EVIDENCE OF SPATIAL PERIODIC FIRING IN THE SUBICULUM OF MICE

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Abstract

The subiculum (SUB) is a region located at the core of the hippocampal formation. The SUB receives inputs from grid cells located in the medial entorhinal cortex (MEC) and from place cells in the CA1 area. Moreover, this structure mediates the output from the hippocampus to cortical and sub-cortical areas involved in the processing of different types of information. Despite the potential relevance of the SUB, its role in memory and spatial coding remains poorly studied. Previous work described a heterogeneous population of SUB spatial neurons, with evidence of its role in coding the geometry of the environment and spatial navigation in darkness. With the aim of understanding further the properties of spatial coding in the SUB, we implanted mice with microdrives carrying tetrodes to target CA1 and the SUB. Apart from the classical place cells described before, we discovered a fraction of SUB pyramidal neurons that generated spatial periodic firing. SUB spatial neurons presented lower spatial resolution and spatial stability than CA1 place cells. The role of spatial periodic neurons in the SUB might be relevant in several computations through the interaction of this region with CA1, the pre-parasubiculum and the MEC.

Keywords:

Spatial navigation, subiculum, place cells, grid cells, subiculum, hippocampus, periodic firing.

Significance Statement:

Spatial information coding and memory formation relies in a complex circuit that involves the hippocampal formation and multiple spatial neuron types. We found for the first-time evidence of pyramidal neurons showing grid and periodic firing in the SUB. Our data support the idea that the SUB might be involved in complex spatial information processing beyond the integration of CA1 unit inputs.

Introduction

Since the discovery of hippocampal place cells -neurons that code for space being active in specific locations of the environment (O'Keefe and Dostrovsky, 1971)- multiple efforts have aimed to understand the computational bases of spatial navigation. As result, different types of spatially modulated neurons have been described across brain regions: head direction cells, (HD); border and boundary vector cells (BVC), coding for the geometry of the environment in medial entorhinal cortex (MEC) and the SUB; and grid cells (GC) in the medial entorhinal cortex (MEC) (Taube et al., 1990; Sharp and Green, 1994; Hafting et al., 2005; Fyhn et al., 2008; Solstad et al., 2008; Lever et al., 2009; Brotons-Mas et al., 2017a). These neurons are either strongly involved in the codification of external information, such as BVC coding for geometry, or in egocentric related information. This is the case of GC, which tessellating regular firing was related with the representation of the metrics of space and therefore necessary for path integration, the capability of navigate in the absence of external cues (McNaughton et al., 2006). The integration of both streams of information, allocentric and egocentric, is instrumental for the generation of an efficient spatial representation.

Multiple computational models have aimed to unveil the possible mechanisms underlying the integration of these different types of spatial signals. The BVC model suggested that place cells require the existence of BVC neurons coding for the geometry of the environment (Barry et al.,2006; Hartley et al., 2000; Solstad et al., 2008; Stewart et al., 2014; Brotons-Mas et al., 2017). Other models, suggested that place cell required of GC activity (Hafting et al., 2005; O'Keefe and Burgess, 2005; Solstad et al., 2006). However, single unit recordings during neurodevelopment, have demonstrated that HD, place cells and boundary neurons appear before GC during neurodevelopment (Langston et al., 2010; Wills et al., 2010). Thus, the dependence of place cells activity on grid cell firing seems to be unclear.

Further research has considered the heterogeneity of the different spatial cell types, and their distributed anatomical localization. In this sense the SUB might be a critical anatomical hub for spatial navigation (O'Mara et al., 2009; Aggleton and Christiansen, 2015). Strategically located between the EC and CA1, the SUB supports spatial information in a complex and heterogeneous manner. While spatial neurons in CA1 typically exhibit spatial reliability, the behavioral correlates of SUB neuron activity include location, speed, direction, and the geometry of the spatial context, while providing a reliable representation of space across light-dark transitions (Barnes et al., 1990; Sharp, 1997; Brotons-Mas et al., 2010; Ledergerber et al., 2021). Interestingly, the SUB is one of the areas to be first affected in Alzheimer's diseases, it propagates

epileptogenic activity in temporal lobe epilepsy (TLE), and is involved in schizophrenia (Sharp and Green, 1994; Rosoklija et al., 2000; Menendez De La Prida & Gal, 2004; Stafstrom, 2005; Godsil et al., 2013; Carlesimo et al., 2015). The SUB contains neurons with different electrophysiological characteristics that might have different roles in memory process (Menendez De La Prida, 2006;Jarsky et al., 2008; Eller et al., 2015; Nitzan et al., 2020).

Previous work in rats suggested the existence of grid-like spatially tuned activity in the SUB (Brotons-Mas et al., 2017), whose electrophysiological characteristics were consistent with axons coming from the MEC. This suggested that SUB pyramidal cells can potentially integrate periodic (from EC) and aperiodic (from CA1) signals, however this possibility has not been extensively explored so far. Here, we sought to test for the existence of periodic firing in individual SUB cells of mice using large open field arenas. We found evidence of grid and other forms of spatially-periodic firing (i.e. band-like, (Krupic et al 2012) in SUB neurons which electrophysiological characteristics are compatible with pyramidal neurons. To the best of our knowledge, this is the first evidence of the existence of spatially periodic neurons in the rodent SUB, supporting the unique role of this structure in the definition of the spatial cognitive map.

Methods

Subjects

Male wild-type mice C57, N=9, age p60 to p90 supplied by the UMH “Servicio de Experimentación Animal (SEA)" were used. Mice were maintained on a 12 h light/dark cycle with food and water available *ad libitum* and were individually housed after electrode implantation. Our procedures were approved by the UMH-CSIC ethics committee, and the regional government and met local and European guidelines for animal experimentation (86/609/EEC).

In vivo recordings on freely moving mice.

Micro-drives (Axona ltd) with four independent screws were loaded with tetrodes (12 mm tungsten wire, California Fine Wire Company, Grover Beach, CA, USA). Electrodes were implanted under isoflurane anesthesia and buprenorphine analgesia, through a craniotomy above the hippocampus. Animals with misplaced electrodes or showing lesions were left out of the analysis. Mice were recorded in standard (50x50 com) and in large open field arenas (70 x 70 cm).

Data acquisition.

As in previous studies (Brotons-Mas et al., 2010, 2017) electrophysiological recordings were obtained using 16-channel headstage (gain x1), with an infrared light-emitting diode (LED) to track mouse position (Axona Ltd, UK). Signals were amplified (400 to 1000x), band pass filtered (0.3 Hz to 24 KHz, Dacq system, Axona, UK) and recorded at 48 KHz/24 bit precision. These recordings were initially used to determine electrode location before the implementation of the recording protocols. For single unit recordings, signal was band-pass filtered between 380 Hz and 6 KHz. Spikes were recorded whenever the signal was 3-4 times above background noise, typically 20-30 µV and stored in windows of 1 ms duration (200 ms before threshold and 800 ms after threshold detection). Spike waveforms were sampled at 48 KHz while the same channels were also recorded in continuous mode at 4.8 KHz and band-pass filtered between 0-3-2.4 KHz.

Local field potential recordings.

During recording sessions, animals were examined for their basal activity while they were in their home cages. Ripple power, unit activity and theta power were used as electrophysiological landmarks to determine electrode location.

Place cells recordings.

Animals were recorded using the pellet chasing task. Small pellets of food were thrown in every 20 s to random locations within the open field, keeping the animal in continuous locomotion, thereby allowing a complete sampling of the environment (Brotons-Mas et al., 2010).

Data Analysis.

Local field potential was analyzed using custom-written MatLab codes (Mathworks, Natick, MA, USA). Raw recordings were FIR filtered (<1.2 KHz) and down sample to 2.4 KHz. Similarly, to previous work (Del Pino et al., 2017) data obtained in the open field recordings was used to characterize the local field potential. Running speed was computed based on the coordinates of the animal's position and epochs above 5 cm/s were included for analysis obtaining epochs of similar behaviors in both groups. To visualize the power spectrum in relationship to the speed of movement the spectral power (in decibels 10log10) and spectrogram was built using the Thomson multi-taper method. Then, a sliding window with 50% overlap, yielding a frequency resolution of 1 Hz was used.

Unit isolation and spike train analysis.

Single-unit activity was isolated using cluster cutting techniques developed for data acquired with tetrodes, TINT (Axona, St. Albans, UK). Principal components, peak-trough distance, voltage at time t, peak height, trough, time of peak and time of trough in each electrode were used for unit isolation. Klustakwik was used for initial discrimination of clusters and manual refinement was performed using principal components, spike amplitude and other parameters. To verify cluster quality isolation the probability of cluster overlapping was calculated as described before (Hill et al., 2011; Brotons-Mas et al., 2017; Del Pino et al., 2017). Spike trains were analyzed by generating interval time histograms and temporal auto-correlogram. Only units with no spikes in the refractory period of the inter spike time histogram (1-2 ms), and with spike amplitudes 3-4 times above background noise, typically 20-30 mV, were included. Putative pyramidal cells and interneurons were differentiated following previous criteria (Brotons-Mas et al., 2010, 2017). Further spike train analysis was performed to determine the bursting properties of the recorded neurons using the burst index (BI) proposed by Royer and the propensity to burst (PrtB) proposed by Staff (Royer et al., 2012; Staff et al., 2000).

Firing rate maps.

Firing-rate maps were assembled as described previously (Brotons-Mas et al., 2010, 2017; Del Pino et al., 2017). Pixel maps were converted to a bin matrix with a bin size

of 2.5 cm x 2.5 cm. Firing rate in each bin was determined by a smoothing process using overlapping squares of 7.5 cm x 7.5 cm as described before. Firing fields were plotted as a contour map reflecting the frequency of firing; colors were interpolated from the top firing bins down to the lowest firing area by scaling them in decreasing intervals of the peak firing and giving them a color scale: red highest frequency, dark blue, lowest frequency. The spatial information content was quantified using the Skaggs information indexes for the smoothed map (Markus et al., 1994) .

Spatial coherence is a spatial autocorrelation measurement from which a correlation coefficient is calculated between the rate for each bin, and the average rate of the eight surrounding bins (Muller and Kubie, 1987). Firing fields were defined as a group of 6 contiguous bins, where the firing frequency was above the mean firing frequency plus the standard error of the firing matrix. The maximal firing frequency of this group of bins had to be above 1 Hz. For those units displaying more than one firing field, firing field size was computed as the sum of the existing firing fields and expressed as the percentage of the arena size occupied by the firing field calculated using the smoothed firing matrix. In-field maximum frequency was computed as the maximum firing field frequency of the smoothed firing map. Mean frequency was computed as the total number of spikes divided by the total recording time to provide the average session firing rate expressed in Hz.

To classify units as having spatially related activity, a randomized distribution was calculated using the values obtained in the original unsmoothed firing map and respecting the position matrix (n=1000). For a unit to be regarded as being spatially modulated, it had to display a spatial coherence above 99% of the randomized distribution. This same random distribution was used to obtain stability index between the original and a random distribution. Thus, for units to be regarded as stable they had to be above the 99% of cross-correlation between the original and the randomized distribution.

Grid analyses and periodic firing analysis.

Grid analyses were implemented following similar criteria to previous studies (Hafting et al., 2005). Spatial autocorrelograms were calculated for each firing map.

$$r(\tau_x,\tau_y)=\frac{n\sum\lambda(x,y)\lambda(x-\tau_x,y-\tau_y)-\sum\lambda(x,y)\sum\lambda(x,y)\sum\lambda(x-\tau_x,y-\tau_y)}{\sqrt{n\sum\lambda(x,y)^2-(\sum\lambda(x,y))^2}\sqrt{n\sum\lambda(x-\tau_x,y-\tau_y)^2-(\sum\lambda(x-\tau_x,y-\tau_y))^2}}$$

where r is the autocorrelation between the original firing rate map and its spatially shifted copy. The lag τ is normally 1-dimensional for signal analysis. Because of the 2-

dimensional nature of the firing rate map, the autocorrelation has lags in the x- and y-directions: τx and τy. n is equal to the amount of pixels. To determine the existence of a hexagonal grid firing structure, grid index, size and orientation were calculated as described previously.

Periodic Firing

Then we obtain the sum of values of the 2D resulting map in all its orientation and we displayed the preferred orientation of these neurons in space as a polar plot (Krupic et al., 2012). These values were plotted against the polar plot of the shuffled original firing map, n=1000. Only neurons that showed higher values than this confidence interval were considered as periodic neurons.

Histological Analysis

After completing experiments, animals were sacrificed using sodium pentobarbital (180 mg/kg at a concentration of 100 mg/ml) and transcardially perfused with saline and 4% paraformaldehyde. This procedure was authorized by the ethical commission according to the current normative and following veterinary recommendations. The brains were removed, sliced in 40 µm sections, and prepared for Immunohistochemistry against DAPI, NeuN (Merck,ltd) and GFAP (Adigene technologies) for electrode localization.

Statistical analysis

Distribution of dependent variables was tested for normality and for equal variance. Parametric statistical tests were used for normally distributed variables and non-parametric tests were used for variables that did not show normal distribution. All descriptive values are expressed as mean ± the standard error (S.E.). Statistical significance was set at $p < 0.05$. All statistical analysis was performed using SPSS (IBM, V27) software package.

Results

To test for the presence of spatially periodic SUB cells we decided to perform recordings in standard (50 x 50 cm) and large open fields (70x70cm), as compared to mouse body size (6-8 cm). A total of 147 CA1 and 65 subicular neurons were recorded in eight mice (five implanted in the CA1 area, one in the subiculum and two in both regions). Units were classified based on their spike duration, waveform shape and firing frequency (Fig 1A), as previously reported (Sharp and Green, 1994; Brotons-Mas et al., 2010). We isolated 74 CA1 and 28 SUB cells that meet pyramidal and spatially modulation criteria (see methods) and were further analyzed.

First, we looked for difference in the basic electrophysiological properties, including spike amplitude, duration, and firing rate. Thus, we verified that there was no difference in the sorting quality across isolated units in both regions (CA1 vs SUB: Overlapping probability 0.06±0.01 vs 0.10±0.02; U=4290.0, p=0.236, Mann-Whitney). Statistical analysis indicated similar spike amplitude and duration (Fig 1B). Neither there were differences for spike amplitude nor the duration (CA1 vs SUB: spike duration 477.53±7.8 μs vs. 491.02±10.39 μs: U=862.0, p=0.192; spike amplitude 254.1±11.9 μv vs 226.07±15.6 μv: U=943.0, p=0.48) (Fig.1C, D). Instead, we observed significant higher firing rate for SUB neurons (mean firing frequency; 1.2±0.12 Hz vs. 1.8±0.2 Hz: U=745.0, p=0.02, Mann-Whitney) (Fig.1E). These results were consistent with previous descriptions in rats and in mice (Sharp and Green, 1994; Brotons-Mas et al., 2010a, 2017b; Mizuseki and Kitanishi, 2022).

The interaction between the CA1 and the SUB is critical for the organization of inputs and outputs of the hippocampal formation. This dialog depends on the specific timing of the activity of different populations of neurons in different theta cycle windows (Valero and de la Prida, 2018). Therefore, understanding the CA1 and SUB firing relationship to local LFP is fundamental to understand the temporal dynamics of the hippocampal formation. We investigated the relationship between the firing of CA1 and SUB neurons with the locally recorded theta (4-12Hz). We found that CA1 neurons showed a bimodal distribution accumulating neurons in the ascending and descending phases of the local theta oscillations (Fig.1F). In contrast, SUB neurons showed a higher concentration around the peak of the locally recorded theta (Fig.1G).

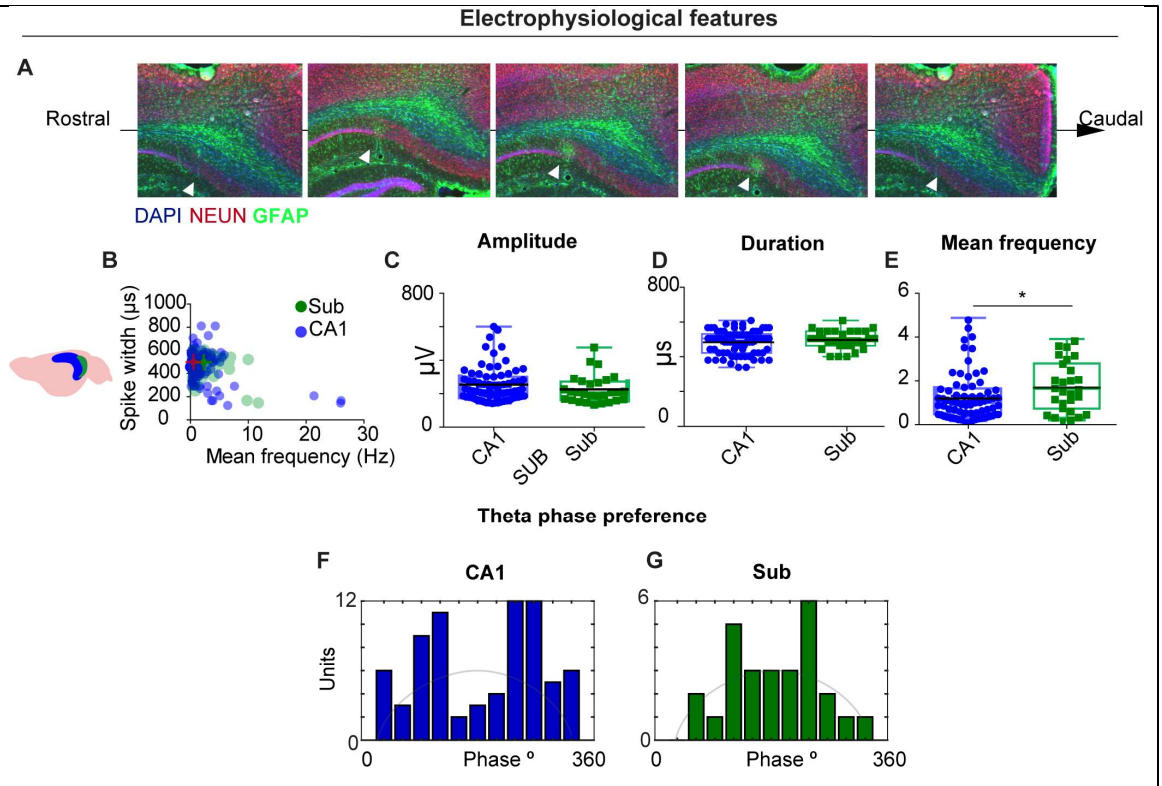

Figure 1. Electrophysiological properties of CA1 and SUB recorded units. **A.** Electrode location indicated by GFAP signal in the proximal SUB. Slices ordered from rostral to caudal. **B** Anatomical organization of the CA1/SUB axis. **B.** Scatter plot of spike width and firing rate for CA1 and SUB. Most units presented pyramidal-like properties. Red and green crosses represent the electrophysiological properties of units in figure 2. **C** and **D**. No significant differences were found for spike amplitude and spike duration between both regions. **E.** Firing rate was significantly higher for SUB units. **F** and **G**. Phase firing preference of theta in CA1 and SUB. Values indicate a bimodal distribution in CA1 and a more concentrate firing of SUB neurons around the peak of theta

Subicular neurons showed grid and spatially periodic firing

In rats, spatial representation in the SUB has been typically defined from the heterogeneous population of place cells and BVC (Lever et al., 2009; Brotons-Mas et al., 2017). Given the rat size as compared with the typically used arenas, the possibility of detecting spatially periodic cells was likely challenging. Instead, using 50x50 cm arenas for mice, we observed that many SUB neurons displayed significant recurrent firing at specific spatial orientations. Remarkably and in contrast to previous reports (Brotons-Mas et al., 2017), these units presented clear electrophysiological characteristics of pyramidal neurons (Fig 2A,B), fired consistently along the entire session (Fig.2C), and cannot be confounded with another overlapping cell (Fig.2D). For quantifying periodicity we obtained the 2D Fourier transform of the unsmoothed firing fields and we calculated the orientation and number of modules (Krupic et al., 2012). We found that about 14% of SUB units actually met grid index criteria (Fig.2C-

E). CA1 pyramidal units recorded in the very same arenas did not exhibit these trends (Fig.2A' to F').

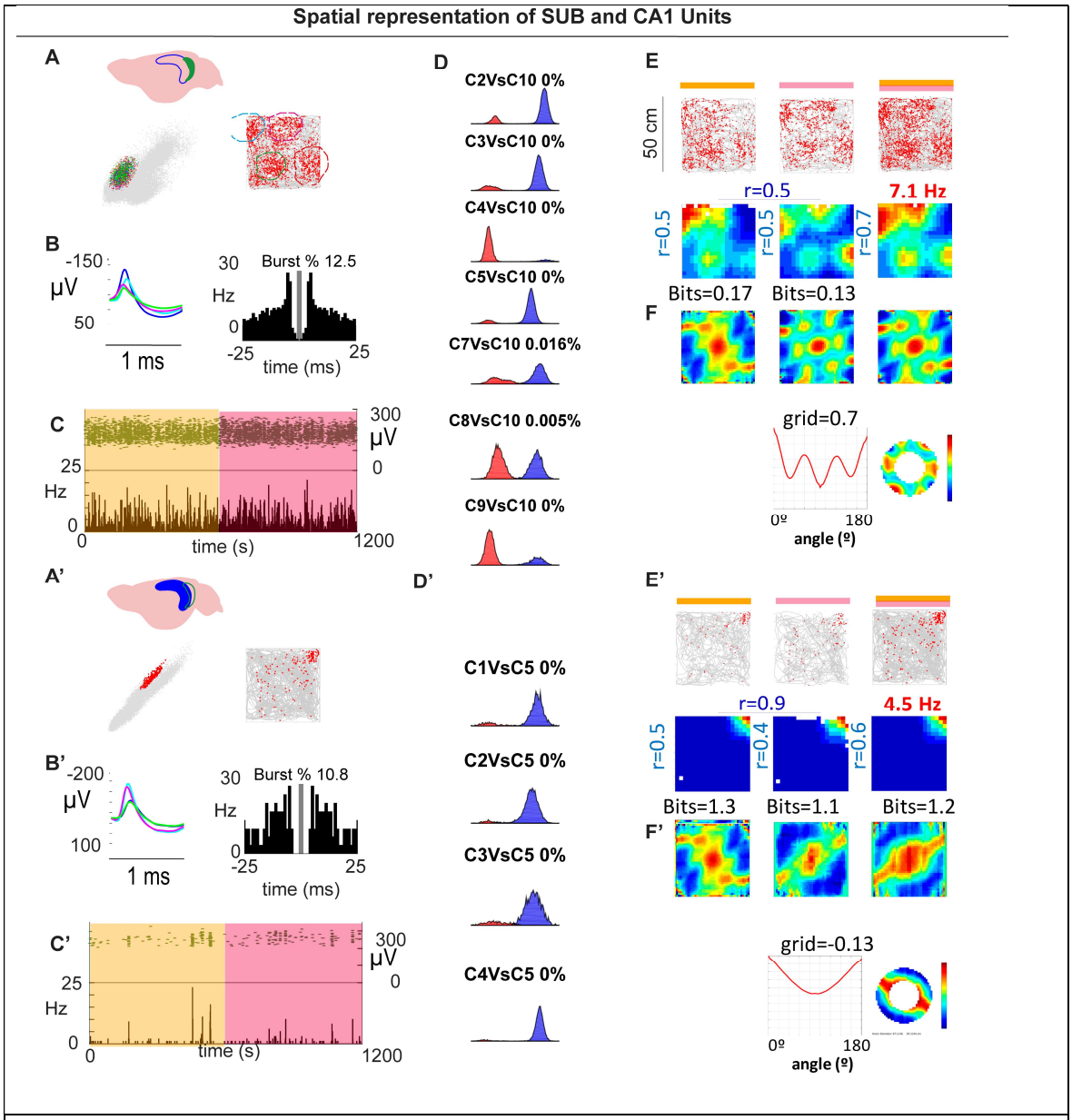

Figure 2. Spatial firing of SUB and CA1 units. **A** and **B.** Isolated unit recorded in the SUB region of a mouse exploring a 50 x 50 cm arena. Multiple peaks in the firing field were contained in the same cluster. Notice different colors in the cluster and in the spike position figure. Waveform of the isolated unit and spike autocorrelogram indicated that this unit was consistent with a pyramidal cell with tendency to fire in burst and with a clean refractory period. **C**. Spike amplitude during the 20 min recording session in the open field 50x50 cm. It can be noticed how the spike properties were stable during the recording. Bottom panel in C indicates the instant firing rate during exploration. The two different colored shadows indicate the two halves represented later in E. **D**. Probability of overlapping unit in A, unit C10 vs other simultaneously recorded units. **E.** Top panel shows the tracking of the mice, gray lines. Red dots represent the position of the mouse when the unit was active. Data was segmented in the two halves, orange, and pink shadows as in C. The full recording is showed at most right panel. Bottom panel

represents the firing frequency matrix. Stability for the two halves was calculated, (r=0.5). Spatial coherence, light blue text color, and maximal firing rate, red text color. **F.** Spatial autocorrelograms helped to reveal the spatial regularity of some units. Bottom panel shows grid index calculation, met by this unit. **A'** to **F'** represent the same information for a unit recorded in the CA1 region. **A'** and **B'**: Note that while the CA1 unit firing frequency was lower than that of the SUB unit shown above, their electrophysiological properties were similar: wide spikes, clean autocorrelograms and propensity to burst.

We challenged the identification of spatially-periodic SUB cells by testing their responses to arenas of different dimensions. Thus, we managed to longitudinally track a subset of individual SUB cells across 50x50 cm and 70x70 cm square boxes, and found consistent grid-like responses (Fig.3A, B versus A',B'). In addition, several SUB neurons that showed discrete place fields in the 50 x 50 cm arena changed their firing generating periodic firing in the large arena (Fig.3C,D versus C',D'). This reinforces the idea that spatially-periodic activity is a genuine characteristic of some SUB cells.

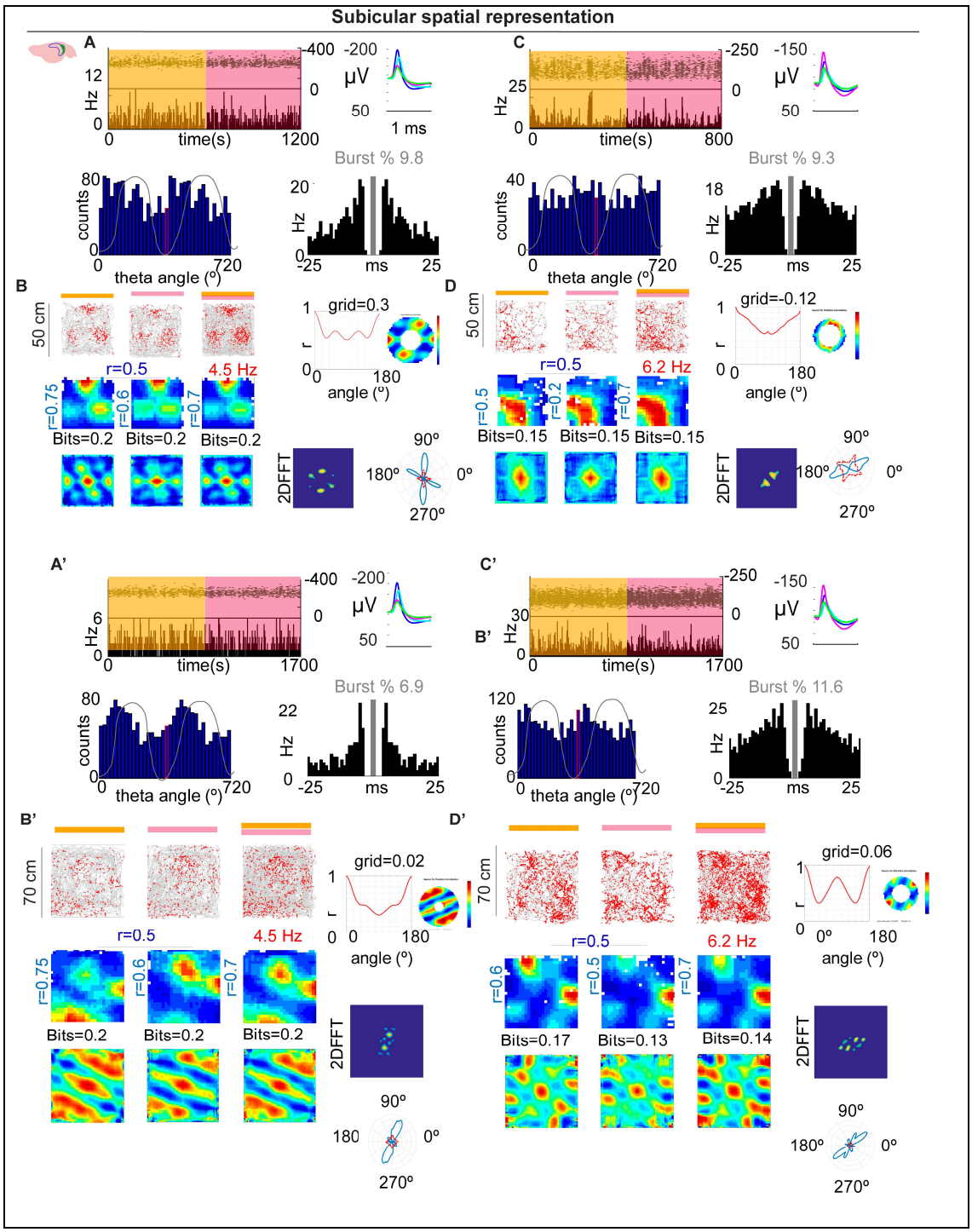

Figure 3. SUB neurons showing different periodic firing patterns. **A, B** and **A', B'** are the same recorded unit in a 50x50 cm arena and then in a 70x70cm arena. This unit showed stable characteristics in both conditions, see spike amplitude in first panel in **A** and **A'**. See, waveform similarity in both recordings, top right panel in A and A'. This neuron tended to fire in the ascending phase of theta and presented a clear bursting activity, see bottom panels in A and A'. **B.** Neuron in this panel displayed a clear grid firing in the 50 x50 cm arena. However, this grid-firing pattern changed in the 70x70 cm arena. The autocorrelogram and the 2D FFT showed that this unit tended to fire in a periodic manner in space. See **B'** most left panels including the 2D FFT and the polar plot derived from this analysis. Blue represents the sum of values of the 2D FFT and the red line the same values for a randomized distribution, n=1000, of the original firing matrix. **C, D** and **C', D'** another SUB unit that did not showed grid firing in the 50x 50 cm arena. However, when the experiment was performed in the 70x70cm arena, a non-grid periodic firing emerged.

Spatial firing of SUB neurons showed lower spatial modulation that CA1

As previously mentioned, spatial coding in the hippocampal formation shows differential spatial characteristics across regions (Barnes et al., 1990; Oliva et al., 2016; Mizuseki and Kitanishi, 2022). To confirm functional specificity of SUB neurons, we next investigated their spatial characteristics as compared with those of CA1 units using standard parameters such as the spatial information per second and per spike, the spatial coherence, and the firing field size. We found significant difference between CA1 and SUB neurons for the spatial information per second, spatial information per spike, and for the spatial coherence (Fig.3A-C), consistent with previous reports. In contrast, no significant difference were observed for the firing field size (CA1 vs SUB : bits/s 0.39±0.01 vs. 0.322±0.06 μs,: U=738.0, p=0.025; bits/spike 0.43±0.04 vs. 0.32±0.06: U=611.0, p=0.001, Mann-Whitney; spatial coherence: (r), 0.61±0.01 vs. 0.54±0.02: $t_{(100)=}$2.442, p=0.016; firing field size (%), 29.02±0.7 vs. 30.0±1.14, $t_{(100)=}$2.442, p=0.016).

A critical element for the representation of space is the stability of the spatial code in the temporal domain. This ensures the consistent spatial tuning during different exploratory episodes, while integrate information about the temporal aspects of experience. Importantly, temporal instability may be associated with different trends for rate remapping (Leutgeb et al., 2005), which could confound detection of spatially periodic signals. Thus, we investigated the temporal consistency of the spatial code in the CA1/SUB axis. We divided each recording session in two halves of 10 min and then we calculated the bin-by-bin, spatial correlation (Fig 3E). We observed that place cells in the CA1 region showed significantly higher spatial consistency (CA1 vs SUB: intra trial stability coherence (r), 0.72±0.02 vs. 0.61±0.04: U=764.0, p=0.041, Mann-Whitney). However, periodic neurons showed high stability within each trial, (See Fig

3), thus, ruling out remapping effects as a confounding factor in the identification of spatially-periodic firing.

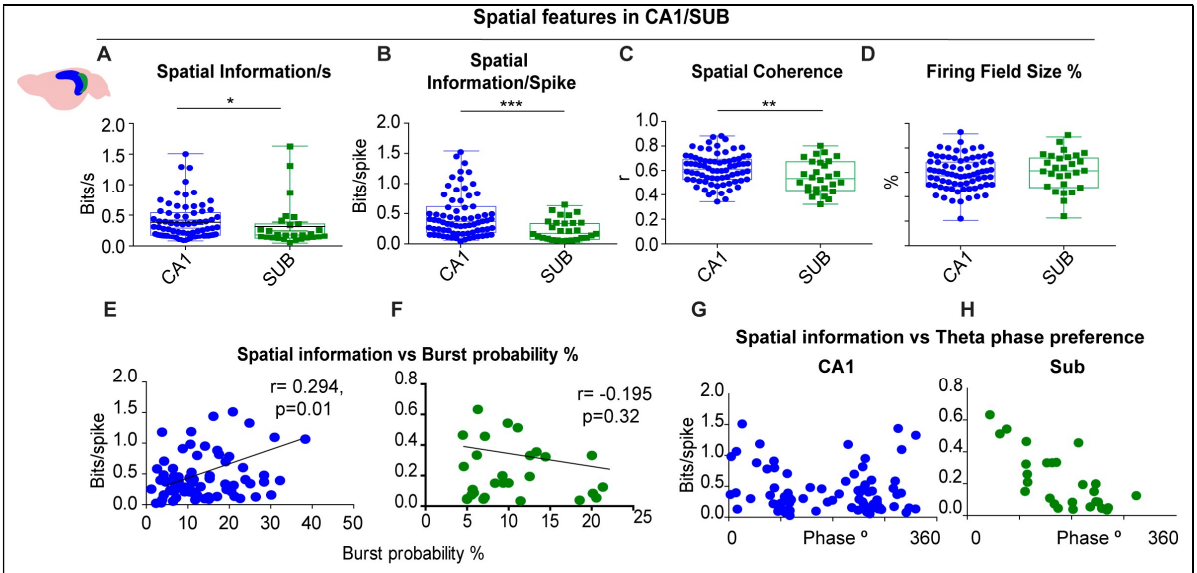

Figure 4. A to D. Different spatial parameters of SUB and CA1 units. **E** and **F** show the correlation between bursting activity and spatial information per spike. Results indicated a significant correlation for these variables only in the CA1 region. **G** and **H**. Spatial information of units in relationship to their preferred firing phase.

Spatial modulation of SUB units was not determined by bursting activity.

The spatial information code is related with the electrophysiological characteristics of hippocampal pyramidal neurons. In this way, it is known that place cells show complex spikes, or bursting, which is characterized by the generation of trains of action potentials within an envelope of 15-20 msec. This bursting pattern is thought to increase the fidelity of spatial information being transmitted across different brain regions (Simonnet and Brecht, 2019). Thus, we sought to determine if there was a specific relationship between bursting patterns and the spatial modulation in the CA1/SUB axis.

We observed that place cells in the CA1 region showed a significant correlation between their bursting characteristics and the spatial modulation (Fig.4F,F'). In this way, the probability to burst was significantly correlated with the spatial information per second, per spike and with the spatial coherence (PrtB vs bits per second, r= 0.485, p=0.0000; PrtB vs bits per spike r= 0.294, p=0.01; PrtB vs spatial coherence, r= 0.35, p=0.002; PrtB). Interestingly, the propensity to burst was also positively correlated with the mean vector length, indicating that bursting propensity was associated with a

stronger relationship between units' activity and the ongoing locally recorded theta (PrtB vs mean vector length, r= 0.35, p=0.002). A similar pattern was observed when we compared the bursting index with the spatial modulation (BI vs bits per second, r= 0.26, p=0.026; BI vs bits per spike r= 0.305, p=0.008; BI vs spatial coherence, r= 0.213, p=0.068; BI vs mean vector length, r= 0.4, p=0.0005).

Remarkably, we observed no correlation between bursting properties and spatial modulation in SUB recorded units (PrtB vs bits per second, r= 0.274, p=0.158; PrtB vs bits per spike r= -0.195, p=0.321; PrtB vs spatial coherence, r= -0.72, p=0.715; PrtB vs mean vector length, r= 0.009, p=0.96). A similar pattern was observed when we compared the bursting index with the spatial modulation (BI vs bits per second, -0.122, p=0.573; BI vs bits per spike r= -0.210, p=0.283; BI vs spatial coherence, r= -0.257, p=0.187; BI vs mean vector length, r= 0.009, p=0.96).

This indicates that CA1 neurons had a higher tendency to burst showed higher spatial modulation, while in SUB recorded neurons, bursting activity was not a predictor of the spatial modulation whether periodic or not.

Next, we looked for the specific phase of maximal information transfer. Remarkably, CA1 cells showed a non-linear relation between their preferred theta-firing phase and the spatial information (CA1 angular mean vs bits per second, r= -0.154, p=0.19; angular mean vs bits per spike r= -0.098, p=0.407; angular mean vs spatial coherence, r= -0.185, p=0.115), while SUB cells rather exhibited a negative linear correlation (Fig.4H,H'). This suggested that neurons with a preferred phase around the ascending phase of theta showed higher spatial information per spike (SUB angular mean vs bits per second, r= -0.429, p=0.023; angular mean vs bits per spike r= -0.581, p=0.001; angular mean vs spatial coherence, r= -0.519, p=0.005).

Discussion

Here, we report for the first-time evidence of grid and spatially periodic firing of pyramidal neurons in mice SUB. SUB neurons were recruited at different phases of the ongoing theta and their electrophysiological properties did not correlate with their spatiality. We verified the quality of unit sorting by observing the significance of refractory period contamination and calculating the overlapping probability of different clusters. Moreover, we compared the probability of false positives (Hill et al., 2011), finding no statistical difference between isolated neurons and meeting similar quality indices as in relevant previous work (Krupic et al., 2015.; Hill et al., 2011; Navratilova et al., 2016; Brotons-Mas et al., 2017; Del Pino et al., 2017).

The role of periodic firing in the SUB in spatial cognition

Speed and direction of movement are critical for path integration and therefore both inputs have been proposed as essential for GC. This could be provided by GC ensembles (Zutshi et al., 2017). From a mechanistic point of view, the computation of speed could be sustained by theta oscillations, known to be strongly correlated with velocity, and by speed cells (Kramis et al., 1975; Chen et al., 2011; Long et al., 2014; Kropff et al., 2021; Abad-Perez et al., 2023). Moreover, the direction of movement is of great relevance (Raudies et al., 2015). This input might be provided by axis cells described in the SUB (Olson et al., 2017). Complementary to these, the speed and directional signal could be shaped by periodic cells in MEC, pre-parasubiculum and SUB units here reported (Mhatre et al.,2012.; Jeewajee et al., 2008; Krupic et al., 2012; Olson et al., 2017; Zutshi et al., 2017). The redundancy of different speeds and direction inputs might provide a more robust spatial integration for the navigation across different conditions, for example across light-dark transitions.

Our new evidence of spatially-periodic cells suggests that the SUB might contribute to the generation of the spatial representation of space by supporting periodic cells and GC in the pre-parasubiculum and MEC. This is supported by anatomical data that confirms the strong afferences sent by the SUB to the pre- and parasubiculum and the deep layers of the MEC (O'Mara et al., 2001, 2009). On the other hand, the SUB might also provide relevant input from BVC and periodic neurons to hippocampal place cells, thanks to the back projection from this region to the CA1 area (Xu et al., 2016). This way, the circuits involving SUB-MEC, SUB-CA1, might support the transformation of the grid produced by alterations of the geometry of space (Krupic et al., 2015). Finally, SUB spatial representation seems to require thalamic inputs, and might meditate changes in GC due to thalamic lesions also known to be relevant for GC in MEC (Winter et al., 2015; Frost et al., 2021).

SUB spatial representation is different to CA1

We observed that place cells in the CA1 showed higher spatial resolution as compared with SUB cells. This apparent lower spatial modulation of SUB neurons is related with their higher firing rate. Such augmented activity seems to serve as a mechanism to improve information routing from the hippocampal formation to other brain regions, a hypothesis sustained by the anatomical location and connections of the SUB with other brain regions (Kitanishi et al., 2021; Mizuseki and Kitanishi, 2022). SUB neurons seem capable to maintain information active during the inter-trial in a working memory task (Deadwyler and Hampson, 2004). This same activity, or changes on this sustained activity, might be relevant for the signaling of the temporal aspects of different episodes. The integration of the what-when information by the same group of neurons would reflect changes in their spatial activity across time. However, this hypothesis would need specific experiments, to pinpoint the existence of the temporal coding by SUB neurons.

SUB neurons tended to fire across different phases of the LFP but clustering towards the center of the theta cycle. This is at odds to previous reports that showed a higher concentration towards the descending phase of theta and earlier than CA1 neurons (Kitanishi et al., 2021). This might be result of different electrode location of the reference electrode at the proximo-distal and deep-superficial layer. The fact that in this work we used tetrodes and not multisite electrodes limits our capability to determine the deep-superficial location of the recorded cells. Interestingly, neurons that fired in the earlier theta phases were more strongly spatially modulated. As previously described this is coherent with a specific temporal organization for different types of information transfer across the theta cycle to different brain structures (Kitanishi et al., 2021).

Conclusion

Here we present evidence of spatial periodic firing in the SUB. However, the properties, response to complex spatial manipulation or their relationship with BVC, GC and place need to be determined. The SUB is of great relevance for the integration of the spatial code, episodic memory and dimensions related to the ongoing tasks. Further work should aim to investigate the role of the SUB in memory consolidation and spatial coding.

Author Contributions:

JRBM designed the experiments. JRBM and PA implemented the experiments, developed and implemented the analysis toolbox. VB and LM supported the project providing resources for its development and discussed the results. JBRM wrote the paper, and the manuscript was further reviewed, and data discussed by JBRM and PA.

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