Dentate gyrus single cell neuronal activity in rats during fixed interval task and subsequent sleep

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Abstract

The hippocampal dentate gyrus is known to be crucial for cognitive tasks involving so-called pattern separation and completion. The exact neuronal mechanisms behind these phenomena are still unclear, but it is hypothesized that sparse firing of dentate gyrus principal cells during exploration corresponds to separate patterns of contextual stimuli. Less is known of the activity of dentate principal cells when the cognitive task does not require the active involvement of external stimuli such as during immobility. Therefore, we recorded the activity of dentate gyrus principal cells, granule and mossy cells, and interneurons while the animal was immobile during a fixed interval task and a subsequent rest period. While interneurons were constantly active, both mossy and granule cells preferentially fired during the immobility period. However, all cell types fired during NREM sleep, especially during dentate spikes. Therefore, we were able to show that both principal cells and interneurons are active during the fixed interval tasks and subsequent sleep in distinct phases. This will allow future studies focusing on the role of different single cell activity of dentate gyrus during immobility.

Introduction

The dentate gyrus (DG) is an important hub of information flow from multimodal sensory cortices to hippocampal areas. Specifically, it is crucial to categorize this information in separate entities, that is, pattern separation [1–5]. It is assumed that this process takes place when an

animal is actively engaging with its environment. However, less is known about the importance of dentate activity while the animal is not actively engaging with its environment but is immobile or even in sleep [2,4,6]. The properties of these latter forms of activity at the cellular level and the role they play in behaviour are largely unknown.

Overall, the DG exhibits low levels of activity under normal physiological conditions and behavior states regardless of whether the animal is immobile or engaging in active behavior, such as, during exploration and mobility. Only about one to three per-cent of principal granule cells are active while the majority of the cells remain inactive, and the cells fire only one to two times in 10 seconds [1,2,6–8] (but see also Stefanini et al. [9]). However, this does not apply to the interneurons of the dentate gyrus which show persistent spiking activity during active periods[10]. Interestingly, during the NREM sleep state both the principal granule and mossy cells and interneurons can be highly active while dentate LFP expresses high amplitude (2-4 mV) and short duration (< 30 ms) dentate spikes [10–12]. Although the activation of granule and mossy cells during awake behaviour is well correlated to the spatial context, so-called place fields [13,14], the functional role of activation of these cells during awake immobility is less clear. It has been proposed that granule cell activity during immobility is not a random activity among cells but these cells form co-synchronous populations that are simultaneously active, and this activity recapitulates activity patterns that occur during activity periods, namely, locomotion[6]. It is tempting to postulate that similar cell population co-synchronization could also take place during NREM sleep and especially during dentate spikes, but this is still unknown.

The hippocampal formation is also involved in cognitive processes other than the location in space, that is, place cells, such as temporal information processing [15–17] and it has been

proposed that the dentate gyrus mediates a temporal pattern separation mechanism [18]. The temporal separation is hypothesized to be based on the labelling of different populations of newly born neurons [18] but the temporal function of dentate gyrus could also be based on sequential activation of dentate neurons[19]. In general, the hippocampus is thought to produce a sequential structure to access and organize sensory experiences distributed across cortical modules over spatial and temporal scales[20]. Whether the activity of neurons of dentate gyrus expresses a temporal scale is still unknown.

Therefore, the main aim of our study was to test whether the activity of dentate granule cells, mossy cells, or interneurons reflects the processing of temporal context during immobility and if representations of temporal scale are expressed by time-locked activation of individual cells. We recorded single cell activity and local field potentials (LFP) from the dentate gyrus while animals performed a behavioral task devoted to the study of how animals perceive and reproduce time, the fixed interval task [21,22]. On the other hand, the fixed interval task does not require the animal to be actively engaged with the environment and mainly requires the animal to stay immobile over a certain period of time. We were able to record the main principal excitatory neurons in the dentate gyrus, granule cells and mossy cells as well as the inhibitory interneurons [10,23–26] while the animal was performing the task, and during the following rest period. Simultaneously, we also recorded the activity of neuronal networks, LFPs, and especially dentate spikes [11,12,27,28]. Granule cells increased their discharge rates during dentate spikes more than mossy cells and, also, granule cells peak earlier than mossy cells, but it is not known whether these cell populations can change these firing properties related to the task previous to the sleep period [10].

Results

Histology

Examination of hippocampal slices stained with cresyl violet under a microscope revealed electrode tracts reaching the upper blade of the dorsal hippocampal dentate gyrus and the hilus in all animals included in the analysis. That is, the cells recorded were located in the dentate gyrus.

Rats performed the fixed interval task at a high accuracy

By the time recordings in the dentate gyrus started, all animals had been trained in the fixed interval task (FI) with a 113 s interval for at least 7 days. In this task, the animal was placed in a plastic cylinder of 20 cm in diameter. The cylinder was equipped with a nose poke port on one side (infrared detector) and a tray for delivering sugar pellets on the opposite side[29]. On entering the cylinder, the animal would poke its nose into the port and receive a reward. Then, it would need to wait for (at least) 113 s to be rewarded again for poking its nose in the port. Additional nose poke attempts during the fixed interval did not change the fixed interval period and were left unrewarded. FI training lasted for 45-60 minutes at a time, followed by a rest period in a separate container for 45-90 minutes. On average, the rats had learned to wait for the fixed interval to pass and obtained the sugar pellet at an interval of 120 ± 9 s (mean ± standard deviation). The experimental set-up is depicted in Figure 1A and an example of performance in the task is presented in Figure 1B.

To determine the quality of performance in each session, the number of nose pokes within a 20% marginal of the established 113-second interval were divided by the total number of nose pokes in the session. Nose pokes in this timeframe were regarded as accurately timed, learned nose pokes. The mean performance across all sessions was 0.8538 (± 0.1165), that is, 85.38% (± 11.65 percentage units) of nose pokes occurred during the 113s ± 20% timeframe. After the fixed interval task session, the animal was placed in a plastic container resembling its home cage. The animal was allowed to rest or sleep for at least 60 minutes and for up to 90 minutes.

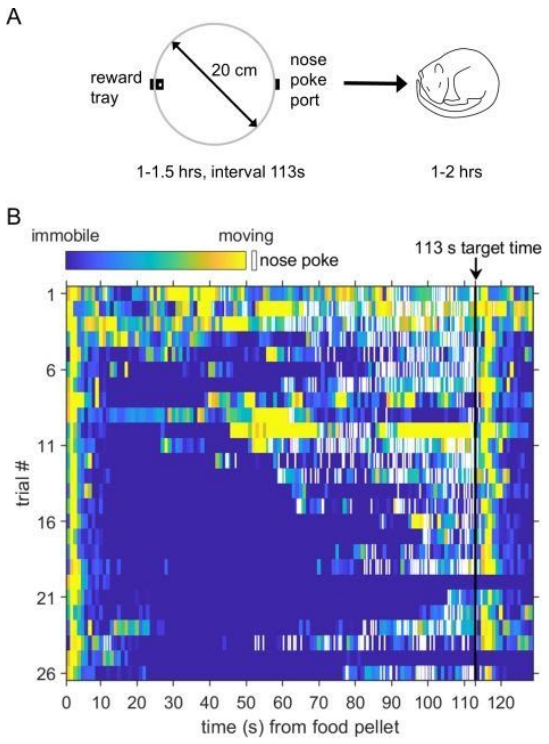

Figure 1. Rats performed a fixed interval (FI) task and then were allowed to rest in quiet while recording neural activity and movement.

A) Rats were trained in a FI task daily. The interval length was 113 s and it remained constant throughout the recordings. After each FI session, the rat was placed in an open plastic standard rat cage and let to rest and sleep there for up to 90 minutes undisturbed. Hippocampal neural

activity (1-20000 Hz) was recorded wirelessly along with an accelerometer signal from the rat's head. Nose pokes into the port and pellet deliveries were recorded as TTL pulses. B) Rats readily performed the FI task. In the example presented, nose pokes are marked with white vertical lines. For plotting and analysis, data are divided into trials based on the delivery of sugar pellets (time point 0). The rat received a single 45-mg sugar pellet if it poked the nose port when at least 113 s had elapsed since the last pellet delivery. Depending on the accuracy of performance, the rat could receive a total of up to 48 pellets during one 90-minute session.

Excitatory cells in the dentate gyrus fired most during quiet immobility and sleep while interneurons fired at all times

A total of 176 units were isolated from the data (rat #002: 20 sessions and 125 units, rat #018: 10 sessions and 51 units). Five units were such that they could not be classified reliably. Of the remaining 171 units, 106 units were classified as putative inhibitory interneurons and the rest as putative excitatory principal cells based on trough-to-peak latency, burst index and the firing rate recorded while the rat was resting. Note that for this, the state of the rat during the rest session recording was categorized into wake behaviour, NREM and REM sleep (Figure 2C,D,E)[10]. Of the putative excitatory principal cells, 43 units were determined to be putative mossy cells and 22 were classified as putative granule cells. For the units classified as interneurons, the trough-to-peak latency was 0.52 ± 0.25 ms (mean ± standard deviation) and the burst index was 0.32 ± 0.32. For the mossy cells, the trough-to-peak latency was 0.72 ± 0.08 ms and the burst index was 11.88 ± 7.84, while for the granule cells these descriptives were 0.74 ± 0.09 ms and 20.60 ± 14.58, respectively. The ratio of firing rate (Hz) during wake behavior vs. NREM sleep was 1.79 ± 3.32 in interneurons, 1.84 ± 1.76 in mossy cells and 0.33 ± 0.08 in granule cells. Paired samples t-tests indicated that interneurons fired significantly more often during REM sleep compared to NREM sleep [two units missing data from REM sleep: $t(103) = 3.62$, $p < 0.001$] and compared to wake behavior [$t(103) = 2.55$, $p = 0.012$] while there was no difference between wake behavior and NREM sleep [$t(105) = 0.24$, $p = 0.809$]. Mossy cells fired at an equal rate during all behavioral states [$t(42) = 0.37 - 0.58$, $p = 0.563 - 0.712$]. Granule cells fired significantly fewer action potentials during wake behavior compared to both NREM

sleep [t (21) = 8.76, $p < 0.001$] and compared to REM sleep [t (21) = 3.75, $p = 0.001$] while there was no difference between the two sleep states [t (21) = 1.32, $p = 0.202$].

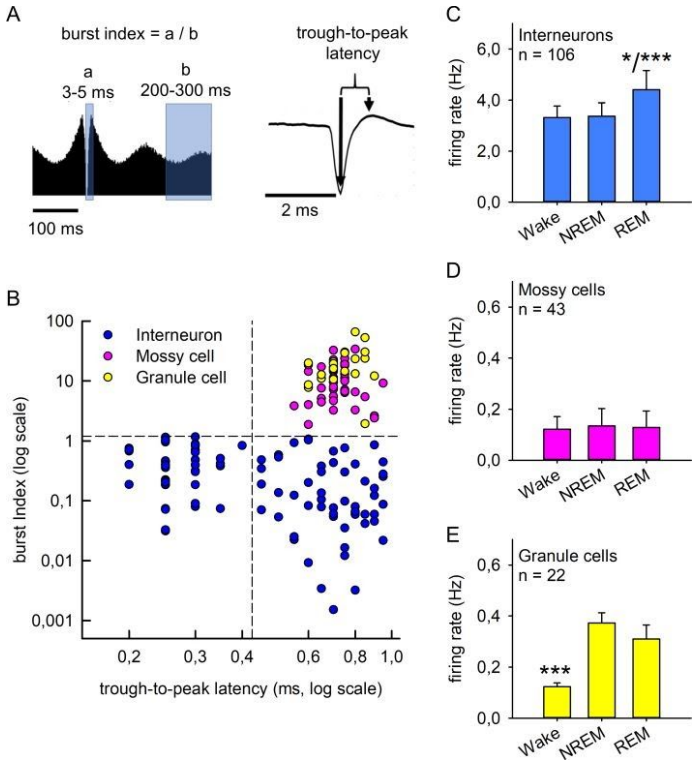

Figure 2. Recordings in two rats over several days yielded a total of 106 putative interneurons, 43 putative mossy cells and 22 putative granule cells.

A) Units were clustered with KiloSort and then manually curated in phy. After that, the units were divided into inhibitory and excitatory based on burstiness and waveform shape similar to Senzai and Buzsaki (2017). Illustration of how the burst index was calculated from the spike-to-spike perievent time histogram and how the trough-to-peak latency was derived from the mean waveform. B) Most of the cells were classified as putative interneurons and the rest as excitatory neurons. The cut-off values for the burst index and the trough-to-peak latency are indicated by the dashed lines. Using data from the rest recording, excitatory cells were further divided into putative mossy cells and putative granule cells based on their firing rate during wake behaviour vs. NREM sleep. C) Interneurons fired significantly more during REM sleep compared to wake ($p < 0.05$) or NREM sleep ($p < 0.001$). D) Mossy cells fired at an equal rate during all three states. E) Granule cells fired less frequently during wake behaviour compared to sleep ($p < 0.001$ for both REM and NREM). Asterisks refer to statistically significant differences between firing rates ($p < 0.05$ = *, $p < 0.001$ = ***).

Interneurons, mossy cells and granule cells responded differently to a nose poke and the delivery of a sugar pellet.

First, we analyzed the firing of the different cell types during the performance of the FI task (Figure 3A). The firing of each unit during the FI task was analyzed first by simply calculating the firing rate during specific time periods: The first period started from the sugar pellet delivery and encompassed the consumption of the sugar pellet (pellet consumption, first 10 s of the trial). The second period lasted from the end of the pellet consumption period (fixed at 10 s) until the first nose poke (idling) and the last period encompassed the rest of the trial from the first nose poke onwards, until the delivery of the next sugar pellet (nose poking). Movement (see Figure 3B) was most vigorous during the pellet consumption period and rats remained rather still during the idling period (rm ANOVA: $F [2, 58] = 127.64$, $p < 0.001$; Bonferroni-corrected pairwise comparisons: pellet consumption vs. idling: $p < 0.001$, idling vs. nose poking: $p = 0.003$, pellet consumption vs. nose poking: $p < 0.001$). Interneurons fired at the same frequency throughout the FI task ($F [2, 210] = 0.62$, $p = 0.479$). Mossy cells did not show an overall change in firing rate across time periods but fired more during the nose poking compared to the idling period ($F [2, 84] = 1.90$, $p = 0.172$; pellet consumption vs. idling: $p = 0.926$, idling vs. nose poking: $p = 0.001$, pellet consumption vs. nose poking: $p = 1.000$). Granule cells were mostly silent during both pellet consumption and nose poking but fired more during idling ($F [2, 42] = 20.37$, $p < 0.001$; pellet consumption vs. idling: $p < 0.001$, idling vs. nose poking: $p < 0.001$, pellet consumption vs. nose poking: $p = 0.715$). See Figure 3C for illustration.

We also analyzed cell firing time-locked to the sugar pellet delivery and the nose poking, as well as time-locked to dentate spikes recorded during the rest session (Figure 3D).

Interneurons tended to increase firing in response to the pellet delivery (1-s period, 81 cells out of 106, 76 %) and nose poking (1-s period, 72/106 cells, 68 %). On the contrary, only 14% of mossy cells (6/43) and none of the granule cells increased firing in response to the pellet delivery. Similarly, only one mossy cell (of 43, 6%) and one granule cell (of 22, 5%) increased firing in response to nose poking.

Dentate cells fired at an increased rate during dentate spikes

Next, we analyzed cell firing during the rest session. We determined whether cells changed firing around the dentate spike peak (see description of statistical procedures in Materials and Methods). Few interneurons (13/101, 13 %) and mossy cells (8/38, 21 %) and a significant proportion of granule cells (9/22, 41 %) decreased their firing rate statistically significantly during a 70-ms period just before the dentate spike (-100 to -30 ms in relation to DS peak timing). For all cell types, the predominant response during the dentate spike (60-ms period from -30 to 30 ms) was to increase firing (interneurons, 62/101, 61 %; mossy cells, 25/38, 66 %; granule cells, 14/22, 64 %). Lastly, most interneurons (61/101, 60 %), mossy cells (28/38, 74 %) and granule cells (16/22, 73 %) continued to fire at an increased rate also during a 70-ms period after the DS, from 30 to 100 ms after the DS peak. To summarize, dentate spikes in the hilus were associated with increased firing of interneurons, mossy cells and dentate granule cells in the upper blade of the dorsal hippocampal dentate gyrus granule cell layer.

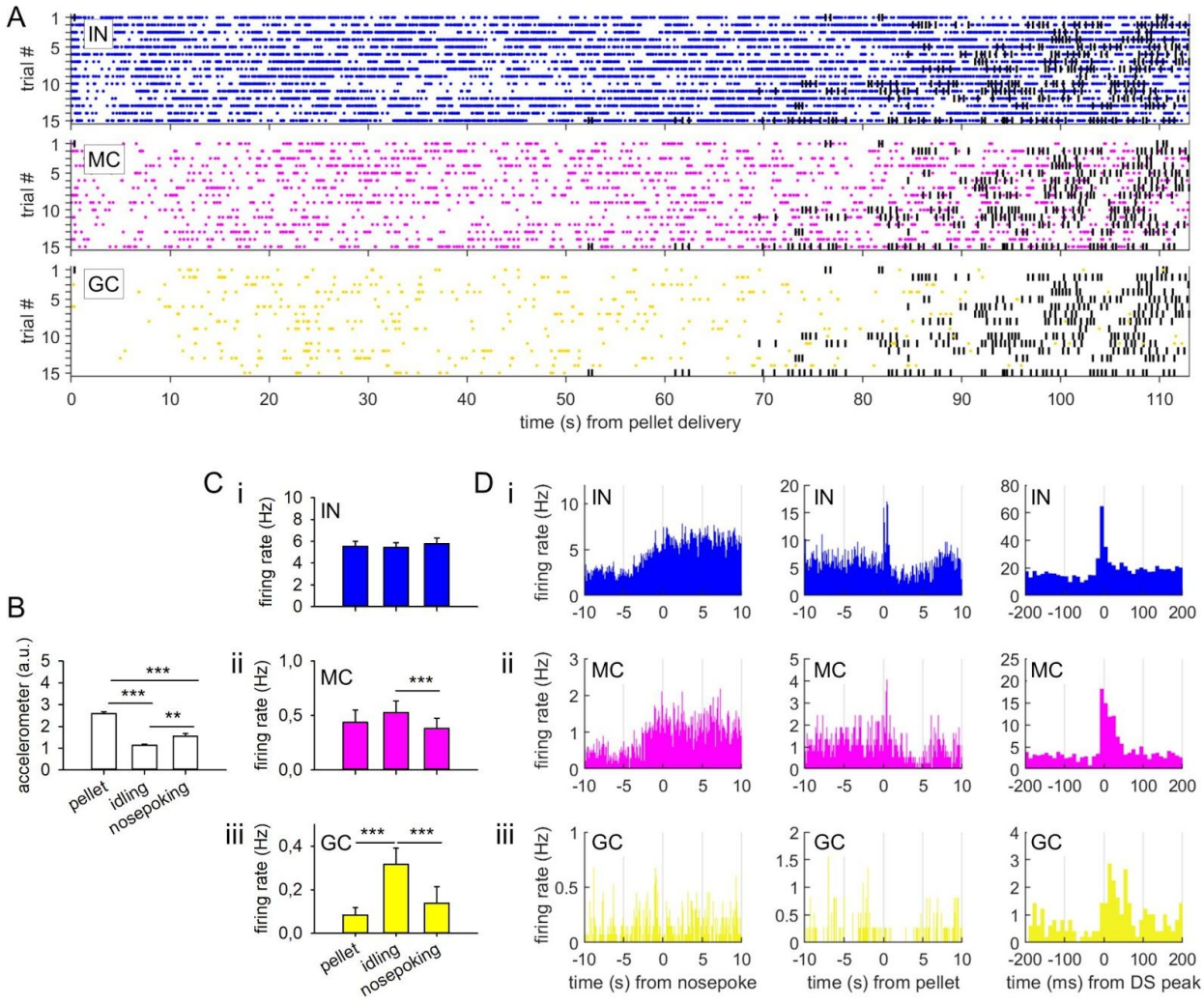

Figure 3. Interneurons, mossy cells and granule cells showed different firing patterns during the FI task, but all cell types fired during dentate spikes recorded at rest.

A) Nose pokes (black vertical lines) and the firing of an interneuron (IN, blue, top), a mossy cell (MC, pink, middle) and a granule cell (GC, yellow, bottom) as a function of time from pellet delivery during the FI task. B) Movement was most vigorous during pellet consumption and rats remained still during the idling period. C) i) Interneurons (blue) fired throughout the FI task, while ii) mossy cells (pink) fired more during idling and iii) granule cells (yellow) during the idling period. Asterisks refer to statistical significance of pairwise comparisons (Bonferroni-corrected): $p < 0.001$ = ***, $p < 0.01$ = **. D) Responses during the FI task varied depending on behaviour. Examples of an i) interneuron (blue), ii) a mossy cell (pink) and iii) a

granule cell (yellow) are depicted. Interneurons and mossy cells increased firing both prior to and during nose poking (left) and in response to pellet delivery (middle). Granule cells remained mostly inactive during nose poking and pellet delivery. All cell types fired during dentate spikes (right) recorded during the rest session following training on the FI task.

Discussion

The dentate gyrus is an important hub of information flow from multimodal sensory cortices to memory related hippocampal areas. Specifically, it is crucial to categorize this information in separate entities that is pattern separation. It is assumed that this process takes place when an animal is actively engaging with its environment. However, less is known of the importance of dentate activity while the animal is not actively engaging with its environment but is immobile or even in sleep [2,4,6]. The properties of these latter forms of activity at the cellular level and the role they play in behavior are largely unknown.

Therefore, we recorded a single cell and LFP of dentate gyrus while the animal was performing a fixed interval task. By using a silicon probes recording technique with accompanied clustering analysis methods, we were able to detect and separate a total of 106 interneurons, 43 mossy cells and 22 granule cells from the granule cell layer and hilar region of the dentate gyrus while the animal was performing the fixed interval task and subsequent sleep period[10]. In this task, the animal needs to wait a certain period of time to receive a food reward[22]. We found that movement is significantly reduced in the period of idling after consumption of the food reward and before releasing the next food reward by a nose spoke providing a suitable model to study dentate gyrus activity in awake immobile animals. Interestingly, the firing of interneurons took place during the whole task but both mossy and granule cells preferentially

fired during idling, that is, immobility period. This finding supports the previous findings that mossy and granule cells are active during rest periods but interneurons do not show firing preference related to the behavior [2,6,10,30]. However, we were not able to detect sequential activation of any of the dentate cell types (see Fig. 3A) during the idling period implying that dentate cells do not present temporal scale as a sequential activation or as a time locked activation of individual cells [23–25].

We also recorded the firing of dentate granule and mossy cells, and interneurons during rest periods that took place immediately after the FI task session. During NREM sleep periods we observed irregularly occurring dentate spikes with the synchronous discharge of granule cells, mossy cells, and interneurons [10–12].

Interestingly, during the idling period (immobility) dentate cells showed activity, proving that this task could be used to study the function of dentate gyrus during immobility in restricted and constant spatial contexts without any external stimuli. We were able to show that the fixed interval task is suitable to study the role of the dentate gyrus in cognitive processes while immobility and consequently sleep, and this paradigm allows us in the future to study, for example, if the synchronous activity expressed during dentate spikes replays previous or future experience as sharp wave ripples do in the CA1 area [31].

Materials and Methods

Subjects and ethical guidelines

Two adult male Sprague-Dawley rats (Envigo, Netherlands) were used in the experiment (permit ESAVI-6718-04.10.07-2015). The experiments were approved by the Finnish National Animal Experiment Board (ESAVI/6718/04.10.07/2015). All the experimental procedures, care and handling were carried out in accordance with Directive 2010/63/EU of the European Parliament and of Council on the protection of animals used for scientific purposes and in compliance with the ARRIVE guidelines. The animals were housed in groups at the Laboratory Center of the University of Jyväskylä. Food and water were freely available during the group housing stage, and room temperature and humidity were controlled. At the start of the behavioral experiment, the animals were moved to individual cages and food intake was restricted. The animals were maintained on a 12/12-hour light/dark cycle, with lights on at 8:00 a.m. All experiments were carried out during the light part of the cycle. Animal handling was performed only by trained personnel and the animals were introduced to human contact and handling for a sufficient amount of time before the surgery.

Surgery

Prior to the surgery, the animal was placed under anesthesia with isoflurane gas. The flow of gas was maintained at 600-700 ml/min at 4-5% concentration during the induction phase, after which it was lowered to 350 ml/min at 2.2-2.5% concentration for maintenance of anesthesia. Hair on and around the incision area was removed. The rat was then injected with

antibiotics cefuroxime (Cefuroxime Orion Pharma 750 mg/ml, Orion Pharma, Finland, 0.22 mg/g s.c.) as well as painkiller carprofen (Rimadyl vet 50 mg/ml, Zoetis, Belgium, 5 µg/g s.c.). To prevent brain swelling, dexamethason (Oradexon 5 mg/ml, Aspen Pharma, Ireland, 2 µg/g s.c.) was administered. Before the incision, the skin was disinfected using a combination of 70% ethanol solution and povidone (Betadine 100 mg/ml, Takeda / Leiras, Finland). Bupivacaine Bicain 5mg/ml, Orion Pharma, Espoo, Finland, 0.1–0.3 ml s.c.) was injected for local anesthesia. The exposed surface of the skull was thoroughly cleaned and disinfected with a 3% hydrogen peroxide solution. A screw (M1x3, DIN84, Screwsandmore, Germany) for the reference was attached on the cerebellum and another similar screw worked as a ground on the frontal area. In addition, two support screws were implanted into the skull bones. The craniotomy was performed at the coordinates AP = -3.4 mm, ML = 0.8-1.2 mm from bregma. During surgery, the recording probe was slowly inserted into the left dorsal hippocampus and lowered into the brain to an initial depth of 2250 µm from the dura. After the recovery period over several weeks, the probe was gradually lowered further into the hippocampus by using a microdrive (3Dneuro, Nijmegen, the Netherlands) , typically reaching a depth of 3300-3600 µm corresponding to the dentate gyrus.

Fixed interval task (FI)

The food intake of the animals was restricted prior to the start of the training in order to increase motivation to seek a sugar-pellet reward. This is standard practice in animal experiments (Rowland, 2007). Body weight was monitored and maintained at no less than 85% of the initial body weight. All animals were pre-trained and habituated to the task by using a short (17s)

reward interval for 3-4 sessions. After this initial training, those rats that showed learning (20-30 rewards over 30-40 minutes of training) underwent surgery to implant the recording probe. After recovery, the animals were trained in the fixed interval task (FI)[29]. The animal was placed in a plastic cylindrical container. A tube dispensing sucrose pellets was inserted on one side of the container. The pellets (45 mg) were dispensed by a Med Associates Pellet Dispenser. On the other side of the container was a hole for nose pokes. Upon poking the hole, the rat received a sucrose pellet as a reward. During pre-test training, the rats were conditioned to associate the nose poke with the reward. After the surgery, FI training lasted (113 s reward interval) for 45-60 minutes at a time, followed by a rest period in a separate container for 45-90 minutes. This set of behaviour/rest recordings was executed a maximum of two times for each rat per day.

Neural signal recording

The probe used in the study was a passive custom-tailored Atlas Neuroengineering probe (E32Nokia+R-20-50-S4-L6-200). It has four shafts, three of which have 8 electrodes along the tip for single cell separation, as well as one shaft that records LFP along its entire length, a so-called linear probe. This allows the tightly packed electrodes on shafts 2-4 to accurately measure single-unit activity in the dentate gyrus, while the electrodes in the LFP shaft allows recording of brain oscillations from the CA1 area to the dentate gyrus.

During both the behavioral task and the sleeping period, a continuous neural signal was A/D converted using a wireless, 32-channel W2100-HS32-ES2-EXT-0.5mA headstage with a sampling rate of 20 kHz per channel (Multichannel Systems [MCS], Reutlingen, Germany). The signal was transmitted to a W2100-RE-AO wireless receiver and further to an MCS-IFB

interface board. Analog data (nose pokes and received rewards) were simultaneously fed to the interface board. Continuous neural signal and analog data were recorded with MCS Experimenter software.

Histology

After the experiments, the animals were anesthetised with isoflurane. Under anesthesia, 30μA of current was passed through the tip of each electrode shaft for 3-4 seconds to confirm electrode placement in later histological analysis. After initial lesioning, the rat was given 24-48 hours to recover, during which the lesion was able to develop before tissue sampling.

Terminal anesthesia was induced with pentobarbital (Mebunat vet 60 mg/ml, Orion, Finland, minimum dose of 120 mg/kg i.p.). The level of anesthesia was verified with a pedal and eyeblink reflex. The animal was first perfused with saline followed by 4% paraformaldehyde fixation for 15-20 minutes. After fixation, the brains were stored in 4% paraformaldehyde for 24 hours and then transferred to 0.1 M phosphate buffer solution until sectioning [32].

The brains were sliced into 40μm thick coronal slices using a vibrating blade microtome (Leica VT1000 S, Nussloch, Germany). Sections of hippocampal tissue were transferred onto glass slides (Menzel-Gläser Superfrost Plus) and stained with Cresyl Violet. Electrode placement was confirmed from stained sections under a microscope with the help of a stereotaxic atlas[33].

Data analysis

Custom scripts in Python and MATLAB (MathWorks, Natick, MA, USA) were used for data analysis. IBM SPSS Statistics 24 (IBM, Armonk, NY, USA) was used for further descriptive statistics and statistical testing.

Unit sorting and classification

Individual spike data was sorted into clusters of similar features using KiloSort (https://github.com/cortex-lab/KiloSort). Phy (https://github.com/kwikteam/phy) was used to refine the computer-generated clusters. The clusters were marked as 'good' or 'noise' based on their waveforms, refractory period and number of spikes[34].

After sorting, the units were classified into inhibitory and excitatory neurons based on burstiness and waveform[10]. First, the inter-spike intervals for each identified unit were determined and plotted in a histogram. A burst index value indicating burstiness of a unit was then calculated with the following formula:

$$burst\ index = a / b,$$

where a = mean number of spikes in the 3-5 ms window of the inter-spike interval histogram and b = mean number of spikes in the 200-300 ms window of the inter-spike interval histogram (Fig. 2A). Next, mean waveforms of each identified unit were extracted using the KiloSort plugin getWaveForms (https://github.com/cortex-lab/spikes/blob/master/analysis/getWaveForms.m). The waveforms were then used to calculate the trough-to-peak latency, defined as the latency from the lowest

amplitude (trough) to the highest amplitude (peak) within a 200 ms window of the cell firing. Units with a trough-to-peak latency exceeding 0.425 ms and a burst index exceeding 1.2 were designated excitatory and those with the opposite pattern as inhibitory (Fig. 2B).

Single-unit activity during FI task

The firing of each unit during the FI task was analyzed first by simply calculating the firing rate during specific time windows. For the FI task, we defined three periods based on behavior: food consumption (first 10 seconds of each trial), idling (until the first nose poke) and nose poking (the rest of the trial until the rewarded nose poke). We also analyzed unit firing time-locked to the sugar pellet delivery and to the nose poking: Firing rate within a 1000 ms window immediately after the event was compared to randomly jittered sequences (n = 5000) of firing in the same time window. Statistical significance was determined by comparing the real firing rates to the 5% tail ends of the jittered data (alpha 0.05)[35].

Single-unit activity during rest

First, we used the accelerometer data and hippocampal dentate gyrus LFPs to determine periods of wake behavior, rapid eye movement (REM) sleep and periods of non-REM (NREM) sleep. REM sleep was identified based on immobility and a high relative amplitude of theta (3-12 Hz) oscillations. Prolonged immobility in the absence of theta was classified as NREM sleep and periods of movement as awake behavior. The result was used to further define unit classification: Excitatory units were defined as either mossy cells or granule cells based on activity during NREM and awake periods. Granule cells are known to be significantly more active during NREM, whereas mossy cell firing activity is less dependent upon brain state. A wake/NREM

firing rate ratio of 0.5 was chosen as the cut-off for granule cells, based on the average firing rates of both cell types[10].

We also identified specific events from the hippocampal dentate gyrus LFPs recorded during immobility, namely, dentate spikes[11,12,27,27]. We compared sequences of firing in the -100 to +100 ms time window (in respect to the dentate spike) to randomly jittered sequences (n = 5000) of firing in the same time window. Statistical significance was determined by comparing the real firing rates to the 5% tail ends of the jittered data (alpha level 0.05). The dentate spike modulation effect was split into three categories: overall increase in firing rate during dentate spikes (-30 to +30 ms), decreased firing rate before dentate spikes (-100 to -30 ms), and increased firing rate after dentate spikes (-30 to +100 ms).

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Competing interests

The authors report no competing interests.

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