude of the 4 and 8 Hz groups are almost equivalent while the 10 Hz group is lower. In contrast, for +10 Hz beats the 4-Hz group is substantially lower than the 8-Hz cell group, and the 10-Hz cell group has a slightly larger dF value

than the 8-Hz group. Statistical tests verify that there was in fact a significant interaction between dF and cell type (P=0.0001). This indicates that the relative magnitude of dF-dependent spike activity varies between different dF-tuned cell types with stimulus dF. There was a significant effect between dF and individuals (P=0.0055). This indicates that there is individual variation between animals in the change of the magnitude of the dF component with changes in beat dF. When the values for neurons from the same animal within the same dF cell type were averaged together, the interaction between dF and cell type remained significant (P=0.0001). Therefore, the patterns of change in the magnitude of the dF components between cell types with changes in dF most likely do not arise from sampling biases.

Most cell groups had their greatest dF Fourier component for the beat dF they were tuned to; however, other cell groups across the population sometimes had a higher magnitude. For example, in response to 6-Hz beats, the 4-Hz, 8-Hz, and 10-Hz dF cell groups had higher dF Fourier components than the 6-Hz group. This indicates that the tuning of a neuron indicates the stimulus parameters it will most likely respond best to. However, it does not predict which cell type across the population will have the maximum response to a given stimulus parameter. Thus, while the pattern of activity across the entire population in response to a particular dF emerges from the dF tuning of individual neurons, the pattern cannot be directly predicted by this tuning.

The average values of the phase of firing for each cell group across dFs are shown in Fig. 6e, f. Although not so obvious visually, slight differences in the relative phase angles of cell groups were discernible. There was a significant interaction between stimulus dF and cell type for the mean phase of firing of units (P=0.0006). The interaction between dF and individual animals was also significant (P=0.0001), again indicating that there is variation between animals in the dF-dependent phase shifts in their responses. The significant interaction of dF and cell type was upheld when the values for neurons from the same animals within the same cell type were averaged together (P=0.0007). Thus, the relative time of peak of activity during a beat period also varies between dF tuned cell types in response to beats with different dFs.

The average standard deviation in phase angle across all cell types ranged from 13.7° for 10-Hz signals to 17.8° for 2-Hz signals. These values correspond to average variation in time of 3.7 ms for 10-Hz signals and 24.6 ms for 2-Hz signals. The average standard errors in phase angle across cell types ranged from 3.5° for 10 Hz to 5.1° for 2 Hz with corresponding average error in time of 0.94 ms for 10-Hz signals and 7 ms for 2-Hz signals.

In sum, both the magnitude and mean phase of firing of dF-dependent spike activity within a population of midbrain neurons across dF cell types varies with changes in dF. This suggests that specific temporal patterns of activity across the population arise in response to different beat dFs, i.e., a temporal population code.

Discussion

Previous single-unit recording studies of the coding of concurrent vocal signals have shown that the spike outputs of midbrain neurons synchronize to the dFs of beat stimuli and that neurons are tuned to a specific beat dF (Bodnar and Bass *1997*, *1999*). In the present study, we assessed the coding of beat dF and spectral composition at the population level using multivariate statistics for a neuronal sample collected across multiple animals. Our results suggest that the dF tuning of individual neurons produces a population code of dF as defined by varying patterns of the dF component across cell types. In addition, there was a dF-dependent pattern of mean phase of firing across cell types. Together, the data strongly suggest that within the midshipman midbrain there is a temporal population code of the dF of concurrent vocal signals given by patterns of the magnitude and phase of spike activity across

neurons with different dF tuning.

Because the magnitude and mean phase of firing varies between groups for each dF, the relative degree of correlated firing of different dF cell types will differ at any time point. This can be illustrated in a schematic drawing of spike activity within a beat period of the different dF cell groups. Figure 7 shows approximate distributions of dF-dependent spike output for each dF cell group for two dFs (+4 Hz and +6 Hz). The curves represent a combination of the magnitude and phase data for the cell-types (see Fig. 6d, f). The *x*-axis represents the relative phase of firing while the *y*-axis represents the relative magnitude of correlated firing among neurons in a group. The vertical lines designate three different time points at 15° increments. Because the absolute phase of firing is unknown due to frequency dependent phase shifts in the speaker, the responses are aligned along the peak response of the broad units (center line).

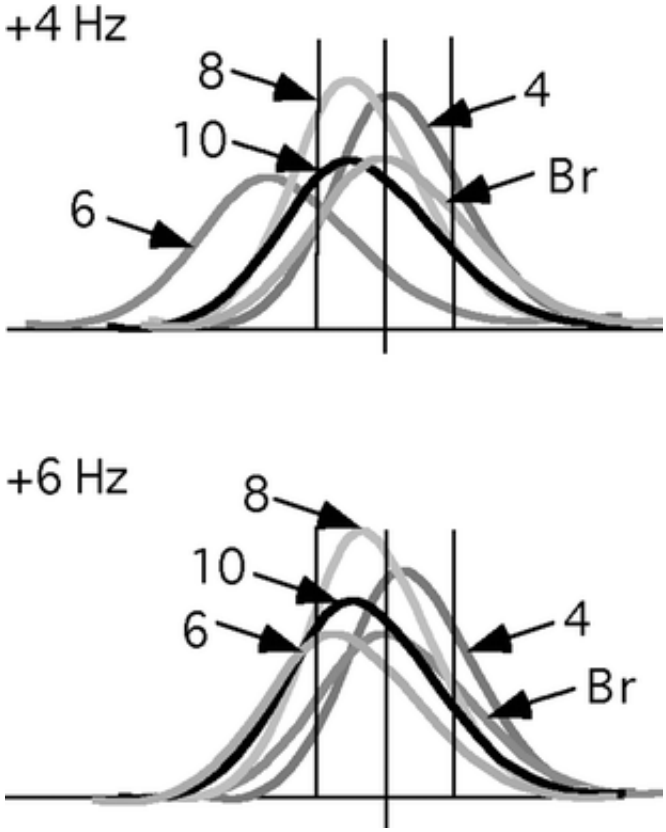

Fig. 7. Schematic of population code for two beats. Each panel shows the approximate distributions and relative phase of firing of different dF cell types for two different beats (+4 Hz and +6 Hz). The peak height of each curve is given by the magnitude of its dF component. The lines indicate three different phases of firing at 15° increments with the center line aligned with the peak value of the Broad (*Br*) dF group. The relative phases of the other dF groups determined the location of the center of each curve. Comparisons of the relative amplitude of each dF cell group along each line shows the relative contribution of each dF group to the synchronous firing pattern at that phase. This pattern is different for the two stimuli at each time slice

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Each curve represents the relative contribution of each dF group to the correlated firing patterns over time. These patterns differ between the two stimuli. For example, at the center time point of the +4-Hz beat, the contribution of each dF group in descending order is 4 Hz, 8 Hz, Broad, 10 Hz, and 6 Hz, while for a +6-Hz beat the pattern is 8 Hz, 4 Hz, 10 Hz, Broad, and 6 Hz. In addition, these patterns change with time. Thus, both the relative number of active neurons within each dF group at any point in time as well as the dynamic change in the activity of each group differ with dF.

Our analysis here for a given set of beat signal parameters (12 dB above threshold, 1 s in duration, 100% depth of modulation) suggests that the dynamic temporal patterns of midbrain neurons can provide information regarding the amplitude modulations of the beat signals. Previous studies have shown that the spike rates and strength of dF synchronization of midbrain neurons exhibit differential changes with variation of the intensity, depth of modulation, and duration of beats (Bodnar and Bass *2001a*, *2001b*). Thus, the temporal patterns of midbrain neuron activity will also differentially change with these signal parameters. Hence, dynamic temporal patterns may code a constellation of signal parameters that define the magnitude and depth of beat amplitude modulations.

Another alternative hypothesis is that a global average of the magnitude and phase of firing across the entire population serves as a code, rather than the relative patterns between different cell types. One advantage that a relative pattern code would have over a global average is that it has the potential to uniquely represent a wider range of changes in the temporal structure of a signal. For example, in response to both increases in intensity and depth of modulation of beats midbrain neurons show increases in their dF Fourier component (Bodnar and Bass *2001a*). Hence, a global average of dF-dependent activity may not be able to distinguish between a change in intensity or depth of modulation. However, given that spike rate and dF synchronization vary differentially for these changes in signals parameters (Bodnar and Bass *2001a*), the resulting temporal patterns of neuron outputs are likely to be different. Hence, a coding mechanism that is sensitive to changes these patterns can uniquely code these different changes in signal parameters. This hypothesis predicts that different dF cell types will show differential changes in the magnitude of their Fourier component with changes in intensity and depth of modulation. Hence, these two hypotheses can be directly compared by assessing the population coding of beats of varying dF at different intensities and depth of modulation.

Temporally patterned population activity would provide a code whereby signal parameters could be ascertained without integration over longer time periods as is required for a code that averages spike synchronization over several beat cycles based on labeled lines. For animals such as midshipman that move about their auditory environment and encounter a constantly changing auditory scene, such a population code would permit the rapid segregation of concurrent vocal signals.

These coding hypotheses can be tested neuroanatomically. A mechanism that relies solely on the tuned spike output of individual cell groups to code dF suggests that dF tuning should remain maintained and likely sharpened within efferent nuclei. This predicts that the spike outputs of different dF cell groups within the midbrain should provide inputs to different neurons in post-synaptic nuclei, i.e., the axonal projections of different dF cell groups should remain largely separate. In contrast, under the population coding model, the patterned information across dF cell groups needs to be decoded. This would require that the inputs from many, or even all, dF cell groups converge onto the same post-synaptic neurons. This predicts that the axonal projections of neurons with different dF tuning should substantially overlap within post-synaptic nuclei. A previous neuroanatomical study has mapped the nuclei that receive inputs from midbrain neurons (Bass et al. *2000*). Potential sites of decoding are pre-toral and pre-glomerular neurons of the thalamus. Thus, the above hypotheses can be tested by labeling individual identified dF tuned neurons within the midbrain and the mapping of the distributions of their efferent projections to these nuclei.

Based on single-unit recording data, it is often presumed that neurons tuned to a particular stimulus feature will have the maximum response to that parameter across the population, e.g., neurons tuned to dF=6 will show the highest response to 6 Hz beats across the population. One surprising result of our analysis was that this supposition is not upheld by the data. At some dFs, the cell group with the highest magnitude of the dF component was not the group tuned to that dF. Thus, analysis of data at the population level may be necessary to discern the actual pattern of responses within auditory nuclei. Post-hoc assessment of single-unit data revealed that the 6-Hz cell group overall had lower spike rates than the other cell types; this is also evidenced by their lower d.c. values. Hence, because on average the fewer spikes are produced, there are fewer spikes available to contribute to dF-related patterns even at 6 Hz.

Comparisons with other population coding studies

Early studies of the temporal relationships of neuron firing utilized pair-wise or small cluster recordings of multiple units (Gerstien and Perkel *1969*, *1972*; Epping and Eggeremont *1987*; Aertsen et al. *1989*). In the auditory system, these studies demonstrated that a large portion of correlated firing among neurons was stimulus dependent. In response to two-tone stimuli, small clusters of neurons and the envelope multiunit recordings showed variation in activity with changes in stimulus frequencies (Nelken et al. *1994*).

More recent studies in a number of sensory systems have identified patterned activity among small groups of neurons that are correlated with stimulus periodicities or intrinsic oscillations (Laurent *1999*; Castelo-Banco et al. *2000*). Decoding analysis of small groups of retinal ganglion neurons has indicated that the stimulus representation by a single neuron is modified by inputs from other cells (Warland et al. *1997*). All of these studies indicate that the correlated and temporally patterned activity of neurons may encode stimulus features.

Ultimately, a complete understanding of temporal population coding of stimuli requires the simultaneous recording of large numbers of neurons from the same nucleus. While current multineuron electrode recording is increasing rapidly, it is still difficult to isolate the activity of a large number of neurons. Furthermore, the locations of many deep brain nuclei are not amenable to recording with current designs of electrode arrays. Yet, in many systems there is a wealth of single-unit data available for analysis of population responses. The use of multivariate statistical analysis enables one to assess the presence of patterns within such data sets.

One caveat of this approach is that analysis should be done carefully to limit the effects of potential sampling biases due to multiple recordings from the same animal. Statistical analysis generally requires that each data point be independent, i.e., sampled from individual animals. The nature of neurophysiological data makes adherence to strict independence difficult. However, measures can be taken to minimize the effects of multiple recordings from that same animal and thus have confidence that the results are not arising from sampling biases. In our analysis, the average number of neurons per animal was 2.3, and there were no more than six neurons from any one animal. We also designated individual animals as a "between-group" effect. This enabled analysis of other effects to be separated out from those arising from individuals. Finally, we also ran an additional analysis where the data for neurons of the same cell type from the same animal were averaged together. Thus, there was only one sample per animal per cell type. Any correlations among animals across cell types would counter the effect of cell type itself. Our precautions therefore indicate that the observed patterns likely emerge from the entire population and are not due to sampling biases within exceptional animals.

Alternative approaches to assessing population coding have been to utilize models, neural networks, or decoding analysis methods to detect temporal patterns in spike train data. Several modeling studies of the coding of auditory scene features such as sound location and target characteristics established the capacity of population spike rates and spatiotemporal patterns of activity to code acoustic signal parameters (e.g., Fitzpatrick et al. *1997*; Palakal and Wong *1999*). More recently, artificial neural network analysis of recorded ensemble activity has shown that both ensemble spike counts and relative spike times can accurately predict sound location (Middlebrooks et al. *1994*; Furukawa et al. *2000*).

The ability of models and networks to extract acoustic signal information from spike trains indicates the presence of statistical patterns within the data sets. Our analysis of single-unit recordings collected across a large population of neurons made direct use of statistical tests that detect patterns of activity to code signal parameters. The advantage of statistical tests is that the discernment of spike patterns is not dependent on model assumptions or neural network training methods, but rather is directly

dependent on tests of the empirical data. However, to utilize this method, one must have some means of grouping neurons to test for patterns such as those based on tuning to some stimulus parameter. Other spike patterns that this method cannot detect may be present. The presence of statistically significant patterns of the magnitude and phase of firing of different groups indicates that the decoding of information by model neurons or neural networks can be successfully achieved.

Across many species, neurons have been identified within the auditory midbrain that show both differential changes in their spike rate and synchronization characteristics for signals with different amplitude modulation rates (for reviews see Langner *1992*; Bodnar and Bass *1999*). Hence, a population code comprised of temporal patterns of activity among differentially tuned neurons, as demonstrated here in midshipman, is likely to be present in the auditory midbrain of other species and, thus, contribute to the temporal encoding of behaviorally relevant signals.

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