# Characterizing *in vitro* Hippocampal Ripples using Time-Frequency Analysis

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## Abstract

We analyze *in vitro* hippocampal activity exhibiting drug induced ripple-like rhythms using time-frequency distributions. The different tradeoffs between time-frequency resolution, interference, and range are examined with reference to the suitability of each for use in analyzing ripple data. An unbiased approach is taken to characterizing ripple rhythms uniquely by determining the parameter choices in which its behaviour is most distinct from baseline activity. Mixture distribution analysis of the two components generated from correlating frequency behaviour allows a clear method for determining the distinctiveness of ripple-like rhythms given a particular parameter choice.

Keywords: Hippocampus; Time-Frequency; Ripples; Mixture distributions; EEG

## 1. Introduction

Electroencephalogram (EEG) recordings of the hippocampus reveal the presence of specific frequency associations with distinct behavioural states. One such association is in the 150-200 Hz range which is present during awake immobility, consummatory behaviour, and slow-wave sleep [2]. These ~200 Hz ripple-like rhythms last approximately 0.1 seconds at a stretch and are associated with sharp-wave activity. This frequency would be suitable for LTP induction, suggesting an important physiological role for ripple-like rhythms [1]. One issue which arises from recordings of these EEG signals is what characteristics other than mean frequency can be used to characterize rhythms, and how rhythms of different frequency will then relate. One particular case is to find how the properties of ripples relate to the properties of the ongoing rhythms present before and after the ripple. An example of a similarity in property might be in the range of activity rather than the mean. If these similarities were present in a continuum between different rhythms, they might suggest a single mechanism underlying otherwise different classes of activity; this is not true of mean frequency data for ripples [2].

Because the rhythms observed are biological, techniques to characterize frequency which assume that that the rhythmic behaviour does not vary with time, known as stationarity, are often inappropriate. One solution to this problem is to divide the signal into short enough pieces that they are essentially stationary; this is known as time-frequency analysis. To generate time-frequency plots, a common choice is to divide the signal up into equally sized pieces in time. A simple example of this is the spectrogram which effectively separates the signal into pieces the frequency of each of which is determined by Fourier analysis. For smaller signal pieces, the time at which the frequency is present is known with greater precision but the precision of the known frequencies declines; the product is constant for a particular transform and parameter choice. The Wigner distribution can provide greater resolution but is more likely to spread noise across the signal transform, a common problem in multi-component signals. The Wigner transform and spectrogram both have unchanging time and frequency resolution. If the resolution changes with frequency, the wavelet transform results. This technique gives good frequency resolution at low frequencies, and good time resolution at high frequencies. Since signals which have high frequency might be thought to change frequency more rapidly with time, this is often an appropriate choice [4]. Ripple-like behaviour is characterized by comparison to surrounding rhythms through the use of these techniques. In this work we propose a simple method for characterizing ripple rhythms with respect to baseline rhythmic activity.

## 2. Method

Ripples were induced in an *in vitro* preparation of the hippocampi of mice by application of 1,3-dipropyl-8-cyclopentylxanthine and eight CA1/3 field EEG recordings of two to three minutes, sampled at 20 kHz comprised the data set (L. Zhang, unpublished data). Time-frequency plots of the data were generated using the spectrogram, the Wigner distribution, and the wavelet transform. The breakdown between time and frequency resolution was also altered in the plots generated, running from 0.01 seconds up to 4 seconds in time resolution by doubling for each power of 10 (e.g. 0.01, 0.02, 0.04, 0.08, 0.1, 0.2. . .). Of course, this also determines the correspondingly better frequency resolutions. Having generated the frequency distributions for each unit time at a given resolution, they were compared for similarities in their frequency distributions. Simply taking the correlation between the different frequency distributions obtained at each time serves to do this, for the most part. In order to reflect trends of different magnitude, a chirp was added to the signal, resulting in a default high correlation. In this way, purely noisy parts of the signal correlated well with one another, as we would like (indicating similar behaviour). This done, each time-frequency representation, and over varying frequency ranges, the frequency distributions were correlated. This was performed for each plot generated, and also for subdivisions in frequency (e.g. 1-30 Hz, 150-200 Hz, etc). Histograms were then constructed from the correlation values obtained. Clearly, the usefulness of any characterization is context dependent, and our analysis of ripple rhythms is explicitly dependent on its context - the baseline rhythmic activity. Because the signal exhibits two clear modes of activity (ripple, baseline), the histograms will be a weighted sum of different component distributions, called a mixture distribution. In our case, we would obtain a mixture of approximately two components, reflecting times where frequency distributions are the same (ripple to ripple, baseline to baseline giving similarly high correlations, but the former is also vanishingly infrequent), or different (ripple to baseline giving a relatively low correlation). Mixture distribution analysis was used to characterize the underlying components giving rise to the distribution, under the assumption that there were two primarily contributing (bounded) normal distributions ([3], [5]). The statistical significance of the separation was determined and was taken to indicate the ability of a given set of parameters to characterize ripples (Figure 1). That is, parameters have been chosen which well characterize ripples when their frequency distribution is distinct. The analysis was also performed on artificial rhythmic data. Errors were inspected both by examining log-likelihood slopes, and by using a range of starting parameters and observing resultant fits.

**3. Results**

Ripples exhibit highly distinctive frequency distributions, and are normally defined by those characteristics in the high frequency domain (Figure 2). Time-Frequency analysis did not show any characteristics preceding ripples, and non-stationarity in ripples was similar across all frequency ranges, eliminating the particular advantage of the wavelet transform. The baseline rhythmic activity varied substantially in frequency and regularity even within individual datasets, but approximated a 3 Hz rhythm. While the baseline rhythm varied substantially, there was no detectable relationship indicating the onset of a ripple. Ripples did exhibit a characteristic frequency distribution in the low frequency range, even after normalization within that range. This could be seen in correlating the low-frequency signal contribution, and observing the clear presence of a mixture distribution in the resultant histograms (Figure 3), reflecting the substantial difference between ripples and baseline in this frequency range. Regardless of the specific frequency range, duration, or times chosen, the correlation of frequencies exhibited qualitatively similar features with a tartan like appearance. This is also reflective of the lack of stationarity associated with ripples at all frequency ranges.

The frequency distributions gave the greatest separation into two distinct components for time resolution of 0.2 second, in the range of the approximate duration of ripples (Figure 2). In general two component mixture distribution fits were quite close with variation in the degree to which they were significant being the important point in determining defining frequencies. There were three clusters of frequency ranges that most distinctly separated ripples from baseline activity, 1-40 Hz, 120-170 Hz, and 150-220 Hz, with only that latter corresponding to the rough range most often used to define ripple behaviour. Each dataset had similar qualitative features with respect to the characterization of ripples by frequency. In each, there were three peaks in ripple separability corresponding to the given frequency ranges, although the relative contribution of each varied from dataset to dataset. Using both of the two latter overlapping regions generated less distinct distributions, suggesting a qualitative change in behaviour during the overlap. Ripples (to ripple correlations) constitute a small enough fraction of the higher-valued distribution so as to be relatively insignificant, even if their frequency distributions do not constitute precisely the same distribution as the same-same values generated by baseline activity. Variation in frequency range chosen did not alter the identification of ripples as determined from the separation of the mixture distribution of correlations. The same analysis performed on artificial spiking did not yield the distinctive mixture distribution of correlation values, since the spikes were not distinctly different in the population of frequency characteristics they displayed.

Overlap between the two distributions depended upon the frequency range and resolutions used, but even with high overlap there was excellent separation and the existence of two classes of activity was very clear. Varying starting values rarely altered the fits obtained for the mixture distribution. Attempting three component fits was rarely successful. In general, the weighting of a third component was very low, or the mean was very close to a pre-existing distribution. Pieces of the signal contributing to intermediate points in the correlation histogram sometimes exhibited ripple-like characteristics in the low frequency band (e.g. $r>0.95$) but no ripple-like character in the high frequency band, and otherwise did not change. That is, while most activity categorized as ripple-like in one parameterization remained ripple-like in another, to the extent there was migration, it was one way: more activity sharing distinctly ripple-like character in lower frequencies.

## 4. Conclusions and Discussion

Ripples are defined largely by their high-frequency behaviour, to the point where other contributing frequencies are filtered out in analysis. We have attempted to determine a way to best characterize ripples with fewer *a priori* assumptions as to their behaviour. So, while the identification of ripples is trivial, the determination of which parameters *best* characterizes them is not. In an *in vitro* hippocampal preparation known to exhibit ripples and a baseline rhythm, we assumed that the distribution of frequencies would exist in broadly two classes, and checked this assumption. Many frequency distributions were generated over time and with different parameter choices, and tested for their statistical separation into two classes, under the reasoning that the parameter which best separated the data into two classes would also be those that best characterize ripples with respect to the baseline rhythm. The primary intent is to develop a single metric for reasonably characterizing ripples with a minimum of bias - distinctiveness from baseline. Of course, one possibility would be to determine the contribution of different measured factors (e.g., factor analysis, component analysis) to our measure, but first it is necessary to have some characterization or output of interest.

Unsurprisingly, we found that the data separated well for temporal resolutions in the range of the duration of ripples. More interestingly, the frequency ranges determined included not only the roughly traditional range (150-220 Hz), but also a slightly lower range (120-170 Hz), and a much lower one (1-40 Hz), surprising for the duration of activity. This last frequency range may represent ongoing activity (baseline rhythms) altered due to the occurrence of the ripples. It is not simply due to change in total power since we are comparing frequency distributions. Further, the low frequency characterization being merely a side-effect of ripples is argued against by the occurrence of an intermediate population which demonstrates the low frequency aspect even where rippling is not present.

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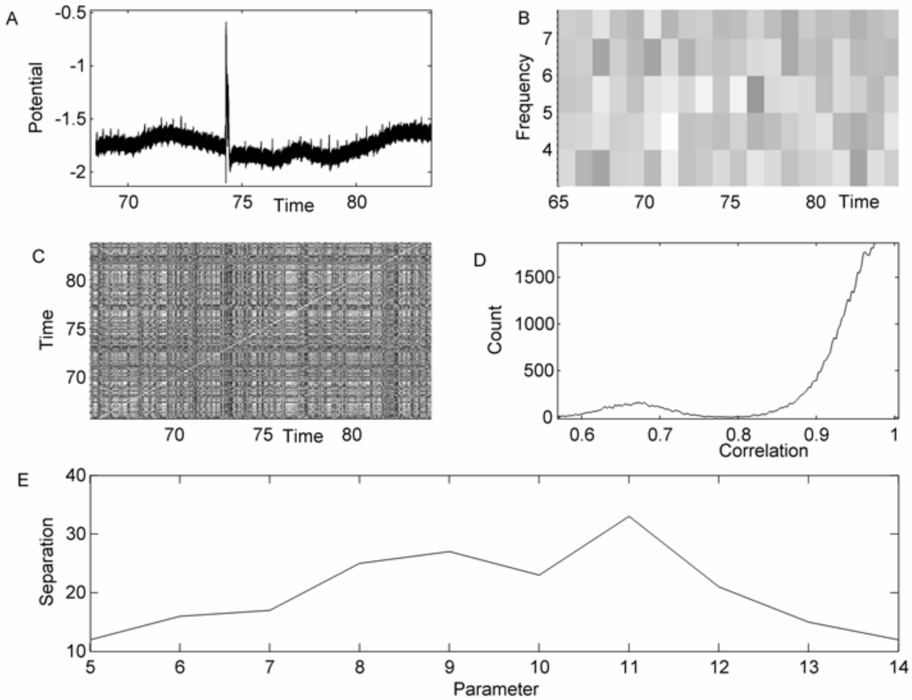
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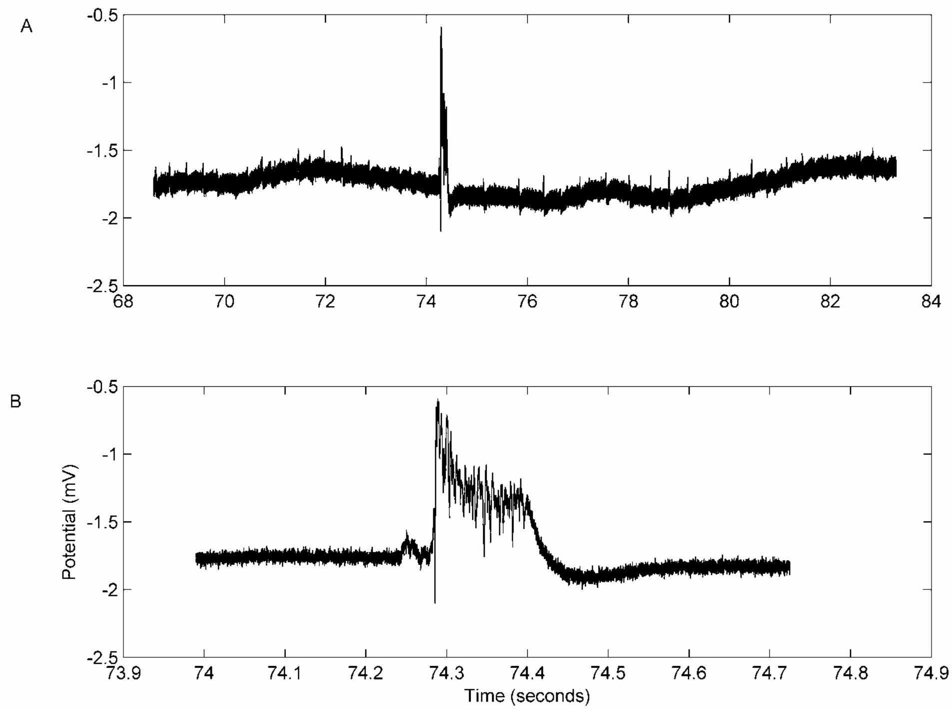
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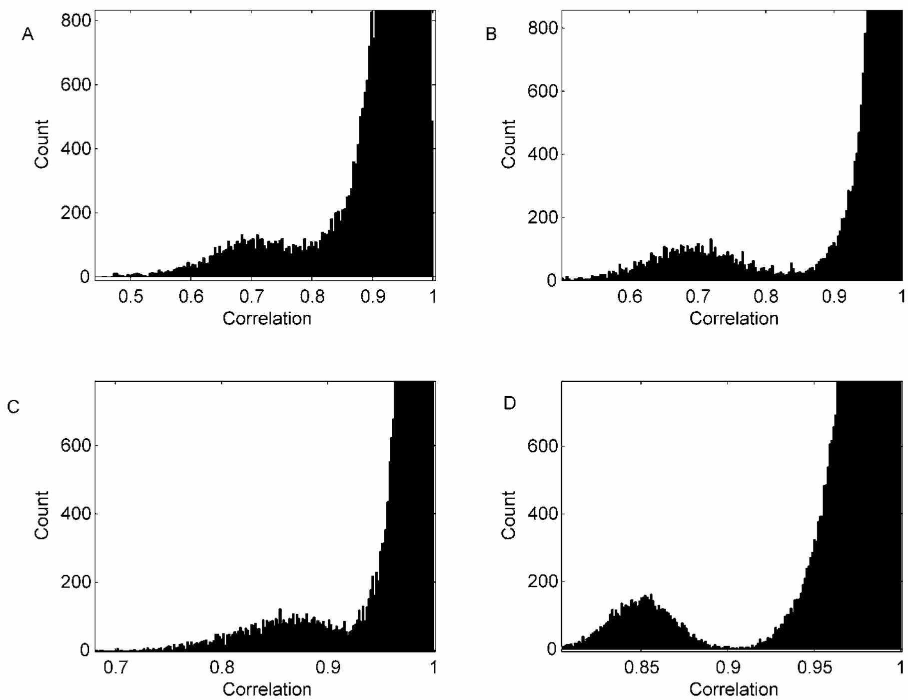
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Figure 1. A flow chart of the methodology is shown; A: The signal, a recording from the hippocampal preparation; B: A specific segment of a Time-frequency representation of the signal with this step being repeated across the entire signal for different resolutions and segments; C: Correlation between each time's frequency activity from the previous plot; D: Histograms for the values of the (adjusted) correlations; E: Measuring separability in those histograms with respect to the parameters varied in step B.

Figure 2. A sample of the data is shown at two scales to observe the two different essential characteristics; A: The baseline rhythm and ripple; C: The ripple in close-up.

Figure 3. Four histograms constructed from correlating frequency distributions as described and showing variation with frequency and transform type; A: Wigner transform in the 140-190 Hz range; B: Spectrogram in the 140-190 Hz range; C: Wavelet transform in the 140-190 Hz range; D: Spectrogram in the 1-40 Hz range.