Research Articles: Systems/Circuits

Burst firing and spatial coding in subicular principal cells

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https://doi.org/10.1523/JNEUROSCI.1656-18.2019

Received: 26 June 2018
Revised: 27 January 2019
Accepted: 8 February 2019
Published: 7 March 2019

Author contributions: J.S. and M.B. designed research; J.S. performed research; J.S. contributed unpublished reagents/analytic tools; J.S. analyzed data; J.S. and M.B. wrote the first draft of the paper; J.S. and M.B. edited the paper; J.S. and M.B. wrote the paper.

Conflict of Interest: The authors declare no competing financial interests.

This work was supported by Humboldt-Universität zu Berlin, Bernstein Center for Computational Neuroscience Berlin, NeuroCure Cluster of Excellence, Deutsche Forschungsgemeinschaft (SPP 1665, BR 3479/12-1), Deutsche Forschungsgemeinschaft Gottfried Wilhelm Leibniz Preis. We thank Edith Chorev, Rajnish Rao, Juan Ignacio Sanguinetti-Scheck, Konstantin Hartmann, and Peter Bennett for discussion and comments on the manuscript. We thank Andreea Neurkirchner, Undine Schneeweiß, Juliane Diederichs, Tanja Wölk, Maik Kunert and Arnold Stern for outstanding technical support.

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Cite as: J. Neurosci 2019; 10.1523/JNEUROSCI.1656-18.2019

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Abbreviated Title
Bursts and spatial coding in subiculum

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3 figures

Abstract: 151 words; Introduction: 599 words; Discussion: 895 words.

Acknowledgements
This work was supported by Humboldt-Universität zu Berlin, Bernstein Center for Computational Neuroscience Berlin, NeuroCure Cluster of Excellence, Deutsche Forschungsgemeinschaft (SPP 1665), Deutsche Forschungsgemeinschaft Gottfried Wilhelm Leibniz Preis. We thank Edith Chorev, Rajnish Rao, Juan Ignacio Sanguinetti-Scheck and Peter Bennett for discussion and comments on the manuscript. We thank Andreea Neurkirchner, Undine Schneeweiß, Juliane Diederichs, Tanja Wölk, Maik Kunert and Arnold Stern for outstanding technical support.
Abstract

The subiculum is the major output structure of the hippocampal formation and is involved in learning and memory as well as in spatial navigation. Little is known about how the cellular diversity of subicular neurons relates to function. Previously, in vitro studies have identified distinct bursting patterns in subiculum. Here, we asked how burst firing is related to spatial coding in vivo. Using juxtacellular recordings in freely moving male rats, we analyzed the bursting behavior of 51 subicular principal neurons and distinguished two populations, i.e. sparsely bursting (~80%) and dominantly bursting neurons (~20%). The two populations had distinct spatial properties, sparsely bursting cells showing strong positional tuning and dominantly bursting cells showing weak positional tuning. In sparsely bursting neurons, bursts defined sharper place fields than isolated spikes. We conclude that burst firing is relevant to subicular spatial coding, possibly by serving as a mechanism to transmit spatial information to downstream structures.

Keywords: Hippocampus; orientation; cluster analysis; multiplexing

Significance statement

The subiculum is the major output structure of the hippocampal formation and is involved in spatial navigation. In vitro, subicular cells can be distinguished by their ability to initiate bursts, being brief sequences of spikes fired at high frequency. Little is known about the relationship between the cellular diversity and spatial coding in this structure. We performed high-resolution juxtacellular recordings in freely moving rats and found that bursting behavior predicts functional differences between subicular neurons. Specifically, sparsely bursting cells have lower firing rates and carry more spatial information than dominantly bursting cells. Additionally, bursts fired by sparsely bursting cells encoded spatial information better than isolated spikes, pointing towards bursts as a unit of information dedicated to space coding.
Introduction

The subiculum is the major output structure of the hippocampus, receiving its main inputs from CA1 and sending divergent outputs to many subcortical and cortical areas (Amaral and Witter, 1989; Witter, 2006). The subiculum is involved in spatial learning and memory (Morris et al., 1990; Galani et al., 1998; Roy et al., 2017; Cembrowski et al., 2018) but has not been the major focus of studies analyzing hippocampal function in spatial navigation.

In vivo, the vast majority of subicular neurons carry positional information – in various discharge patterns such as place fields, irregular spatial cells or boundary cells (Sharp and Green, 1994; Lever et al., 2009). In addition, firing fields of subicular cells do not remap in response to novel environments, nor do they remap in darkness (Sharp, 1997; Lever et al., 2009; Brotons-Mas et al., 2010). A subset of subicular cells maps the current trajectory taken in an environment with well-defined routes rather than in an open-field arena, implying that environmental constraints strongly influence subicular cell coding (Olson et al., 2017).

Subicular cell spatial fields are less well-defined than CA1 place cells (O’Keefe and Dostrovsky, 1971), or the eye-catching medial entorhinal grid cells (Hafting et al., 2005), due to higher basal firing rates in the subiculum compared to other spatial areas such as CA1 or medial entorhinal cortex.

The microcircuitry underlying spatial tuning in the subiculum is largely unresolved. The subicular anatomy is not as clearly stratified as the stratum pyramidale of CA1 (proximal to subiculum) and also lacks the elaborate lamination of the 6-layered cortical structures such as the presubiculum (distal to subiculum). The analysis of cell morphology indicates some internal structure (O’Mara, 2005) as well as laminar or modular organization based on long-range connectivity (Naber and Witter, 1998; Ishizuka, 2001; Witter, 2006; Kim and Spruston, 2012). In vitro, subicular principal neurons may be distinguished by their firing patterns: some are intrinsic bursting (from 45 - 80%) and others are regular spiking cells (Greene and
Totterdell, 1997; Staff et al., 2000). Bursting relates to subicular anatomy: deeper cells as well as cells located on the distal part tend to be the most bursty (Greene and Totterdell, 1997; Harris et al., 2001a; Kim and Spruston, 2012). However, how bursting relates to subicular function remains mostly unresolved, even though a few functional correlates of bursting have been suggested. First, the biophysical properties of subicular cells could be predicted by their efferent target area (Kim and Spruston, 2012; Cembrowski et al., 2018), suggesting that intrinsic bursting or regular spiking cells might generate different streams of information. (Cembrowski et al., 2018) Second, local connectivity and recruitment by sharp wave ripples suggested distinct roles for regular spiking and intrinsic bursting cells in the subicular microcircuit function (Bohm et al., 2015).

Here, we asked how subicular bursting relates to spatial coding in vivo. We took advantage of high-resolution juxtacellular recordings, which enabled us to reliably resolve small amplitude spikes – especially those resulting from sodium-channel inactivation during bursts. Using this technique in freely moving rats we asked: (i) Can bursting patterns be used to classify subicular neurons in vivo as in vitro? (ii) How does the burstiness of discharges relate to spatial coding? (iii) Do bursts and isolated spikes convey different types of information? We could classify cells based on their burstiness and found that sparsely bursting cells are more spatially modulated than dominantly bursting cells. In a large fraction of spatially modulated neurons, we found that bursts encoded position significantly better than isolated spikes. The encoding of spatial position by bursts predicts that such information is transmitted more effectively by facilitating synapses to downstream areas.
Material and Methods

All experimental procedures were performed according to German guidelines on animal welfare.

Juxtacellular recordings in freely moving rats

Experimental procedures for obtaining juxtacellular recordings in freely moving rats were performed similar to earlier publications (Tang et al., 2014). Recordings were made in 28 male Long-Evans rats (150-350 g) maintained in a 12-h light/dark phase and recorded during the dark phase. Surgical procedures were all performed under ketamine (80-100 mg.kg\(^{-1}\)) and xylazine (80–10 mg.kg\(^{-1}\)) anesthesia. Rats were implanted with a head-implant including a metal post for head-fixation, a placement of a miniaturized preamplifier coupled to two LEDs (red and blue) and a protection cap. In order to target the dorsal subiculum, a plastic ring was glued on the skull surface 5.7-6 mm posterior to bregma and 2.9-3.2 mm left to midline. The craniotomy and the positioning of the metal post for holding the miniaturized micromanipulator (Kleindiek Nanotechnik GmbH, Kusterdingen, Germany) were done either during the same surgery or in a subsequent surgery. After implantation, rats were allowed to recover and were habituated to head-fixation for 2-5 days. Rats were trained to forage for chocolate pellets in an open field arena – a 70 x 70 x 50 cm (WDH) box with a white polarizing cue card on one of the walls – prior to and after implantation (3-7 days, multiple sessions of 15-20 min each per day).

For recordings, rats were head-fixed and the miniaturized micromanipulator and preamplifier were secured to the metal posts.

Glass pipettes with resistance 4-6 MΩ were filled with Ringer solution (n = 45/53) containing (in mM) 135 NaCl, 5.4 KCl, 5 HEPES, 1.8 CaCl\(_2\), and 1 MgCl\(_2\); or patch clamping internal solution (n = 6/53) containing (in mM) 130 K-gluconate, 10 Na-gluconate, 10 HEPES, 10 phosphocreatine, 4 MgATP, 0.3 GTP, 4 NaCl. In both cases, pH was adjusted to 7.2, Neurobiotin (1-2%) was added to the solution and the osmolality was adjusted to 285-305 mmol/kg. The patch clamp solution was used to perform juxtacellular stimulations, of which
the results are not used in the context of the current study. The firing rate and the firing pattern was not different between subicular cells recorded with the 2 different solutions; therefore the two subsets have been merged and considered as one group.

The glass recording pipette was advanced into the brain; a thick agarose solution (3.5-4% in Ringer) was applied into the recording chamber for sealing the craniotomy and stabilization.

Animals were then released into the behavioral arena and juxtacellular recordings were established while animals were freely exploring the environment. The juxtacellular signals were acquired with an ELC-03XS amplifier (npi electronic GmbH, Tamm, Germany) and digitized with a Power 1401 data-acquisition interface coupled to Spike2-v7 (CED, Cambridge Electronic Design, Cambridge, UK) where signals were sampled at 50 kHz. The arena was filmed from above with a color camera so the position of red and blue LEDs could be tracked offline to determine animal’s location and head-direction. All signal processing and analyses were performed in Matlab (MathWorks, Natick, MA, USA).

Anatomy

Juxtacellular labeling was performed at the end of the recording session according to standard procedures (Pinault, 1996). A number of recordings were either lost before the labeling could be attempted, or the recorded neurons could not be clearly identified, but the location of all the cells included in the current study was positively assigned to the subiculum. Ten to thirty minutes after the labeling protocol, the animals were killed by prolonged isoflurane exposure and overdose of urethane, and perfused transcardially with 0.1 M PB followed by 4% paraformaldehyde solution. We used standard procedures for histological analysis of juxtacellularly-labeled neurons. Neurobiotin labeling was visualized with streptavidin conjugated to Alexa 488 (1:250 to 1:1000). Fluorescence images were acquired and position of tracks, filled neurons or recording sites were assigned to an area (subiculum, CA1 or presubiculum).
Spike and bursts detection

For spike detection, the raw signals were filtered (0.3 - 6 KHz, zero phase band-pass Butterworth filter of order 8). Transients were then detected using a threshold of 2.5 times the root mean square (rms) of the signal. High amplitude artefacts, due to behaviors like grooming, could increase the rms value significantly and prevent the detection of the smallest transients; the values in a window of 2.5 ms around these artefacts were therefore clipped and replaced by zeros. A second step for separating spikes from noise consisted of calculating the principal components of the transients followed by manually clustering the events to spikes and noise. This cleaning step was first performed on filtered waveforms and subsequently on raw waveforms. Eventually, the accuracy of spike detection was visually checked, scrolling throughout the whole recording. The cleaning step was repeated until the detection was optimal (minimizing false positives and negatives).

Finally, spikes were categorized as belonging to a burst if the interval from the prior spike and/or to the next spike was shorter than a threshold set at 6 ms. One burst was therefore defined as a group of spikes (>=2) interleaved with less than 6 ms. The burst time stamp was set to that of the first spike in a burst. Burst length was the time difference between the last and the first spike in a burst and burst modal interval was the mean inter-spike-interval (ISI) during bursts.

Spike waveform analysis

The raw signals were filtered (6 KHz, zero phase low-pass Butterworth filter of order 8) in order to minimize high frequency noise. Spike shape parameters were determined based on the spike average waveform calculated from these low-pass filtered traces. Prior to the calculation of the average spike, the single waveforms had to be properly aligned. To this end, every spike waveform was oversampled at 1000 kHz using a spline interpolation to better estimate its shape. Signal to noise ratios often differed between recordings and with it, the spike amplitude. To be able to compare spike shape parameters between cells the waveform was normalized so that the rising amplitude was 1 mV. We then calculated the
derivative of each waveform. The threshold was set as the first sample where both the
voltage and its derivative were at least 5% of their maximal value. The rising amplitude (mV)
was set to the difference of potential between the peak and the threshold voltage. The after-
hyperpolarization was set to the point at minimum voltage after the peak, when the derivative
≥ 0. The spike duration was set to the threshold-to-after-hyperpolarization duration. Putative
fast-spiking interneurons (n = 2) were identified based on their high average rate (41 and 73
Hz), their spike duration inferior to 0.5 ms (0.34 and 0.39 ms), their high maximum derivative
(10.5 and 9.2 mV.ms⁻¹) and low minimum derivative (-8 and -12.3 mV.ms⁻¹). Fast spiking
interneurons were not used for the subsequent analyses.

Analysis of burstiness

We estimated the burstiness of each subicular principal cell using a combination of two
different methods, which are both biased by basal firing rates but in opposite directions.
The first method is based on the distribution of inter-spike-intervals and has previously been
used in order to study a cell’s burstiness in the subiculum (Sharp and Green, 1994; Lever et
al., 2009; Brotons-Mas et al., 2010) and other areas such as parahippocampal cortices
(Ebbesen et al., 2016). The ISI interval distribution might overestimate burstiness for cells
with elevated firing rates. This problem is solved by the second method - analysis of the
spike autocorrelograms from 1 to 20 ms (Kim et al., 2012). Here, the bursting index was
calculated as the ratio of the integrated power of the autocorrelogram between 1 and 6 ms
normalized by the overall power between 1 and 20 ms. This method does not bias the
burstiness estimation for high firing neurons. However, we realized that it could overestimate
the bursting probability of neurons with low firing rate and very occasional bursts because
only the first 20 ms of the autocorrelogram are considered in this analysis.
Principal component analysis (PCA) was done on both the log(ISI) probability matrix and for
the 1-20 ms lag probability matrix. The first three components from each PCA were used to
generate a firing pattern vector in a 6-dimensional space. We then generated a cluster tree
using Ward's method on the normalized Euclidean distance between cells. The Ward's method establishes hierarchical clusters by iteratively grouping the two closest observations or groups of observations together. Consequently, cells with very similar firing patterns are primarily grouped together and groups with very different properties are linked at the end of the procedure (Ward, 1963). Two clusters strikingly emerged from the dendrogram, defining two groups of neurons that we named sparsely bursting cells and dominantly bursting cells based on their potency to initiate bursts.

Analysis of spatial modulation

The position of the rat was defined as the midpoint between two head-mounted LEDs. A running speed threshold (1 cm.sec\(^{-1}\)) was applied for isolating periods of rest from navigation. For generating color-coded firing maps, space was discretized into pixels of 2.5 cm x 2.5 cm. For each such pixel the occupancy \(o(x)\) was calculated:

\[
o(x) = \sum_t w(|x - x_t|)\Delta t
\]

where \(x_t\) is the position of the rat at time \(t\), \(\Delta t\) the inter-frame interval, and \(w\) a Gaussian smoothing kernel with \(\sigma = 5\) cm. Then, the firing rate \(r\) was calculated for each pixel \(x\):

\[
r(x) = \frac{\sum_i w(|x - x_i|)}{z}
\]

where \(x_i\) is the position of the rat when spike \(i\) was fired.

For recordings in which the animal's trajectory covered at least 60 % of the open field (n = 41/51), we calculated the spatial information rate, \(I\) (bits / spike), from the spike train and rat trajectory as follows:

\[
I = \frac{1}{T} \int [r(x) \log_2 \frac{r(x)}{\bar{r}}] o(x) dx ,
\]

where \(r(x)\) and \(o(x)\) are the firing rate and occupancy as a function of a given pixel \(x\) in the rate map. \(\bar{r}\) is the overall mean firing rate of the cell, and \(T\) is the total duration of a recording session (Skaggs et al., 1993).
Statistical analysis

A cell was declared to have a significant amount of spatial information if the observed spatial information rate exceeded the 95th percentile of a distribution of values of I obtained by circular shuffling. Shuffling was performed a 1000 times by a circular time-shift of the recorded spike train relative to the rat’s trajectory by a random time $t' \in [0,T]$ (von Heimendahl et al., 2012; Bjerknes et al., 2014), $T$ being the total duration of the recording session.

The size difference between the two clusters of subicular neurons and the small size of the group of dominantly bursting cells used for spatial information calculation brought us to test the significance of the difference with a bootstrapping procedure. N (number dominantly bursting cells) values of spatial information were randomly selected from the sparsely bursting population. Repeating the procedure 1000 times, we then obtained a bootstrapped distribution of the median spatial information for sparsely bursting cells. The difference was significant if the rank of the median spatial information of dominantly bursting cells was within the 5th percentile of the bootstrapped distribution ($P \leq 0.05$).

We wanted to test whether the spatial information encoded by bursts was significantly different than the spatial information encoded by isolated spikes. A direct comparison of spatial information values would not be appropriate as the total number of events and smoothing parameters used for generating the rate maps can introduce bias in information-theoretic measures (Harris et al., 2001b). Consequently, we used a randomization method similar to Harris et al. (2001b). In instances where there were less bursts than isolated spikes, we would compare information given by the N bursts to the information given by 1000 random subsets of N isolated spikes. Bursts were significantly more informative than isolated spikes if the rank of the burst spatial information was within the 95th percentile of the distribution of the spatial information given by the random subsets of isolated spikes.
In some instances where highly bursting cells had less isolated spikes than bursts (n = 2/51), we compared the information given by N isolated spikes to the information given by 1000 random subsets of N bursts. In this case, bursts were significantly more informative than isolated spikes if the rank of the isolated spike spatial information was within the 5th percentile of the distribution of the spatial information given by the random subsets of bursts.

In many cases, the significance of the difference observed between distinct groups was assessed with non-parametric tests only. Two-tailed Mann-Whitney U tests were used to determine whether two groups of unpaired observations were significantly different from each other (e.g. comparing sparsely bursting and dominantly bursting cells). Two tailed Wilcoxon signed rank tests were used in case of two groups of paired observations (e.g. comparing bursts and isolated spikes).
Results

We performed juxtacellular recordings in rats foraging for food in a 70 x 70 cm open field arena. Our data consists of 51 subicular principal cells recorded in 28 rats. Neurons were assigned as subicular cells histologically and as principal cells based on firing rates and spike waveforms.

Sparsely and dominantly bursting cells: distinct firing patterns in vivo

Previous in vitro work (Greene and Totterdell, 1997; Staff et al., 2000; Harris et al., 2001a; Kim and Spruston, 2012) indicated the existence of distinct patterns of bursting in different types of subicular principal cells. In our in vivo recordings, we also noted distinct bursting patterns of subicular principal cells during navigation. We categorized cells according to their burst discharge pattern. Clustering neurons using the Inter-Spike-Interval (ISI) histograms and spike autocorrelograms (Figure 1; see methods) resulted in two distinct groups: sparsely bursting cells (n = 41 / 51 cells, ca. 80 %) and dominantly bursting cells (n = 10 / 51 cells, ca. 20 %; Figure 1)

A trace from a recording and spikes of a sparsely bursting cell are shown in Figure 1A and B. Burst firing (i.e. spikes interleaved with ISI ≤ 6 ms) occurred only sparsely. Both the ISI histogram (Figure 1C) and autocorrelation function of spikes (Figure 1D) confirm that short ISIs were present but rare in this cell group.

A trace from a recording and spikes of a dominantly bursting cell are shown in Figure 1E and F. A large fraction of spikes was organized in bursts. A prominent peak at short ISIs is obvious in the ISI histogram (Figure 1G) and autocorrelation function of spikes (Figure 1H).

The proportion of ISIs ≤ 6 ms of the total number of spikes was calculated as a bursting index. The median bursting index was significantly higher for dominantly bursting cells than for sparsely bursting cells (Figure 1I, median: SB = 0.137, DB = 0.0.514, Mann-Whitney U test, P = 1.10^-6). The average number of intra-burst spikes tended to be higher in dominantly bursting cells than in sparsely bursting cells (median: SB = 2.1, DB = 2.23, Mann-Whitney U
test, $P = 3.10^{-4}$). In addition, the mean intra-burst intervals were shorter in dominantly bursting cells than in sparsely bursting cells (median: $SB = 4.3$ ms, $DB = 3.6$ ms, Mann-Whitney U test, $P = 2.10^{-6}$). Dominantly bursting cells had higher bursting rates than sparsely bursting cells (medians: $SB = 0.7$ Hz, $DB = 4.8$ Hz; Mann-Whitney U test, $P = 3.10^{-6}$). Firing rates were variable and rather high as previously reported for subicular neurons (Sharp and Green, 1994; Lever et al., 2009; Kim et al., 2012). Firing rates of dominantly bursting cells were higher on average than sparsely bursting cells during navigating periods (speed > 1 cm.sec$^{-1}$; Figure 1J; medians: $SB = 13.5$ Hz, $DB = 20.1$ Hz; Mann-Whitney U test, $P = 0.0043$). Spike duration (from threshold to after-hyperpolarization) of sparsely bursting cells and dominantly bursting cells were not different from one another (Figure 1K; median: $SB = 0.84$ ms, $DB = 0.92$ ms; Mann-Whitney U test, $P = 0.6954$).

**Sparsely bursting cells provide more spatial information than dominantly bursting cells**

Our initial analysis showed that subicular cells can be clustered into two distinct cell populations based on their bursting activity in vivo. Next we asked if these populations also differ in their spatial tuning properties. Figure 2 shows the animals’ running trajectory with the superimposed spike positions and the resulting rate maps for one sparsely bursting cell (Figure 2A, B) and one dominantly bursting cell (Figure 2C, D). The sparsely bursting cell is robustly spatially tuned, while the dominantly bursting neurons is far less tuned. We calculated the spatial information for cells with sufficient coverage (>60%) of the open field area. This analysis (see methods, Figure 2E) revealed that sparsely bursting cells are far more spatially tuned than dominantly bursting neurons (Figure 2E, median: $SB = 0.19$ bits/spike ($n = 35$), $DB = 0.03$ bits/spike ($n = 6$), Mann-Whitney U test, $P = 0.028$). Given the size difference between groups and the low number of dominantly bursting cells ($n = 6$), we further tested the significance of the difference using bootstrapping (see methods). The spatial information of dominantly bursting
cells was significantly different from the distribution of spatial information values of the
randomly selected subsets of the sparsely bursting cells (P = 0.01).

Approximately 65% (25/35) of sparsely bursting cells and 50% (3/6) of dominantly bursting
cells were significantly modulated by the animal’s position and could therefore be defined as
spatial neurons. Sparsely bursting spatial neurons encoded more spatial information than
dominantly bursting neurons (median: SB = 0.42 bits/spike, n = 25; DB = 0.13 bits/spikes, n
= 3, bootstrapping P = 0.04).

The differences in spatial coding between sparsely bursting and dominantly bursting cells
reinforced the idea that the classification of cells according to bursting discharge patterns
captures significant functional differences between subicular neurons.

**Bursts provide spatial information in sparsely bursting cells**

Our previous analyses revealed that subicular cells can be clustered according to bursting
discharges and that such a classification reveals functional differences. While dominantly
busting cells burst most of the time, sparsely bursting cells do so occasionally and we
wondered, how such occasional bursts contributed to the transmission of spatial information.

In the example shown in Figure 2A and B, it can be seen that many spikes occurred outside
of the main spatial firing field. In order to evaluate how the firing patterns contributed to the
coding of spatial information at the single cell level, we separated isolated spikes and bursts
into distinct plots (Fig 3A-F). A first example from a sparsely bursting cell, shown in Figure
3A-C, suggested that isolated spikes occurred in numerous locations, even though a
preferred location is still evident in the rate map (Fig 3B). A strikingly different picture
emerged when we only plotted bursts. A well-defined firing field akin to a CA1 place cell
emerged by looking at the bursts’ positions on the trajectory and the corresponding rate map
(Figure 3C). Applying the same analysis to further cells strengthened the idea that sparsely
bursting cells provide spatial information through bursts. As shown for a second sparsely
bursting cell in Figure 3D-F, bursts were confined to the north border of the environment (Fig
3F) whereas isolated spikes were widely distributed throughout the arena (Fig 3E).
The difference in the number of events per group (bursts or isolated spikes) can bias spatial information values; it tends to be higher while computed on a lower number of events (Harris et al., 2001b). Typically, there was more isolated spikes than bursts. We compared the value of the spatial information encoded by the N bursts to the average of the spatial information encoded by 1000 random subsets of N isolated spikes (or the opposite if there was more isolated spikes than bursts). In sparsely bursting cells, burst transferred higher spatial information than isolated spikes (medians: 0.51 bits / burst, 0.41 bits / isolated spike; Wilcoxon signed rank test, P = 0.0001; Figure 3G). In contrast, bursts did not encode sharper spatial information in dominantly bursting cells (medians: 0.03 bits / burst, 0.07 bits / isolated spike; Wilcoxon signed rank test, P = 0.563; Figure 3G). Here the median difference appears to be low. However, this analysis considered all neurons of our dataset, including non-spatial neurons. The median difference between bursts and isolated spikes was higher if we considered spatially significant sparsely bursting cells only (n = 23, 0.80 bits / burst, 0.44 bits / isolated spike; Wilcoxon signed rank test, P = 0.0001).

At the single cell level, information encoded by bursts was determined to be significantly more informative than isolated spikes if it was within the 95th percentile of the distribution of spatial information values random subsets of N isolated spikes (See methods for when there are fewer isolated spikes than bursts). The difference between isolated spikes and bursts was significant for 17 of the 23 spatially modulated sparsely bursting cells and 2 of the 3 spatially modulated dominantly bursting cells (Figure 3G, H). For sparsely bursting cells with a significant difference (n = 17), burst spatial information (median = 0.912 bits / burst) was on average 2.4 times higher than isolated spike spatial information (median, 0.42 bits / isolated spike, Figure 3I). Similarly, it was 2.4 and 1.6 times higher for the two dominantly bursting spatial cells with a significant burst effect, nevertheless the burst spatial information remained among the lowest from our dataset (0.33 and 0.17 bits / burst, Figure 3I).
Discussion

We studied how burst firing related to spatial coding in the subiculum of rats. We first classified subicular neurons according to their bursting patterns and distinguished two classes of subicular neurons, a large fraction (80%) of sparsely bursting cells and a small fraction (20%) of dominantly bursting cells. Most sparsely bursting cells were spatially modulated cells and carried more spatial information than dominantly bursting cells. Finally, we found that bursts in sparsely bursting neurons carry more spatial information than isolated spikes, which account for the ubiquitous firing seen in most of the subicular spatial cells.

The initial impetus for our study came from in vitro studies, which identified bursting in subicular neurons. Most interestingly, bursting was shown to be correlated with the projection target of the respective neuron (Kim and Spruston, 2012) suggesting a functional relevance of the bursting phenotype. In previous studies, bursting relationship to space coding has been investigated in subiculum without establishing a clear picture (Sharp and Green, 1994; Lever et al., 2009; Brotons-Mas et al., 2010; Kim et al., 2012). Previous reports on CA1 place cells suggested than intrinsic bursting cells, rather than regular spiking cells, were more likely to be spatially modulated (Epsztein et al., 2011). Unlike previous studies on the subiculum, we observed marked functional differences between cell classes defined by their bursting behavior, however quite opposite to CA1. Indeed, we found that dominantly bursting cells fire at higher rates and their spikes carry little spatial information, greatly strengthened the idea that bursting is of functional significance for subicular space coding. We believe that the high resolution of the juxtacellular recordings as well as our method to cluster subicular cells in distinct groups reflecting burstiness were key element in our findings.
However, it is not yet clear how our in vivo classification of sparsely bursting and dominantly bursting cells is related to various classifications of bursting based solely on intrinsic properties. Different in vitro studies on subicular neurons reported varying estimates for the fraction of intrinsically bursting neurons, ranging between 45 to 80% (Mason, 1993; Stewart and Wong, 1993; Behr et al., 1996; Greene and Totterdell, 1997; Staff et al., 2000; Harris et al., 2001a; Kim and Spruston, 2012; Joksimovic et al., 2017). Only about 20% of the neurons observed in our study were of the dominantly bursting subtype. These numbers do not match previous reports and it seems unlikely that the dominantly bursting cells observed here correspond to the broad definition of intrinsic bursting cells used in in vitro studies. It seems possible that the dominantly bursting cells observed by us correspond to a subgroup of neurons with a particular strong tendency for intrinsic bursting described in vitro. In contrast, generating bursts does not appear to be a default mode of firing for most sparsely bursting cells. These cells might be weakly bursting or regular spiking cells requiring more complex mechanisms such as the interaction of intrinsic mechanisms and synaptic inputs for bursting (Larkum, 2013).

Matching numbers of bursting cells between distinct studies is complicated because experimental conditions might be different from one study to another, especially since burst generation depends on many factors, which are not easy to control in vivo. Indeed, a variety of cellular mechanisms of burst generation have been suggested for subicular neurons. For instance, bursting requires T-type voltage gated calcium currents (Joksimovic et al., 2017) that can be affected by neuromodulatory signals, such as serotonin, which was shown to downregulate T-type channels and burst generation (Petersen et al., 2017).

As the output structure of the hippocampus, the subiculum sends high frequency, but rather unprecise, spatial coding to downstream areas. Indeed, peak frequencies of subicular spatial neurons are rather high compared to CA1, as are their baseline ((Sharp, 1997, 2006; Kim and Spruston, 2012). Nevertheless, spatial signals can be refined if one take the precise
firing pattern of subicular neurons into consideration. Indeed, isolated spikes and bursts are functionally distinct units of information in most sparsely bursting spatial neurons (ca. 70%). While bursts were often fired in well-defined place fields, isolated spikes were spatially dispersed. Such differential coding by isolated spikes and bursts is similar to information processing in sensory systems (Krahe and Gabbiani, 2004). Nonetheless, our finding is remarkably different from CA1, where bursts sharpen spatial information in only ca. 20% of place cells (Harris et al., 2001b). Such a difference shows the relevance of burst firing in noisy spatial cells such as subicular cells, compared to sharply tuned CA1 place cells. Bursts and isolated spikes, as two units of information could be readout by the interaction between short-term plasticity and postsynaptic integrative properties (Lisman, 1997; Izhikevich et al., 2003). The spatial information conveyed by a burst could be decoded by the summation of excitatory events at facilitating synapses whereas poorly tuned spatial inputs could be better decoded through depressing synapses (Lisman, 1997). This should be the case for long range projections and could as well define functional sub-circuits within the local microcircuit (Simonnet et al., 2017). The ongoing activity and resonating properties of targeted neurons could define the response to these signals (Izhikevich et al., 2003). However, the neuronal targets of subicular spatial neurons and how these integrate and convert multiplexed signals at the cellular and microcircuit levels are unknown elements. These will need to be resolved for a better understanding of the subicular role in distributing hippocampal output spatial codes.
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Figures & Legends
**Figure 1.** Firing pattern features of sparsely and dominantly bursting cells in vivo.

(A) Bandpass filtered (300 - 6000Hz) trace of recording from a sparsely bursting cell. Spikes occurring in burst are labeled with an orange dots and isolated spikes with a green dots.

(B) Magnification of the burst (orange, left) and the isolated spikes (green, right) indicated with a star.

(C) Plot of the probability of Inter-Spike-Intervals (ISIs) for sparsely bursting subicular cells on a logarithmic scale. Note that short ISIs occur rarely in these neurons. The vertical dashed line is placed at 6 ms.

(D) Autocorrelograms of sparsely bursting subicular cells.

(E, F) as B and C for a dominantly bursting subicular cell.

(G) Inter-Spike-Intervals as in C for dominantly bursting cells. Note the prominent initial peak.

(H) Autocorrelograms of dominantly bursting subicular cells.

(I) ISI based bursting index corresponding to the proportion of inter-spike-intervals lower than 6 ms is significantly higher for dominantly bursting cells. Two-tailed Mann Whitney U-Test.

(J) Spiking rate (Hz) while the animal is navigating (see methods) is significantly higher for dominantly bursting cells. Two-tailed Mann Whitney U-Test.

(K) Spike duration is not different between sparsely bursting and dominantly bursting cells. Statistics: two-tailed Mann Whitney U test.

**Figure 2.** Sparsely bursting cells provide more spatial information than dominantly bursting cells.

(A) Trajectories of the rat with the spikes of a sparsely bursting cell superimposed in red.

(B) Corresponding rate map with the peak rate (above) and spatial information and significance (below).

(C) As A for a dominantly bursting cell.

(D) As B for a dominantly bursting cell.

(E) Spatial information calculated for sparsely bursting and dominantly bursting cells. Sparsely bursting cells provided significantly more information than dominantly bursting cells. Statistics: two-tailed Mann Whitney U test.

**Figure 3:** Sharp spatial tuning of burst firing in sparsely bursting cells.

(A) Spikes (red dots) superimposed to the animal’s trajectory (gray line).

(B) Left, isolated spikes (green dots) superimposed to the animal’s trajectory (gray line) and corresponding rate map; peak rate above and spatial information and significance below.

(C) Left, bursts (orange dots) on animals’ trajectories (gray line) and corresponding burst rate maps; peak rate above and spatial information and significance below.

(D-F), as A-C for another sparsely bursting cell.

(G) Spatial information is significantly higher for bursts than isolated spike spatial information within the population of sparsely bursting cells but not for dominantly bursting cells. $P$, two-tailed Mann-Whitney U test.
(H) Information per burst versus information per isolated spikes for spatially modulated neurons (n = 25 sparsely bursting cells, black circles; n = 3 dominantly bursting cells, blue circle). Solid circles indicate cells with a significant increase of spatial information between burst and isolated spike (n = 17 / 25 for sparsely bursting cells and n = 2 / 3 for dominantly bursting cells).

(I) Same as G, showing only spatial cells with a significant difference between information encoded by bursts and isolated spikes, with sparsely bursting cells in black and dominantly bursting cells in blue. In G-I spatial information values have been calculated using similar numbers of bursts and isolated spikes in order to obtain unbiased and comparable results (see methods).

Figure 4. Anatomy of sparsely bursting cells and dominantly bursting cells

A-G, reconstructions of 6 subicular principal cells. Dendrites are in black (sparsely bursting cells) or blue (dominantly bursting cells) and axons are in orange. Some of the anatomical outlines have been drawn, such as CA1 stratum pyramidale (sp), the stratum lacunosum moleculare (slm) and the limit of the subiculum with the dorsal hippocampal commissure (dhc). dg: dentate gyrus. All cells are oriented as indicated in panel E; prox: proximal; dist: distal; sup: superficial. In A, scale bar = 200 μm.

H. Distribution of sparsely bursting and dominantly bursting cells along the proximo-distal axis of the subiculum. Fisher’s exact tests, with level of significance corrected to be equal 0.05 in total: ns: p > 0.05/3; proximal vs middle, p = .7211; middle vs distal = 0.5457; superficial vs distal = 0.2183.

I. Distribution of sparsely bursting and dominantly bursting cells along the radial axis of the subiculum. Fisher’s exact tests (ns: p > 0.05/3); superficial vs middle, p = 0.593; middle vs deep = 0.0231; superficial vs deep = 0.1588.

Figure 5. Sparsely bursting cells provide more spatial information than dominantly bursting cells.

A-C, left, trajectories of rat in gray with superimposed spikes in red. Middle, corresponding rate maps with their color-map ranging from 0 to the maximum firing rate of the cells (Hz). Right, cell-by-cell spatial significance analyses. Each cell’s spatial information (red vertical line) was ranked within the distribution of spatial information values (black histogram) calculated from rate maps generated by the cell’s spiking activity aligned on bootstrapped trajectories (see methods). For each cell, spatial information was declared significant if the cell’s information exceeded the 95th percentile of the random distribution obtained after circular shuffling (see methods). All the cells showed in A-C are spatially significant (p < 0.05, see methods). Neurons in A-B are sparsely bursting cells, the neuron in C is a dominantly bursting cell. The neuron in B was categorized as a boundary cell (boundary score = 0.73, p = 0.01, see methods).

D. Spatial information and significance were calculated for subicular cells recorded while the animal explored (speed > 3 cm/sec) at least 60% of the open field arena (n = 84 / 102). The bar graph shows the percentage of spatially modulated cells from all subicular cells (n = 51 / 84, gray), and then from sparsely bursting cells (n = 46 / 69, black) and dominantly bursting cells (5 / 15 cells, blue). Sparsely bursting cells are more often spatially modulated than dominantly bursting cells (p = 0.022: significant Fisher’s exact test). E. Percentage of each cell type within spatial cell categories. Left, sparsely bursting cells represent the majority (n = 46 / 51) of subicular spatial units. Right, all boundary cells were sparsely bursting cells F. Spatial information calculated for
spatially significant sparsely bursting and dominantly bursting cells (p = 0.032, bootstrapping, significant). G. Spatial information calculated for all sparsely bursting cells and dominantly bursting cells (p = 0.001, bootstrapping, significant).

Figure 6. Sharp spatial tuning of burst firing in spatially modulated sparsely bursting cells
A-F. Example plots of sparsely bursting spatial neurons. spikes: spikes (red dots) superimposed to the animal’s trajectory (gray line). isolated spikes: isolated spikes (green dots) superimposed to the animal’s trajectory (gray line) and corresponding rate map; corrected spatial information below. bursts: bursts (orange dots) on animals’ trajectories (gray line) and corresponding burst rate maps; spatial information below. Each color-map ranges from 0 to the rate map maximum rate (Hz). significance: results of the bootstrapping used to determine significance of burst spatial information compared to isolated spike information. The orange line represents spatial information calculated from the N bursts fired by each cell and the green histogram shows the distribution of spatial information calculated from 1000 random samples of N isolated spikes. Cells in C-F are boundary cells. G, fraction of cells where burst spatial information is significantly higher than isolated spike spatial information. Within groups: proportion of all spatial cells, sparsely bursting spatial cells and dominantly bursting spatial cells with a significant difference. From group: proportion sparsely bursting cells and dominantly bursting cells in cells showing a significant difference. Boundary cells: proportion of boundary cells with a significant difference. H. Information per burst versus information per isolated spike for spatially modulated neurons (n = 46 sparsely bursting cells, black circles and purple circles corresponding to boundary cells; n = 5 dominantly bursting cells, blue circle). Solid circles indicate cells with a significant increase of spatial information between bursts and isolated spikes (n = 31 / 46 for sparsely bursting cells including (n = 12 / 17 boundary cells) and n = 3 / 5 for dominantly bursting cells). I. Spatial information per isolated spike and bursts only for spatial cells with a significant difference, with sparsely bursting cells in black and dominantly bursting cells in blue and boundary cells in purple. Corrected info: spatial information calculated using similar numbers of bursts and isolated spikes (from the bootstrapping).
ACB logISI histograms cluster tree autocorrelations norm. Euclidean distance

SB - n = 82
DB - n = 20

ISI bursting index
0
0.2
0.4
0.6
0.8
1
p = 9.10^{-12}

spiking rate (Hz)
0
10
20
30
p = 0.012
A sparsely bursting 100 ms

B 2 ms 2.5 mV

C dominantly bursting 100 ms

D 2 ms 1 mV

E p = 0.25

F p = 1.10^{-8}

G p = 1.10^{-11}

spike duration (ms)

bursts with >2 spikes (%)

mean ISI in bursts (ms)

SB n = 82

DB n = 20
