Classifying interneurons of the dorsal CA1 hippocampus from extracellular recordings

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Introduction

A variety of interneuron types has been identified in the rodent hippocampus based on differences in their post-synaptic targets, their expression of molecular markers and their spike timing relative to rhythmic fluctuations of the local field potential (Klausberger et al., 2003, *Nature*; Varga et al., 2014, *eLife*). Such interneuron types are thought to have distinct contributions to the temporal organization of principal cell firing. However, progress in testing the role of each interneuron type has been hindered by the difficulty to assign interneurons to anatomically defined types when solely recorded with extracellular recordings (i.e., without labelling) in behaving rodents. Here, we present results from a dataset of 801 putative interneurons recorded using tetrodes from the dorsal CA1 region of the hippocampus of 47 mice. We employ an unsupervised clustering framework to attempt sorting interneurons into distinct types based on their (a) spike train dynamics, (b) spike waveform, (c) theta phase coupling (d) estimated recoding location and (e) firing response to sharp wave-ripple (SWR) events.

Numerical firing measures

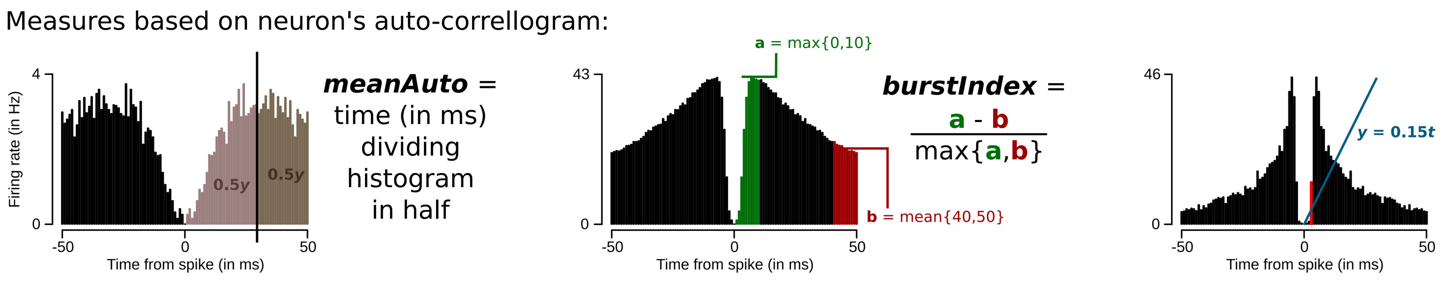
(a) Spike train dynamics

aveRate = mean firing rate over full day

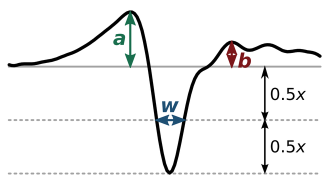
$$CV = \frac{\sqrt{\frac{1}{N-2}\sum_{i=2}^{N}(\Delta t_i - \langle \Delta t \rangle)^2}}{\langle \Delta t \rangle}$$

where Δt_i is the ISI of spike i and $\langle \Delta t \rangle$ is the mean ISI over all spikes

Measures based on neuron's auto-correllogram:



(b) Spike waveform



spikeSymmetry = $\frac{a}{a+b}$

spikeWidth = w

(c) Theta phase coupling

The recorded signal is band-pass filtered (5-12 Hz), after which the instantenous phase is extracted using the Hilbert transform. All spikes during "good theta cycles" (*) are assigned a theta phase. Let a_i be the phase (in radians) assigened to spike i, and N the total number of spikes assigned a phase. Then:

$$\boldsymbol{phase}_{\mathbf{Theta}} = \mathrm{atan2}\left(\frac{\sum_{i=1}^{N}\sin a_i}{N}, \frac{\sum_{i=1}^{N}\cos a_i}{N}\right) \qquad \boldsymbol{r}_{\mathbf{Theta}} = \sqrt{\left(\frac{\sum_{i=1}^{N}\sin a_i}{N}\right)^2 + \left(\frac{\sum_{i=1}^{N}\cos a_i}{N}\right)^2}$$

These Polar coordinates are then converted to Cartesian coordinates:

$$\boldsymbol{x}_{\mathbf{Theta}} = \boldsymbol{r}_{\mathbf{Theta}} \times \cos(\boldsymbol{phase}_{\mathbf{Theta}}) \qquad \boldsymbol{y}_{\mathbf{Theta}} = \boldsymbol{r}_{\mathbf{Theta}} \times \sin(\boldsymbol{phase}_{\mathbf{Theta}})$$

(*) a "good theta cycle" is defined as a peak-to-peak cycle of at least 83 ms, with both peaks and and the trough exceeding 0.5 SD of the filtered signal, with both peak-to-trough intervals between 33 and 167 ms, with theta-power exceeding delta-power at both peaks and at the trough, and with the locomotion speed of the animal above 2 cm/s. For the phase-assignment, the signal of the tetrode with the most good theta cycles is used.

(d) Recording location

To estimate how central a neuron is in the pyramidal layer, the amount of ripple power on that tetrode is quantified. Its signal is band-pass filtered (140-200 Hz), after which the instantaneous amplitude is calculated using the Hilbert transform and z-scored over all sleep sessions. Then, ***rippleEnv*** is defined as the average based on 40 ms window around all SWR peaks.

(e) SWR-response measures

SWR events (and their peak power) are identified offline as described before (McNamara et al., 2014, *Nat Neurosci*). For each neuron, the following seven measures are extracted based on its firing rate time-locked to the SWR peak, after smoothing by a Gaussian kernel with SD of 5 ms:

baseRate = mean rate [-500 ms, -250 ms] & [250 ms, 500 ms]

SWRpeak = peak rate* / *baseRate*

SWRthrough = lowest rate* / *baseRate*

* the peak and lowest rate within 100 ms from the SWR peak-power are taken

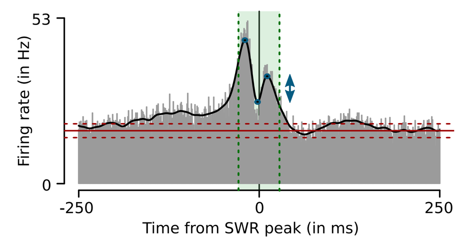
SWRcoupling = mean rate [-10 ms, 10 ms] / *baseRate*

SWRwideCoupling = mean rate [-50 ms, 50 ms] / *baseRate*

SWRsymmetry = $\frac{\text{mean rate [-50 ms, 0 ms]}}{\text{mean rate [-50 ms, 50 ms]}}$

SWRwideSymmetry = $\frac{\text{mean rate [-200 ms, -50 ms]}}{\text{mean rate [-200 ms, -50 ms] \& [50 ms, 200ms]}}$

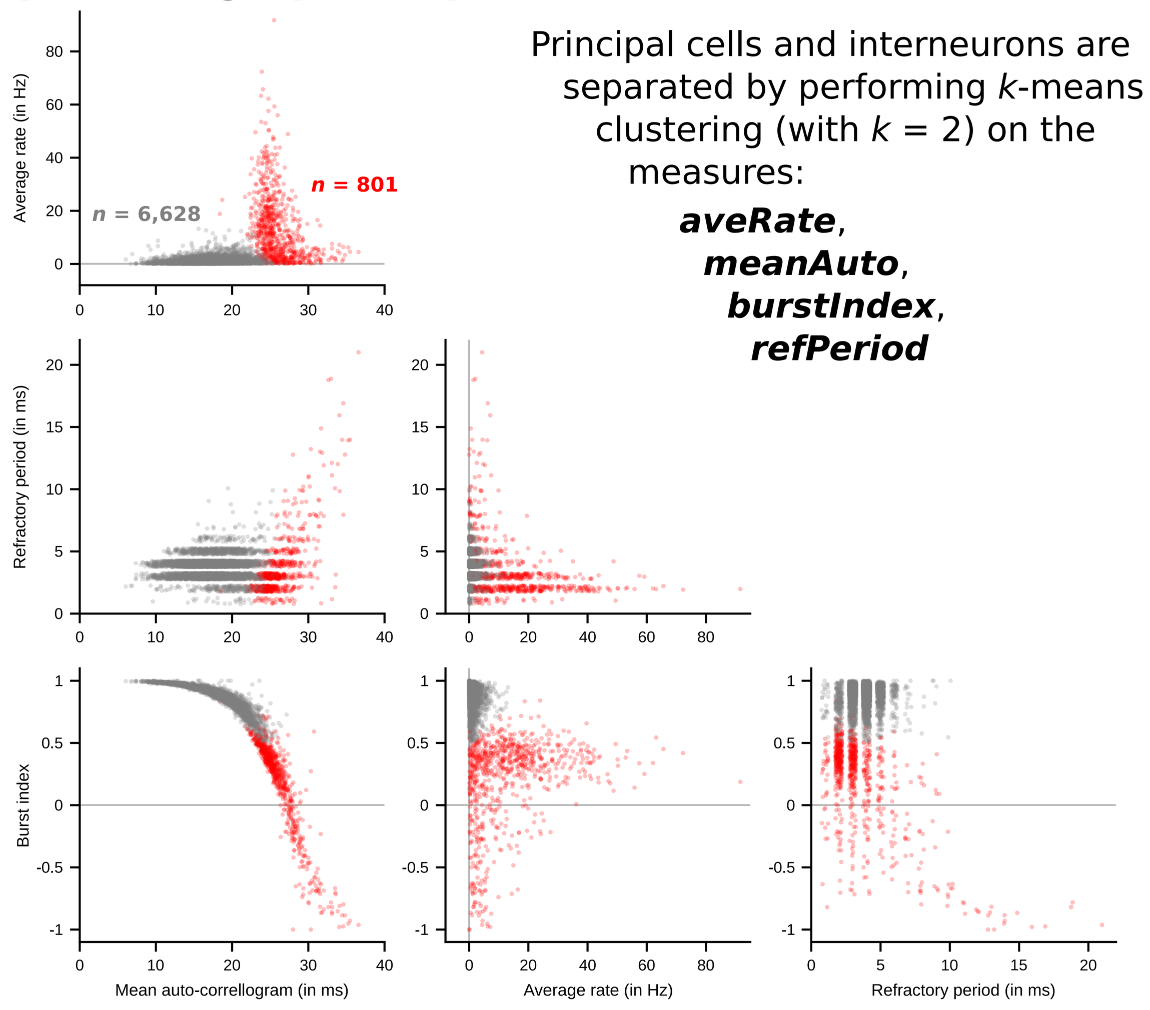
SWRvalley (see example on the right):
All peaks in the interval [-30 ms, 30 ms] that are more than 3SD above the *baseRate* are extracted. If there are two such peaks, this measure is equal to the height of the smallest peak subtracted by the lowest point between both peaks (divided by *baseRate*). If there are more than two such peaks, this subtraction is performed for all adjacent peak-pairs, and this measure is set to the maximum of these subtractions (divided by *baseRate*). If there are less than two such peaks, this measure is 0.



(Because some of these SWR-response measures can have erratic, outlying values if a neuron has too few spikes during SWRs, neurons with an average rate below 0.5 Hz in a window of 500 ms around the SWR peak are excluded for the interneuron-clustering. According to this criterion, 44 out of 801 putative interneurons are excluded.)

Clustering results

(1) Separating "principal cells" and "interneurons"

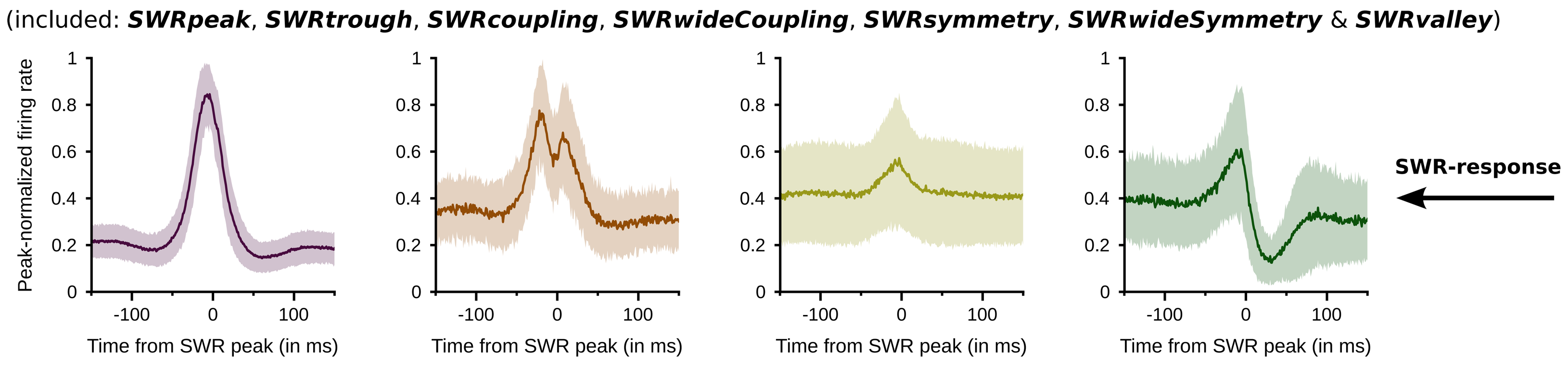


Principal cells and interneurons are separated by performing k-means clustering (with k = 2) on the measures:

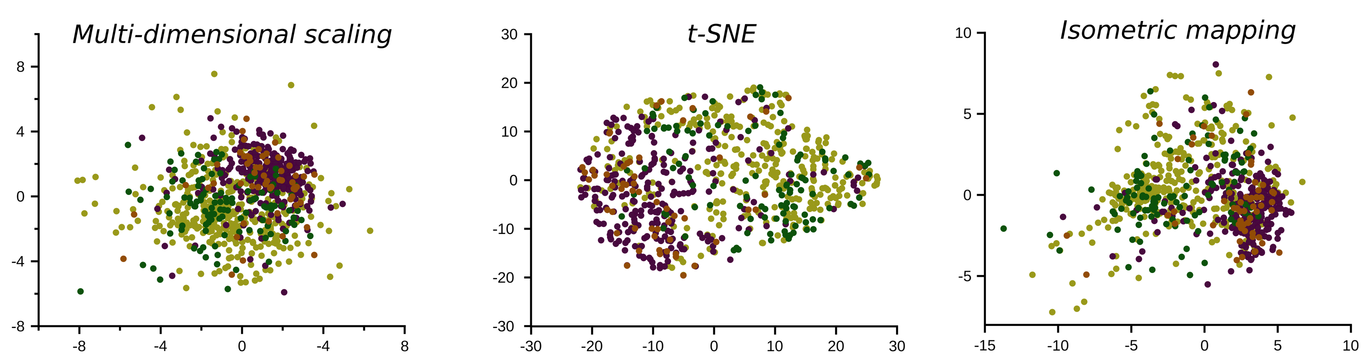
aveRate, ***meanAuto***, ***burstIndex***, ***refPeriod***

(2) Interneuron clusters identified based on their SWR-response also differ in other characteristics ...

Interneurons are subdivided into 4 clusters with k-means on the 7 SWR-response measures:
(included: ***SWRpeak***, ***SWRtrough***, ***SWRcoupling***, ***SWRwideCoupling***, ***SWRsymmetry***, ***SWRwideSymmetry*** & ***SWRvalley***)

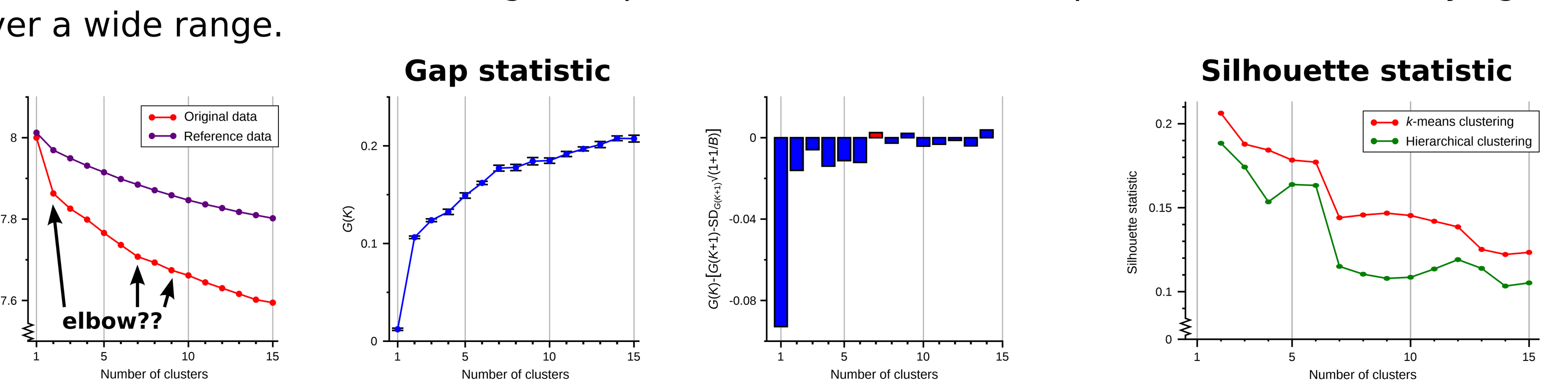


Manifold learning is performed on 9 "other" firing measures (not used for clustering):
(included: ***aveRate***, ***burstIndex***, ***refPeriod***, ***CV***, ***spikeWidth***, ***spikeSymmetry***, ***rippleEnv***, $\boldsymbol{x}_{\mathbf{theta}}$ & $\boldsymbol{y}_{\mathbf{theta}}$)



(3) ... but there appear to be no discrete, clearly separable clusters based on firing properties alone

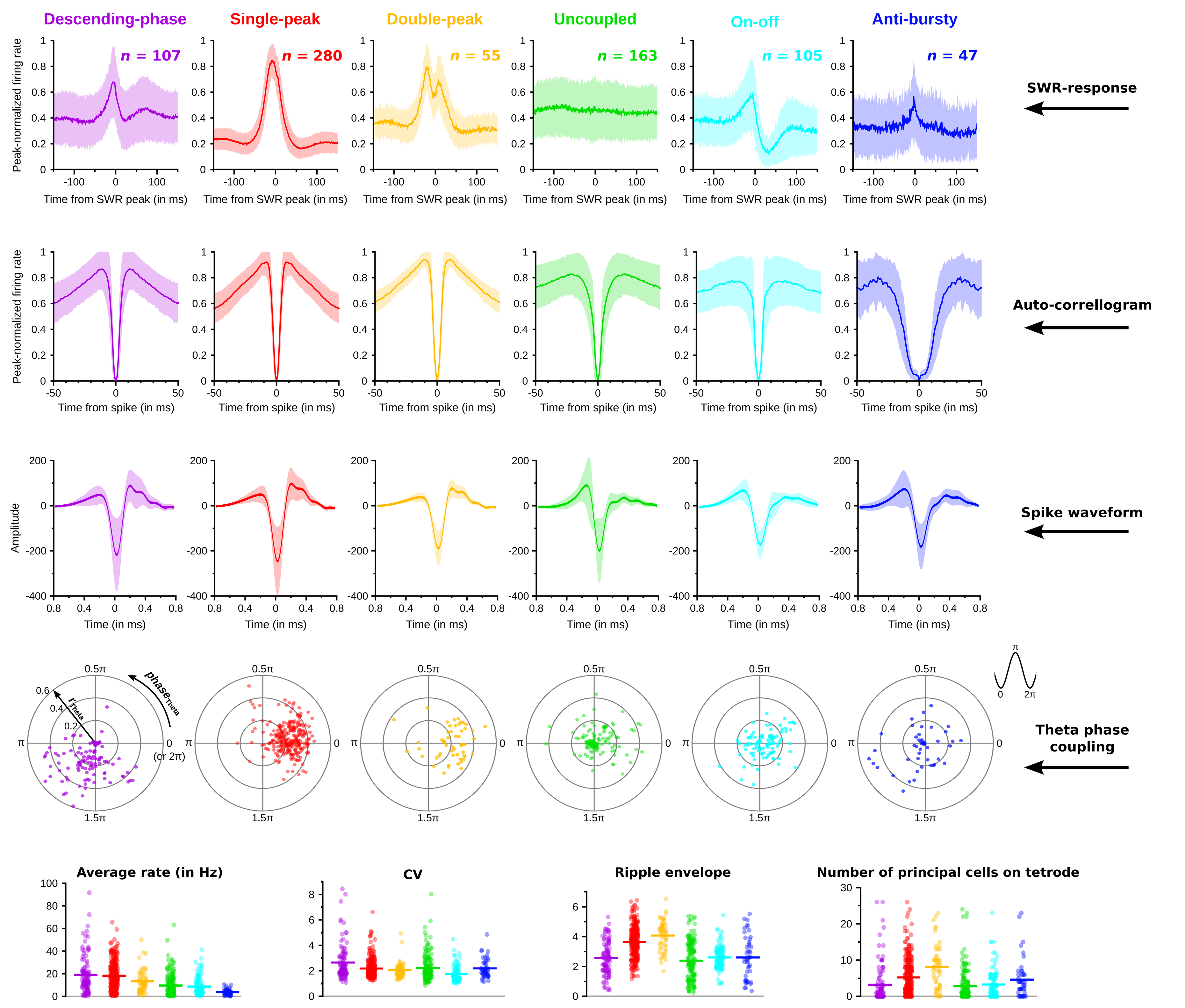
Different methods for estimating the optimal number of clusters provide estimates varying over a wide range.



Elbow method: unclear (with k-means: *red curve in left panel*)
Gap statistic: 7 (with k-means: *left three panels*)
9 (with agglomerative hierarchical clustering)
Silhouette statistic: 2 (both with k-means and with agglomerative hierarchical clustering: *right panel*)

(4) Interneuron classes identified from extracellular recordings

Interneurons are subdivided into 6 clusters with k-means on the 7 SWR-response measures and the 9 "other" firing measures:



(Shaded areas indicate +/- 1 SD)

Discussion

Although we do not find support for the possibility to identify discrete types of hippocampal interneurons solely based on extracellular recordings, we do find structure in our large interneuron dataset indicative of clusters of interneurons with overlapping firing properties. We suggest that our framework for an unsupervised interneuron clustering, although not absolute, nevertheless provides a useful way of classifying hippocampal interneurons that could contribute to further our understanding of their diverse roles in network dynamics and behaviour.

Future work

* Replace the manually crafted numerical firing measures by an automated feature extraction from neurons' SWR-response, auto-correllogram and spike waveform.
* Use data from juxtacellular recordings to evaluate the here proposed classification, which could also help to map these interneuron classes to anatomically defined cell types.

Acknowledgement

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COI statement

None of the authors has a conflict of interest (COI) with regard to this presentation.