Intrinsic biophysical diversity decorrelates neuronal firing while increasing information content.

Krishnan Padmanabhan and Nathaniel N. Urban

Supplemental Analysis

Spike Train Decomposition:

Spikes from each trial were resampled to 1 ms resolution and that trial was assigned a corresponding cell/condition ID in response to the same fluctuating stimulus. For each trial a vector of spike times $x$ of 1s and 0s was generated. To project the spike trains into the space of principal components, the covariance matrix for all spike trains and the eigenvalues were calculated to all repeated presentations of the same fluctuating noise stimulus across all cells.

For classification of spike patterns, we performed principal component analysis on all trials from all cells we recorded from. There were no slow covarying elements in the first three principal components (Fig S2a), suggesting that differential responses in mitral cells were not due to slow changing processing like spike frequency adaptation or decorrelation. To further explore the individual cell responses to the same stimulus, we first took the mean of all the trials for each cell condition (Fig. S2b). From this, we calculated the pair-wise distances of these responses projected into the space defined by their first three principal components (Fig. S2c). The distances allowed us to cluster the mean responses based on the pattern of spike output. Mean spike patterns close together in their principal component space (for instance, cell 1 and cell 9, Fig. S2c white box - red arrow), clustered together in a dendrogram of all mean spike responses (Fig. S2d). By contrast, those mean responses that were far apart in the principal component space (cell 3 and cell 15, Fig. S2c, white box - blue arrow) were far apart in the clustered space of their dendrograms (Fig. S2d, colored labels below dendrogram correspond to highlighted cells in the distance matrix).
Correlation calculation

To further explore similarities and differences in the individual spike train responses among neurons, we examined the pair-wise correlations between each of the spike trains we recorded (N=589). A correlation matrix of all pair-wise correlations to an identical stimulus showed that spike trains were most correlated with spike trains from the same cell (Fig. S3a). What was surprising was the low off diagonal correlations (those correlations between spike trains from different cells, Fig. S3a) that we found.

To account for millisecond level jitter in the spike trains, we resampled spikes in various non-overlapping bin sizes ranging from 1 ms to 16 ms. In each bin example, if a spike occurred at any time within the window of the bin, then the value of the bin was recorded as 1. If no spike occurred, then the value of the bin was 0. This procedure was identical to that used for the calculation of entropy. Following this, we examined the correlation histograms across these various bin sizes (Fig. S3b). Not surprisingly, as the bin sizes increased, the histogram distributions shifted to progressively higher correlation values, demonstrating that if allowances were made for spike jitter (with progressively larger bin sizes) then the correlations between spike trains became larger (Fig. S3c).

Although there was an increase in the mean correlations between all the spike trains as the bin size was progressively increased (Fig. S3c), even the largest bin of 16 ms resulted in a correlation of only 0.34±0.15. As the mitral cells from which our spike trains were recorded were not linked together by any networks due to the blocking of both excitatory and inhibitory synapses, these low correlations existed independent of active decorrelation that may have occurred if mitral cells were linked with granule cells through reciprocal dendrodendritic connections (1-6).

Two recent publications of in vivo recordings in primates (7) and rats (8) have show that nearby neurons with similar tuning properties show virtually no correlations. From this, the authors argue that neurons must either be sharing only a small number of inputs, or their activity is decorrelated though network mechanisms (7). Our demonstration that intrinsic biophysical diversity alone is sufficient to cause spike patterns to be different are consistent with these results, but suggest that even when populations of cells share inputs, their outputs spikes may only be nominally correlated.
Low correlations therefore did not depend on any active network properties such as those found in the bulb (5) or the cortex (8).

**Spike-triggered average**

The spike triggered average was calculated by identifying spikes in each cell (Fig S4a, bottom trace spikes) that occurred (Fig. S4a, top trace) and taking the mean of all current inputs that preceded the spikes in a fixed time window (Fig S4b)(9). To ensure that we had acquired enough spikes to properly estimate the STA, we divided spikes from all the trials in two ways and calculated the STAs using these subsets of spikes. First, we divided spikes from the first half of all trials and the second half of all trials to ensure that there was no systematic drift in our recordings. The invariance of the STA shape (Fig S4b, light red=STA from subsampled spikes, black = STA from all spikes) coupled with the stability of our recordings ensured that the trials we recorded from were equivalent across the entire session. Additionally, we divided all recorded spikes into two random groups independent of trials (Fig S4b, dark red=STA from random spikes, black = STA from all spikes) to further ensure that our data sets were sufficient to provide adequate estimates of the STA. In both cases, the STAs from sub-sampled data sets were highly correlated to the STA (r = 0.98) calculated from the complete data set, confirming that there was little trial-to-trial variability in the STAs and that sufficient numbers of spikes were used to accurately estimate the STA.

To classify the STA diversity within the space of principal components, we first divided the STAs by their input variance to allow for comparison between noise current injections of different σ. From all the STAs collected (N=35), a covariance matrix (Fig S4c) was generated from which the eigenvalues and eigenvectors were extracted to calculate the principal components of the STA.

In order to ensure that the diversity in STA shapes we observed was not due simply to differences such as firing rate, we color coded the STA projections in the space defined by the principal components based on their firing rates (Fig. S4d). Although there was a weak trend (high firing rates neurons= red) for mitral cells wherein
the cells driven at the fastest firing rate clustered in one part of the principal component space, we nonetheless found remarkable diversity across a number of firing rates relevant not simply for activity in the bulb, but throughout the brain (10-12). We divided STAs based on the mean firing rates (Fig S4e) into four frequency ranges (below 10 Hz, between 10 and 25 Hz, between 25 Hz and 40 Hz, and between 40 Hz and 60Hz), with each frequency corresponding to relevant phenomenon in the olfactory bulb and throughout other regions of the brain (4, 12).

We were careful to drive neurons over a large range of firing rates because mitral cell activity can vary between 0 Hz and 80 Hz both spontaneously and when a odor is presented (13). The classes chosen to delineate the different STAs were done so to highlight the preserved diversity of mitral cell STAs across broad ranges of activity. Indeed, if for instance, STA shapes became homogeneous at certain firing rates, it would alter our model of the STA as a feature of diversity and affect the calculations of information content. However, our data suggest that the intrinsic differences among mitral cells which manifest in their STA shapes are diverse throughout a broad spike frequency range. Finally, as the STA reflects differences in the biophysical composition of channels(14), and corresponds to the phase resetting curve (PRC), we could use this single metric as a proxy for the intrinsic features of neuronal diversity, including firing rate.

To further ensure that the differences in mitral cell responses were not due to systematic errors in our recordings, or passive membrane properties of these neurons, we calculated the membrane time constants (tau) (Fig. S5a) and the input resistance (Fig. S5b) for all the cells we recorded from. Here too we found no systematic relationship between either the time constant or the input resistance of the cell and the STAs. Finally, to ensure that the STA was in fact an accurate reflection of the cells unique response to the stimulus, we multiplied the STA by the stimulus at each time t and then transformed this into a predicted firing rate and compared these firing rates to a smoothed firing rate of the mitral cell. The predicted firing pattern was well correlated with the recorded firing pattern for the cell and significantly better than the firing patterns
predicted if other STAs were used (R² = 0.60±0.06 correct STA, R² = 0.43±0.18 other STAs, P = 4.15X10⁻⁵, N =13).

**Information analysis**

To calculate the information relayed in our population code, we employed the method described previously (15, 16). First, we collected all the spike patterns recorded over repeated (30-40) trials for a population of mitral cells (N=15 DC offsets to 8 cells) to an identical stimulus (**Fig S1a**). Another population of mitral cells (N=11) was presented with a different stimulus (**Fig S1b**). To ensure that the diversity of the mitral cells we used in our entropy calculations was analogous to the total diversity we found in our mitral cell population, we plotted the distribution of the selected mitral cell STAs (**Fig. S6a**, red) and all mitral cell STAs (black) in the space defined by the first three principal components. In addition, we wanted to ensure that the firing rates of the mitral cells selected for our entropy calculations were analogous to the firing rates of all the mitral cells we recorded from (**Fig. S6b**). Again, we found no significant difference between the firing rates of our selected mitral cells (24 ±12 Hz, N= 15) and the total population of mitral cells (28 ±20 Hz, N= 27, P = 0.47, ANOVA).

From the binary strings generated using the technique described in the methods section, we could determine the distribution of words (the patterns of 1s and 0s), both over the entire simulated population response (in both homogeneous and heterogeneous cases) at all times (total entropy, **Fig. S7a**) and the population response over multiple trials at time t (noise entropy, **Fig. S7b**). The bits of information carried by the population of cells were calculated as the total entropy minus the mean of the noise entropy for different diverse simulated populations (**Fig. S7c**).

To ensure that bin size did not affect the interpretations of our entropy calculations, we performed this same calculation while changing bin sizes between 4 msec and 12 msec (**Fig. S8**). Across all bin sizes, heterogeneous populations carried more information than their homogeneous counterparts (**Fig. S8a1-b1**). Additionally, in
all bin size examples, the diversity (as measured by the STA distance) predicted the information that the population would carry (Fig. S8a2-b2).

Furthermore, to confirm that the gains in information of heterogeneous populations were not due to increases in the number of cells in the population, we plotted STA distance vs. bits of information for heterogeneous populations comprised exclusively of 10 cells (Fig. S9). To span the complete space of STA distances, we created different populations of 10 cells that had 2 to 10 unique cells. In the case of a 10 cell network with 2 different cells for instance, 5 spike trains in the population were drawn for random trials of each of the two different cells to generate the 10 cell population. In the case where the network had 10 unique cells, the spike train for each cell in the population was drawn from a random trial of a unique recorded cell. This approach allowed us to decouple the effect of the number of cells in the network from the diversity of the population code. We found a strong correlation ($R^2 = 0.61$, n=1313 networks) between our measure of intrinsic diversity, the sum STA distance/number of cells, and the bits of information being transmitted by the 10 cell populations (Fig. S9).

**Physiologically relevant stimuli**

The sampling of odors in mammals is not passive, instead depending on a number of rhythmic processes, including breathing and active sniffing (17-20). Rodents for example, breathe at 1-3Hz but quickly alter their respiratory frequency to between 4-12 Hz when sampling odors (21, 22). This sniffing process has consequences for the neural representation of odors, both at the sensory periphery (22-24) and in the mitral cell layer of the bulb.

To explore the role of physiologically relevant inputs governed by a periodic sniff for mitral cell coding, we modeled ORN inputs (Fig. S10) to the mitral cell layer in both the passive breathing condition and with an 8Hz oscillation corresponding to sniffing using previous work on recordings from ORNs and mitral cells both *in vivo* and *in vitro* (21, 22, 25-29). To generate model input currents, a random spike train convolved with an alpha function (having a 3 ms time constant -Fig. S10a - right insert) was used (Fig. S10a, black trace). To generate the underlying oscillation, we added a prominent 8Hz rhythm upon which the model synaptic inputs rode (Fig. S10a, red trace).
For the mitral cells we recorded from (N=11 8Hz, 23 total), interleaved trials of a frozen noise current input (Fig. S1a), a synaptic input (Fig. S10a, black trace), and a synaptic input with an 8Hz oscillation (Fig. S10a, red trace) were presented for 2.5 seconds over multiple trials (N=27-40 trials/cell). From the output spike responses, we created model homogeneous and heterogeneous populations as described previously (Fig. 6, (15)). The degree of heterogeneity in the mitral cell population was measured by calculating the STAs from the noisy input trials (Fig. S4) and then projecting those STAs onto the space defined by their first three principal components. In both the cases of synaptic input (Fig. S10b) and synaptic input driven by an 8Hz oscillation (Fig. S10c), we found a strong correlation ($R^2 = 0.90$ synaptic input, $R^2 = 0.94$ synaptic input + 8Hz oscillation) between the degree of diversity (as measured by the STA distance) and the information carried by the population of heterogeneous networks, ranging in size from 2 neurons to 8 neurons.
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Figure S1: Mitral cells all receiving rapidly fluctuating inputs produce markedly different spike train outputs. (a-b) Representative examples of two different input noises (top black trace) and cell output spikes to that noise for two different populations of mitral cells (bottom traces - color corresponds to a cell, each row is a single spike raster). (c) The amplitude of the DC offset does not affect trial-to-trial correlations within a population of mitral cells (N = 35, 30-40 trials/cell). (d) Correlations are highly variable across a broad range of the input noise variance (Percent variance = σ (pA)/DC (pA)).
Figure S2. Classification of mitral cell spike patterns. 

a) First three eigenvectors (PC1 - PC3) of the covariance matrix. 

b) Mean firing pattern for each of the 15 recordings used to generate the PCs in a. 

c) Pair-wise distance matrix of mean spike patterns projected into a space defined by the first three eigenvectors of their covariance matrix. 

d) Dendrogram distance tree showing clustering of cell responses based on the mean output spike train.
Figure S3 Intrinsic diversity among mitral cells affects the correlations of spike trains to one another even when the input fluctuations are identical. **a)** Correlation matrix of all spike trains to all other spike trains from mitral cells probed with an identical noisy input. The diagonals are autocorrelations ($R^2=1$). **b)** Histogram of the effect of bin size (allowing for spike jitter) on correlations among all the spike trains. As bin size is increased, the histograms are shifted to progressively higher correlation values. **c)** Mean correlation as a function of bin size (error bars are standard deviation).
**Figure S4**: Spike triggered averages are calculated using sufficient spikes and the diversity of STAs persists over broad firing ranges. **a)** Input noise trace (top) and output spikes from a mitral cell (bottom). The STA is calculated by **b)** averaging all the input current fluctuations that precede the spike by a time window yield the spike triggered-average. Bottom - each colored trace reflects a subsample of all the recorded spikes. The correlation of the subsampled STAs to the STA calculated using all the spikes in all the trials from a single cell shows that there was no systematic drift in the recordings. **c)** Covariance matrix of the STA. **d)** STAs projected onto the space defined by the first three principal components color coded by firing rate. **e)** STA diversity is preserved across a range of firing rates.
Figure S5: STA diversity is not due to differences in input resistance or membrane time constant. Mitral cell STAs projected into the space defined by PC1-PC3 and color coded by a) membrane time constant (tau) or b) input resistance.
Figure S6: Mitral cells used in the information calculation are drawn from a distribution of diversity similar to the total diversity observed in the mitral cell population. **a)** Mitral cells used in the information calculation that all received identical input (red) and all other mitral cells recorded (black) projected onto a space defined by the first three principal-components of their covariance matrix. **b)** Firing rates are not different between mitral cells used in the information calculation and those from the total population of mitral cells recorded.
Figure S7: Calculation of entropy in a biophysically diverse population of mitral cells.  

a) Total entropy of the population of mitral cells (10 bit) ranked by frequency of word occurrence. 

b) Noise entropy to at a single time t of the same population of mitral cells (10 bit).

c) Total entropy (dark red), noise entropy (light red) and information (black) from different networks of biophysically diverse mitral cells (each point represents a different biophysically diverse population of mitral cells)
Figure S8: Heterogeneous populations carry more information over their homogeneous counterparts independent of the binning of the spike train. (a1-b1) Bits of information carried by homogeneous and heterogeneous populations of different numbers of cells over bin sizes of 4 ms (a1) and 12 ms (b1). (a2-b2) Bits of information as a function of STA distance for heterogeneous populations at different bin sizes of 4 ms (a2) and 12 ms (b2).
Figure S9: Increases in information are not due to increases in the population size of the mitral cell network but are actually correlated with the intrinsic biophysical diversity of the population. a) Bits of information as a function of intrinsic biophysical diversity in populations of mitral cells made exclusively of 10 neurons. (N= 1313).
Figure S10: Physiological relevant stimuli are better coded for by heterogeneous populations of mitral cells. **a)** Synaptic model input with no respiratory modulation (black trace) and synaptic model input modulated by a 8 Hz oscillation (red trace) with an enlargement of the synaptic input current (right). Bits of information correlates with degree of diversity when populations receive **b)** synaptic input ($R^2 = 0.90$) or **c)** synaptic input modulated by an 8 Hz oscillation ($R^2 = 0.94$).