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1 **Dentate spikes and learning: Disrupting hippocampal function during memory**
2 **consolidation can improve pattern separation**

3 Running head: Dentate spikes and learning

4

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16

17

18 **Abstract**

19 Hippocampal dentate spikes (DSs) are short-duration, large-amplitude fluctuations in
20 hilar local-field potentials and take place while resting and sleeping. During DSs, dentate
21 gyrus (DG) granule cells increase firing while CA1 pyramidal cells decrease firing. Recent
22 findings suggest DSs play a significant role in memory consolidation after training on a
23 hippocampus-dependent, non-spatial associative learning task. Here, we aimed to find out if
24 DSs are important in other types of hippocampus-dependent learning tasks as well. To this
25 end, we trained adult male Sprague-Dawley rats in a spatial reference memory task, a fixed
26 interval task and in a pattern separation task. During a rest period immediately after each
27 training session, we either let neural activity to take place as usual, timed electrical
28 stimulation of the ventral hippocampal commissure (vHC) to immediately follow DSs, or
29 applied the vHC stimulation during a random neural state. We found no effect of vHC
30 stimulation on performance in the spatial reference memory task or in the fixed interval task.
31 Surprisingly, vHC stimulation, especially contingent on DSs, improved performance in the
32 pattern separation task. In conclusion, the behavioral relevance of hippocampal processing
33 and DSs seems to depend on the task at hand. It could be that in an intact brain, offline
34 memory consolidation by default involves associating neural representations of temporally
35 separate but related events. In some cases this might be beneficial for adaptive behavior in the
36 future (associative learning), while in other cases it might not (pattern separation).

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43 **New & Noteworthy**

44 The behavioral relevance of dentate spikes seems to depend on the learning task at hand. We
45 suggest that dentate spikes are related to associating neural representations of temporally
46 separate but related events within the dentate gyrus. In some cases this might be beneficial for
47 adaptive behavior in the future (associative learning), while in other cases it might not
48 (pattern separation).

49

50 **Keywords**

51 hippocampus, dentate spike, learning, memory consolidation

52 **Introduction**

53 Hippocampal electrophysiological activity reflected in the local-field potential (LFP) is
54 characterized by alternating epochs of large-amplitude, irregular activity (LIA) and regular
55 slow activity also called theta (3-12 Hz). Two types of hippocampal events are evident during
56 LIA: Sharp-wave ripples (SPW-Rs) and dentate spikes (DSs). SPW-Rs are ~100-ms bursts of
57 high-frequency (110-200 Hz), high-amplitude oscillations riding on a sharp slow wave, and
58 visible in the CA1 (Buzsaki, 1986; 2015). DSs are short-duration (up to 40 ms), large
59 amplitude (up to 4 mV) spikes visible in LFPs recorded from the hilus of the dentate gyrus
60 (DG) (Bragin et al. 1995; Headley et al. 2017; Penttonen et al. 1997). While SPW-Rs take
61 place during a variety of behavioral states including slow-wave sleep, immobile awake state,
62 grooming, eating and drinking (Buzsaki, 1986), DSs are almost exclusively limited to slow-
63 wave sleep and awake immobility (Bragin et al., 1995). A recent study by Headley and
64 colleagues (2017) in rats found that both SPW-Rs and DSs take place preferentially during
65 neocortical UP-states in slow-wave sleep. Further, DSs occur especially when cortical
66 activity is highly synchronized, indicated by increased power at the delta-band (0.5-4 Hz)
67 (see also Bragin et al., 1995). Headley and colleagues (2017) also report that the probability
68 of a DS is tripled immediately (~50 ms) following hippocampal SPW-Rs. In addition, DSs
69 seem to suppress SPW-Rs, as coincidence of both results in smaller-amplitude SPW-Rs. This
70 is in line with the original report by Bragin et al., (1995) stating that SPW-Rs virtually never
71 took place within 200 ms of a preceding DS. On the other hand, DSs that take place right
72 before an SPW-R are smaller in amplitude (Headley et al., 2017) and thus possibly not very
73 effective in suppressing the SPW-R. Interestingly, eliminating entorhinal input to the
74 hippocampus reduces DSs but increases the occurrence of SPW-Rs (Bragin et al. 1995)
75 suggesting that normally entorhinal input to the hippocampus evokes DSs and suppresses

76 SPW-Rs. In conclusion, the majority of both SPW-Rs and DSs take place during slow-wave
77 sleep UP-states and their occurrence is bi-directionally related.

78 Upon closer inspection, during SPW-Rs, synchronous neuronal activation spreads from
79 the pyramidal cells of the CA3 to the pyramidal cells of the CA1 and from there on to
80 principal cells in the subiculum and the neocortex [for a review, see (Buzsaki 2015)]. On the
81 contrary, during DSs, entorhinal cortical activation arrives to the DG via the perforant path
82 and evokes firing of both DG granule cells and interneurons. Coincidentally, pyramidal cells in
83 the CA3 and CA1 decrease firing rate for a period of up to 100 ms (Penttonen et al. 1997).
84 That is, whereas SPW-Rs result in increased firing of CA1 pyramidal cells, the effects of DSs
85 are opposite. Considering the relation of SPW-Rs and DSs to neocortical activity, both are
86 associated with an increase in the firing rate of neocortical neurons and an increase in
87 neocortical gamma-band (35-100 Hz) oscillatory power and coherence that peaks roughly 40
88 ms after the hippocampal event (Headley et al., 2017). However, interregional neocortical
89 coherence at the gamma-band is increased more during DSs compared to SPW-Rs. This
90 increase in gamma-band synchronization across the neocortex is time-locked only to the DSs
91 but not to the SPW-Rs (Headley et al., 2017). To summarize, both the intra-hippocampal and
92 neocortical activity patterns that accompany SPW-Rs and DSs are different.

93 Little doubt remains about the importance of SPW-Rs in memory consolidation.
94 Namely, it has been repeatedly demonstrated (using different learning paradigms and
95 different SPW-R manipulation methods) that interrupting the normal course of SPW-Rs in
96 both sleep and awake rest impairs learning (Girardeau et al. 2009; for a recent review, please
97 see Girardeau et al. 2017). On the other hand, boosting hippocampo-neocortical
98 communication related to SPW-Rs enhances learning (Maingret et al., 2016; see also Tang &
99 Jadhav, *in press*). On the contrary, the meaning of DSs for behavior is still largely unclear. A
100 recent study from our group suggests DSs might be important for memory consolidation

101 (Nokia et al. 2017): Disrupting DS-related silencing of hippocampal CA1 pyramidal cell
102 firing consistently after the training session impaired the learning of trace eyeblink
103 conditioning, a hippocampus-dependent pavlovian conditioning task (Kim et al. 1995). Here,
104 to further probe the importance of DSs for neural plasticity and behavior, we trained adult
105 male Sprague-Dawley rats in hippocampus-dependent learning tasks probing spatial
106 reference memory, pattern separation and temporal interval learning. After each training
107 session, during a period of supposed memory consolidation in rest or sleep, we either
108 disrupted the neural processing that normally follows DSs, disrupted neural processing at
109 random or let the neural activity take its normal course. For the disruption, we used electrical
110 stimulation of the ventral hippocampal commissure (Nokia et al. 2017). We expected
111 disruption of DS-related neural processing to impair learning in all three tasks.

112 **Materials and Methods**

113 *Ethical Approval*

114 All experimental procedures were approved by the Animal Experiment Board of
115 Finland and implemented in accordance with directive 2010/63/EU of the European
116 Parliament and of the Council on the care and use of animals for research purposes. The
117 study complies with the ARRIVE guidelines (Kilkenny et al. 2010).

118 *Subjects*

119 The subjects were 36 healthy adult male Sprague-Dawley rats (Envigo, Netherlands)
120 weighing ~300 g at surgery. Animals were single-housed on the premises of the animal
121 research unit at the University of Jyväskylä. Food (R36, Lantmännen, Sweden) and tap-water
122 were freely available, and room temperature and humidity were controlled at $21 \pm 2^\circ\text{C}$ and 50
123 $\pm 10\%$, respectively. All rats had aspen chips (Tapvei, Kaavi, Finland) and paper towels at
124 the bottom of the cage as bedding and nesting material. Rats were maintained on a 12 h–12 h
125 light–dark cycle, with lights on at 08.00 h. All procedures were conducted during the light
126 portion of the cycle, and the training was done in random order to prevent the possible effects
127 of circadian rhythm on the outcome. A subset of the animals was trained in the 8-arm radial
128 maze task only, and the rest were trained first in the context-object discrimination (COD) task
129 and then in the fixed interval (FI) task. At least a week of rest was given in between the tasks.
130 The timeline for the experiments is presented in Figure 1.

131 *Surgery and recording*

132 Two bundles of 4 recording electrodes were implanted unilaterally to record
133 hippocampal local-field potentials (LFPs) from the hilus (3.6–4.5 mm posterior, 1.5–2.2 mm
134 lateral and 3.6–4.0 mm below bregma) and stimulation electrodes were placed bilaterally on
135 the ventral hippocampal commissure (vHC, 1.3 mm posterior, 1.0 mm lateral and 4.0 mm

136 below bregma) to stimulate the hippocampus (see Figure 2A and 2B) (Paxinos and Watson
137 1998). Recording electrodes were made of Formvar insulated nichrome wire (bare diameter
138 50 μm , #762000, A-M Systems Inc., Carlsboro, WA, USA) glued together using
139 cyanoacrylate with a tip separation of 200 to 250 μm . Bipolar stimulation electrodes were
140 made of Formvar coated stainless steel dual-wire with a diameter of 100 μm and a tip
141 separation of \sim 500 μm . For a detailed description of the surgery, please see (Nokia et al.
142 2017).

143 To acquire neural measures, a wireless headstage [bandwidth 1-5 kHz, W2100-HS8,
144 MultiChannel Systems (MCS), Reutlingen, Germany] was attached to the electrode connector
145 anchored with dental acrylic to the rat's head. The filtered and digitized data was conveyed to
146 a wireless W2100-System (MCS) for storage. A flexible, insulated cable was used to connect
147 the animal to a stimulator (model STG4008, MCS). Movement was detected by an
148 accelerometer (EVAL-ADXL335Z, Analog Devices Inc., Norwood, MA, USA) attached to
149 the cable going from the animal to the stimulator. In the W2100-System, the LFP signals
150 were further low-pass filtered (1st order IIR, cutoff frequency 800 Hz) and all signals were
151 recorded with Experimenter software (MCS) using a 2-kHz sampling rate. Labview (National
152 Instruments Corporation, Austin, TX, USA) was used for online signal analysis and
153 triggering of events.

154 *Behavioral training*

155 Eight-arm radial maze task

156 Hippocampal SPW-R involvement in spatial learning was demonstrated by Girardeau
157 and colleagues almost a decade ago (Girardeau et al. 2009). They used a reference memory
158 protocol in the 8-arm radial maze task. Specifically, rats were trained to search for reward in
159 three stable locations over the course of several days. Disrupting SPW-Rs by using electrical

160 stimulation impaired the learning of this task (Girardeau et al. 2009). Here we used a similar
161 protocol to study the involvement of DSs in spatial reference memory. First, the rats received
162 ~10-20 pieces of 45mg sugar pellets (Sandown Scientific, Hampton, Middlesex, UK) to their
163 home cage every day for at least a week, to habituate them to eating them. Then, one day
164 prior to habituation to the maze, the animals were put on a restricted diet of 15 g of feed per
165 day. The rats remained on the restricted diet Sun-Fri throughout the maze task, which was
166 conducted on weekdays for three weeks. Each animal was weighed daily.

167 Habituation to the maze consisted of an initial 10-minute exploration trial during which
168 all arms were baited with three pellets scattered throughout the arm. Next, each animal was
169 allowed three 3-minute trials of exploration with three pellets in each arm. The intertrial
170 interval was 12 min. The next day, training was started. Four trials per session per animal
171 were conducted, the inter-trial interval again being 12 minutes. During each trial, all arms
172 were open and the same three arms were baited, each with one 45-mg sugar pellet. The
173 animal was placed in the center facing a random direction and let to search for the sugar
174 pellets until it ate all of them or up to three minutes. After each trial, the maze was turned at
175 least 45 degrees and cleaned with 70% ethanol. All sessions were recorded with a standard
176 web cam facing the maze from above. The behavior of the animal was scored on-line, during
177 the session but the videos were stored as back-up. Altogether 15 sessions were conducted
178 adding up to 60 trials.

179 Context-Object Discrimination (COD) task

180 Dentate gyrus granule cells are capable of creating sparse (Senzai and Buzsaki 2017),
181 non-overlapping representations of highly similar yet distinct events. This ability to make
182 distinctions between places or events alike is often referred to as pattern separation and is
183 thought to be governed mostly by the DG (Knierim and Neunuebel 2016). COD is a task that
184 relies on efficient pattern separation and the formation of non-overlapping representations of

185 each context-objects entity. Performance in this task is dependent on an intact hippocampus
186 in rats (Mumby et al. 2002). Thus, we studied the involvement of DSs in pattern separation
187 using a COD protocol modified from Czerniawski and colleagues (Czerniawski et al. 2015).
188 The basic set-up is illustrated in Figure 1.

189 For COD we used two different arenas in three different rooms and six different pairs
190 of identical objects. One type of arena was a square, 74 cm x 74 cm, with 30-cm-high,
191 plexiglass walls and the other type was round (~74 cm diameter, 30-cm high walls) with
192 walls of matt white metal sheet. To modify the arenas to provide six different variations, the
193 walls of both arenas were decorated with prominent visual cues. The floor was also modified,
194 the material being either plexiglass, black rubber, brown plywood or grey plastic carpet. The
195 distal visual cues and lighting in the rooms were also varied. Note, that no two identical
196 contexts were used for the same animal. The objects used were halogen lamps, metallic
197 saltshakers, and objects made of glass (transparent bowl, dark brown bottle with white,
198 plastic cap, green cube) or stone (gray, round candle holders). The pairs of objects used in
199 each context was pseudorandomized. Objects were placed side-by-side, 20 cm from the wall.
200 After each exposure, the arena and the objects were cleaned with 70% ethanol solution.

201 As in Czerniawski et al. (2015), on two consecutive days, the rat was placed in contexts
202 A and B and allowed to freely explore the objects and the arena for 5 minutes. Between visits
203 1 and 2 (Day 1) and 3 and 4 (Day 2), each rat was returned to its home cage for 20 minutes.
204 The daily sessions were conducted with an interval of ~24 hrs. The order of visits to A and B
205 was pseudorandomized. The test session took place on Day 3 and consisted of 5 min in either
206 context A or B (pseudorandomized). This time, one of the objects in the arena was consistent
207 with training (in context) but the other one was one that had previously been presented in the
208 other context (out of context). All sessions were recorded with a standard web cam. The
209 behavior of the animal was scored off-line (see Data analysis).

210 Fixed interval (FI) task

211 To evaluate the animal's ability to learn a temporal interval, a fixed interval (FI) task
212 was used. Learning to time responses accurately in a fixed or peak interval task is dependent
213 on the dorsal hippocampus: Animals with lesions tend to respond too early [see for example
214 (Yin and Meck 2014)]. Here we used a protocol in which the rat was trained to poke its nose
215 for a reward every 113 seconds. We wanted to be sure the length of the interval is not
216 divisible and thus picked a prime number. A very long interval (compared to that used in Yin
217 and Meck 2014) was selected because we have plans to record DG single-unit activity during
218 the FI task, and the DG granule cells are known to fire at a very low rate (~0.1 Hz) in awake
219 state (see Senzai & Buzsaki, 2017). Thus, long recordings are needed to catch cells.

220 FI training took place inside a cylinder (diameter 20 cm, height 40 cm) made of clear
221 acrylic and placed inside a sound insulated cubicle (ENV-018V, Med Associates Inc.,
222 Fairfax, VT, USA) dimly lit. A nose poke port (diameter ~2 cm) was on one side of the
223 cylinder, approximately 5 cm from the bottom. On the opposite side, there was another hole
224 for the delivery of the reward pellet onto a black concave acrylic block tray (1.5 cm x 2 cm).
225 Reward was delivered using a standard pellet dispenser (ENV-203-45, Med Associates Inc.,
226 Fairfax, VT, USA) connected to the pellet tray with silicone tubing. The experiment was
227 controlled by an Arduino[®] microcontroller and nosepokes were detected using a reflective
228 optical sensor with transistor output (TCRT5000L, Vishay Intertechnology, Inc., Malvern,
229 PA, USA). More precisely, the infrared LED and the phototransistor were positioned on
230 opposite sides of the opening in the cylinder wall, outside the cylinder, meaning that the
231 animal had to purposely extend its neck to break the light beam for a reward. Only the first
232 nosepoke that started after the target interval had elapsed was rewarded and one 45-mg pellet
233 was delivered at a time.

234 During the FI task, animals were maintained on a restricted diet of 15 g of food pellets
235 per day starting from the day prior to the first FI session. The rats were not habituated to the
236 task to keep them completely naïve prior to training. Our pilot studies did not show marked
237 effects of pre-training using a shorter, 17-s interval. In line with this, the rats were performing
238 the maximum number of trials from the 3rd session onwards. Each session lasted 1 h allowing
239 for ~30 trials. Altogether 10 sessions were conducted, one per day. Nosepokes and pellet
240 deliveries were recorded as TTL pulses using the Analog inputs of the W2100 system.
241 Performance was analyzed offline using Matlab (see Data analysis).

242 *Hippocampal stimulation via vHC*

243 After each training session the animals were let to rest in plastic cages placed inside
244 sound insulated cubicles (ENV-018V, Med Associates Inc.). This procedure was carried out
245 for 2 h following each training session in the 8-arm maze task and the COD-task, and for 1 h
246 following the FI task. Note that after the FI task, the animals remained in the same training
247 cylinder but bright lights were switched on to signal the end of the FI-session.

248 In the experimental condition (EXP), the hippocampus was stimulated via bilateral
249 stimulation electrodes, immediately following the peak of the DS (see also Nokia et al.,
250 2017). In the yoked control (YC) condition, stimulations were delivered during immobility
251 and at a similar rate as for the EXP animal, but irrespective of neural state. To deliver
252 stimulation based on the DSs, signal from a recording electrode showing clear DSs and a
253 large (> 1 mV) positive response to the vHC stimulation was conveyed to Labview. DSs were
254 detected based on a fast rise in signal amplitude and a simple peak amplitude threshold. It
255 was also required that the animal was still, i.e. that the accelerometer signal showed minimal
256 activity. When the conditions for a DS were met, the Labview produced a TTL pulse. This
257 triggered the delivery of a bipolar electrical pulse (STG4008, MCS) to the vHC of the EXP
258 animal (~5-20 ms variable delay). Stimulation duration and amplitude (0.2 to 0.24 ms, max.

259 160 μ A) were adjusted for each animal so that the amplitude of the hippocampal response in
260 the electrode showing spontaneous DSs was 1 to 1.5 mV in amplitude.

261 In the normal control (NC) condition, no hippocampal stimulation was given. Note that
262 stimulation was omitted also in the EXP and YC groups during recordings performed after
263 the 8th (middle) and 15th (last) 8-arm maze training session and after the 10th (last) FI task
264 training session. Data from these recordings were used to study possible link between DS
265 occurrence rate and learning.

266 *Histology*

267 Rats were euthanized by exposure to a rising concentration of CO₂, and death was
268 verified by rapid decapitation. The locations of the electrode tips in the brain were marked by
269 passing a DC anodal current (200 mA, 5 s) through them. The brain was then removed and
270 stored in 4% paraformaldehyde solution (pH 7.4) for at least 48 h at +4 °C. After that, the
271 brain was kept in phosphate buffered saline solution in +4 °C until sectioning. The brain was
272 coronally sectioned with a vibratome (Leica VT1000) into 40- μ m slices. The slices were
273 attached to slides, dried, and stained with Prussian blue and Cresyl violet. The electrode tip
274 locations were determined with the help of a conventional light microscope and a brain atlas
275 (Paxinos and Watson 1998).

276 *Data analysis*

277 Behavior

278 *Eight-arm radial maze task*

279 During maze training, behavior was scored on-line and verified later from video, if
280 needed. The animal was considered to have entered an arm when the whole body (excluding
281 tail) was inside an arm. Entries to empty arms were considered errors, as were entries to a
282 baited arm and not eating the sugar pellet, and re-entries to a baited, already emptied arm.

283 Correct response (maximum 3) was scored when the animal entered a baited arm and ate the
284 pellet. A performance index was calculated as in Girardeau et al. (2009) based on the number
285 of errors and correct responses as follows: Performance index = $2 / \pi * \arcsin(\sqrt{(\text{correct} /$
286 $(\text{correct} + \text{errors})))$. Perfect score is 1 (one) and indicates no errors and one (and only one)
287 visit to each baited arm while eating all the available pellets.

288 COD task

289 For the context-object -discrimination task, behavior was evaluated off-line from the
290 recorded videos by an experimenter blind to animal identity. Exploration was defined as
291 pointing the nose towards the object within < 2 cm distance or otherwise physically
292 interacting with the object (i.e. leaning on it, climbing over it etc.). The time spent exploring
293 each object during the first minute of the test session was scored in seconds. Then the
294 percentage of time spent exploring the out-of-context object was calculated relative to total
295 time spent exploring either object.

296 FI task

297 To evaluate timing performance in the FI task, data was analyzed off-line using
298 MATLAB (MathWorks Inc., Natick, MA, USA). First, the probability of a nose poke was
299 determined for the first half of the fixed interval (false response) and for the period exceeding
300 90% of the fixed interval (correct). Then the ratio between the correct response probability
301 and the summed probability of both false and correct responses was calculated (0 = only false
302 responses, 1 = only correct responses). A value of 0.7 was set as a criterion for learning.

303 Dentate spikes

304 Dentate spikes were detected off-line using a custom-made script in MATLAB. Data
305 was analyzed from recordings during which no vHC stimulation was applied. Only periods of
306 immobility were included in analysis, when accelerometer signal was available. The mean
307 and standard deviation (SD) of the hilar LFP signal during immobility were derived. A

308 threshold was set at mean + x*SD. The factor x varied between 3 and 4, depending on
309 electrode location, i.e. how large were the DSs in relation to background activity. Then,
310 positive deflections in LFP amplitude that exceeded the threshold were detected using a 20
311 ms window split in half: Both the maximum LFP amplitude during the latter half (peak) and
312 the difference between the maximum LFP amplitudes during the former and latter halves
313 (rise) had to exceed the threshold to qualify the event as a DS. Accuracy of DS detection was
314 verified by qualitative analysis of detected events by a trained human eye.

315 Statistics

316 IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY, USA) was used for statistics.
317 Sigmaplot (Systat Software Inc., San Jose, CA, USA) was used for data visualization.
318 Analysis of variance for repeated measures (ANOVA-RM) was used to analyze changes
319 across training and differences between conditions. Whenever a significant interaction
320 emerged, separate repeated measures ANOVAs were conducted for each group. Paired
321 samples t-test was used for comparing two related measures. Greenhouse–Geisser corrected
322 p-values are reported when the sphericity assumption was violated according to Mauchly's
323 test. Bonferroni-corrected p-values were used for post-hoc comparisons when appropriate.
324

325 **Results**

326 *Recording electrodes were placed in the hilus and stimulation electrodes in the vHC*

327 In all animals assigned to the YC or the EXP conditions, stimulation electrodes were
328 verified to be located in the vHC and recording electrodes in the hilus (Figure 2, panels A and
329 B). In animals only used as normal controls, the electrodes were either misplaced in different
330 hippocampal cell layers or the headstage was damaged in some way. Representative
331 examples of dentate spike and vHC stimulation responses in the hilus are illustrated in Figure
332 2, panel C.

333 *Stimulation of the vHC had no effect on spatial reference memory*

334 Fourteen animals successfully completed the 8-arm radial maze -task and were included
335 in the analyses. There were six animals in the normal control group (NC), four animals in the
336 yoked control group (YC) and four animals in the experimental group (EXP). No significant
337 weight loss was detected due to the restrained diet (data not shown). For a graphical
338 presentation of the results, please see Figure 3 panels A (behavior) and D (DSs and vHC
339 stimulations).

340 To study the rate of occurrence of DSs at baseline and during spatial learning we
341 analyzed data from 7 of the 8 animals with hilar recording electrodes (EXP and YC). One
342 animal in the YC group was excluded as the dentate spikes were lower in amplitude than in
343 the others (i.e. recording electrode was not optimally placed). The number of DSs per
344 immobile minute was 4.7 ± 1.1 (mean \pm standard error of mean) at baseline, 3.6 ± 1.0 in the
345 middle of training (8th session) and 3.2 ± 0.4 in the end of training (15th session). On average
346 4.5 ± 0.3 vHC stimulations per minute were delivered to the YC and the EXP animals during
347 the 2-hr recordings conducted after training sessions 1-7 and 9-14. ANOVA-RM showed no
348 change in the rate of stimulation between the first and the 14th session ($n = 8$, $F [1, 7] = 0.52$,

349 $p = 0.496$). That is, DS-contingent vHC stimulation rates were comparable or even slightly
350 higher than that expected based on the rate of DSs detected off-line.

351 To examine behavioral performance in the 8-arm radial maze, the performance index
352 from three consecutive sessions was averaged into five blocks. ANOVA-RM revealed a
353 significant main effect of block ($F [4, 44] = 51.82, p < 0.001$), no interaction of block and
354 group ($F [8, 44] = 0.80, p = 0.607$) and no main effect of group ($F [2, 11] = 0.14, p = 0.875$).
355 Statistical analysis was also performed on the number of errors made and the time (s) taken to
356 retrieve all three pellets. The results were the same, i.e. robust learning and no difference
357 between groups (data not shown). That is, animals in all groups learned the 8-arm maze task
358 equally well. In the end of training, the fastest animals were able to retrieve all three reward
359 pellets in less than 10 seconds.

360 *Stimulation of the vHC contingent on dentate spikes had a positive effect on pattern*
361 *separation*

362 Nine animals were trained in COD using a within-subjects design and were included in
363 the analysis. Each animal was trained and tested three times, once without stimulation
364 (normal control, NC), once with DS -contingent stimulation (experimental, EXP) and once
365 with random stimulation (yoked control, YC). The order in which the animals were assigned
366 to NC, YC and EXP was randomized, and there was at least one week between repetitions.
367 For a graphical presentation of the results, please see Figure 3 panels B (behavior) and E
368 (DSs and vHC stimulations).

369 First, we analyzed the occurrence of spontaneous DSs and vHC stimulations. In the NC
370 condition, the number of DSs per minute was 2.2 ± 0.3 after the first training session and 2.8
371 ± 0.3 after the second training session. Note that during these recordings no movement data
372 was acquired so detection was based on LFP quality only. This means that the rate per minute
373 is an underestimation compared to all other conditions where time periods with movement

374 have been excluded. On average 7.2 ± 0.8 and 8.4 ± 1.1 vHC stimulations per minute were
375 delivered to the animals in the YC and the EXP conditions while immobile during the 2-hr
376 recordings conducted after the two training sessions, respectively. ANOVA-RM revealed no
377 effect of training day on either the rate of DSs or the rate of vHC stimulations. That is, DS-
378 contingent vHC stimulation rates were again higher than that expected based on the rate of
379 DSs detected off-line (even if taking into account the underestimation due to inclusion of
380 awake movement periods).

381 Analysis of behavioral performance in the COD task indicated that even though
382 animals seemed to perform best during the first exposure to the COD task, this was not a
383 statistically significant effect (ANOVA-RM, three rounds of COD: $F [2, 16] = 2.99$, $p =$
384 0.079). However, ANOVA-RM indicated a clear effect of experimental condition (NC – YC
385 – EXP: $F [2, 16] = 3.80$, $p = 0.045$). Paired comparisons (t-test) further revealed that
386 performance on the COD task was better after DS -contingent vHC stimulation compared to
387 no stimulation (NC vs. EXP: $t (8) = 2.66$, $p = 0.029$; EXP vs. YC: $t (8) = 0.76$, $p = 0.467$; NC
388 vs. YC: $t (8) = 1.84$, $p = 0.104$) (see Figure 3, panel B). In fact, rats only performed above
389 chance level (50 %) after the experimental manipulation (one sample t-test, EXP: $t (8) = 3.03$,
390 $p = 0.016$; NC: $t (8) = 0.31$, $p = 0.765$; YC: $t (8) = 1.85$, $p = 0.101$). To summarize, contrary
391 to expectation, DS-contingent vHC stimulation during the memory consolidation period
392 improved pattern separation performance on the context-object –discrimination task.

393 *Stimulation of the vHC had no effect on temporal interval learning*

394 Eleven animals completed the FI task. Five of these animals were assigned to the EXP
395 group, four to the NC group and two to the YC group. For a graphical presentation of the
396 results, please see Figure 3 panels C (behavior) and F (vHC stimulations and DSs). All
397 animals lost some weight due to the restricted diet (start vs. end of training, ANOVA-RM:
398 interaction of group and time: $F [2, 8] = 0.84$, $p = 0.47$; main effect of time: $F [1, 8] = 17.98$,

399 $p = 0.003$; main effect of group: $F [2, 8] = 0.13, p = 0.880$). Animals weighed 461 ± 7 g
400 (mean \pm standard error of mean) in the beginning and 448 ± 6 g in the end of FI training.
401 Thus, the weight loss was on average 2.85 ± 0.57 %.

402 During the 1-hr recordings conducted after the first nine training sessions, 6.1 ± 0.6
403 vHC stimulations per minute were delivered to the YC and the EXP animals. ANOVA-RM
404 showed no change in the rate of stimulation across training (first vs. 9th session, $n = 7, F [1, 6]$
405 $= 0.32, p = 0.593$). To study the rate of occurrence of DSs, we analyzed data from five
406 animals from the last (10th) recording during which no vHC stimulation was performed. The
407 number of off-line detected DSs was 3.0 ± 0.4 per immobile minute. In sum, DS-contingent
408 vHC stimulation rates were again higher than that expected based on the rate of DSs detected
409 off-line. To study learning, FI performance index values from two consecutive sessions were
410 averaged to produce five blocks (see Figure 3 panel C). ANOVA-RM indicated equal
411 learning in all groups (interaction of block and group: $F [8, 32] = 0.59, p = 0.780$; main effect
412 of block: $F [4, 32] = 48.95, p < 0.001$; main effect of group: $F [2, 8] = 0.07, p = 0.932$). That
413 is, hippocampal stimulation via the vHC during a period supposed to support memory
414 consolidation had no effect on learning of a simple temporal interval task.

415

416 **Discussion**

417 Our aim was to study whether DSs are relevant for memory formation in three different
418 hippocampus-dependent learning tasks. To this end, we trained animals in a spatial reference
419 memory task, a temporal fixed interval task and in a pattern separation task. Immediately
420 after training, we interfered with the normal course of DS-related neural processing (Bragin
421 et al. 1995; Headley et al. 2017; Penttonen et al. 1997) by stimulating the hippocampus using
422 a weak electrical pulse to the vHC. This interference had an effect only on performance in the
423 pattern separation task. Contrary to our assumptions, our manipulation improved performance
424 compared to a non-stimulated normal control condition. Thus, while DS-related neocortico-
425 hippocampal processing that takes place during rest and sleep after the initial experience
426 seems crucial for associative learning (Nokia et al. 2017), disrupting it actually might
427 sometimes be beneficial. This may happen in situations that require the preservation of
428 separate representations of similar events.

429 It is somewhat surprising that hippocampal stimulation either contingent on DSs or
430 irrespective of neural state had no effect on learning the location of the reward pellets in the
431 eight-arm radial maze, a task measuring spatial reference memory. A previous study shows
432 that performance in this task is dependent on the normal occurrence of hippocampal SPW-Rs
433 (Girardeau et al. 2009). As in our current experiment, Girardeau et al. (2009) also used
434 electrical stimulation of the hippocampus via the vHC as the method to interfere with SPW-
435 Rs. Thus, the difference in results between our current study and that of Girardeau and
436 colleagues (2009) should not be due to methodological differences. The vHC stimulation
437 method and rate, as well as the rate of DS occurrence, in our present experiments are also
438 comparable to those in our earlier study (Nokia et al. 2017), in which we *did* find a clear
439 effect of disrupting DS-related processing on learning. If anything, in our current studies, the
440 on-line criteria for stimulation of the vHC contingent on DSs seemed to be somewhat looser

441 than the off-line criteria for detecting DSs. That is, stimulations were delivered upon
442 detection of all notable DSs in the experimental condition. Based on these observations and
443 our current results, it seems that for simple place learning or spatial reference memory, SPW-
444 Rs but not DSs are needed. Further studies could probe the effects of interfering with DSs
445 during a certain phase of the training. However, it is unlikely that effects would be seen then,
446 either.

447 We also found no effect of vHC stimulation on learning the fixed interval task. Earlier
448 studies show that accurate performance in tasks requiring evaluation of temporal duration is
449 dependent on an intact hippocampus: Animals with hippocampal lesions tend to seek for
450 reward earlier or more often than normal animals (Meck et al. 2013; Yin and Meck 2014;
451 Bannerman et al. 1999). However, in previous studies, the intervals used have been much
452 shorter (from 2 to 45 s) compared to what we used here (113 s). Although most definitely
453 hippocampus-dependent, it might be that our paradigm was not sensitive enough to show
454 differences between stimulated animals and normal controls. In further studies, it might be a
455 good idea to use a shorter interval for FI training. Nevertheless, our finding is in line with the
456 fact that direct stimulation of the CA1 pyramidal cell layer has no impact on learning or
457 performance of an operant conditioning task (Jurado-Parras, Gruart and Delgado-García
458 2012). To our knowledge, apart from trace eyeblink conditioning (Nokia et al. 2012), the role
459 of SPW-Rs in temporal interval learning or timing ability has not been studied. Thus, further
460 research probing the role of different hippocampal oscillations in fixed interval learning using
461 variable interval lengths could be feasible. Regardless, our results clearly suggest that
462 disrupting the normal course of DS-related neocortico-hippocampal neural processing does
463 not dramatically impair the ability to acquire an appetitive operant conditioning task and to
464 learn the duration of a temporal interval.

465 Last, we did observe a clear effect of vHC stimulation on performance on a context-
466 object –discrimination task that measures a phenomenon termed pattern separation. Our
467 assumption was that after the experience, offline memory consolidation would involve mostly
468 the strengthening of an association between objects and the context in which they were
469 encountered. Much to our surprise, animals in which hippocampal stimulation was targeted to
470 DSs showed better recognition for the out-of-context object in the testing situation, that is,
471 improved pattern separation. Thus, contrary to our assumption, it seems that normal offline
472 memory consolidation involves either formation of “false” associations between entities that
473 are similar but not related or the degradation of accurate associations. As vHC stimulation
474 improved pattern separation especially when targeted at DSs, we suggest that some of this
475 processing takes place during or right after DSs. This proposed process might be especially
476 important during tasks that require either the formation of associations between temporally
477 separate events (Nokia et al. 2017) or retaining separate representations of similar events such
478 as during pattern separation – a function often assigned to the DG (Knierim and Neunuebel
479 2016).

480 Some aspects of our results merit further discussion. First, of all the hippocampus-
481 dependent learning tasks that we have probed, the effects of DS-contingent vHC stimulation
482 were limited to trace eyeblink conditioning (Nokia et al. 2017) and COD (study at hand). It is
483 not very clear what makes both of these tasks so special from the viewpoint of the
484 hippocampus let alone the DG. However, as discussed above, performance on both tasks
485 relies on accurate formation of associations. Related, saturation of the CA3-CA1 synapse by
486 inducing long-term potentiation impairs learning of trace eyeblink conditioning (Gruart,
487 Muñoz and Delgado-García 2006). In addition, selective inactivation of DG granule cells has
488 a remarkably deleterious effect on acquisition and performance of the learned response
489 during trace conditioning but only if the inactivation overlaps with conditioning (Madronal et

490 al. 2016). Madronal and colleagues (2016) propose that the entorhinal input via the perforant
491 path to the DG potentiates the connection between the CA3 and the CA1, while the direct
492 input from EC to CA1 depotentiates this connection. According to them, the role of this
493 depotentiation from the EC via the PP to the CA1 would be to weaken old associations to
494 clear space for new learning. This is at least partially in line with an idea that DSs, together
495 with SPW-Rs, might be crucial for learning due to their possible role in maintaining the
496 excitation/inhibition balance in the hippocampo-neocortical circuit. This notion is related to
497 the more general idea of homeostasis as the necessary basis for adaptive synaptic plasticity
498 (Tononi and Cirelli 2016).

499 Second, for the pattern separation task, there was also a modest but not statistically
500 significant positive effect of random stimulation of the hippocampus. This is not surprising
501 taken that non-specific electrical interference with hippocampal function during the memory
502 consolidation period has profound effects on long-term memory (Kesner and Wilburn 1974).
503 These findings underline the obvious fact that hippocampal processing crucial for memory
504 consolidation also takes place outside DSs. Third, for the pattern separation task, we used a
505 within-subjects design as opposed to a between-subjects design. This might have made it
506 possible to detect differences between conditions more reliably than in the FI and maze tasks
507 in which the effects might have been partially masked by inter-subject variability.

508 In further studies, it might be interesting to more closely investigate hippocampal
509 neural activation during a pattern separation task and the following rest period. Specifically,
510 it would be interesting to see if different DG granule cells (and possibly mossy cells) fire in
511 the different contexts, and whether these two neural assemblies are then co-activated during
512 DSs that are observed at rest following the behavioral exposure. It is possible that DSs reflect
513 the reactivation of a large population of recently activated DG cells belonging to separate cell
514 assemblies, and that this is the mechanism by which associations between neural

515 representations are modified off-line to produce associations between events that in fact did
516 not take place in the exact same moment.

517 In conclusion, in some cases DSs seem beneficial for accurate memory formation
518 [associative learning, (Nokia et al. 2017)] while in other cases (pattern separation) the
519 performance is actually enhanced if the DS-related processing is disrupted.

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529

530

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601

602

603 **Figure Captions**

604 **Figure 1. Experimental protocol.** A) One group of animals was used for examining the role
605 of DSs in spatial reference memory using the 8-arm radial maze task. Surgery (S) to implant
606 electrodes was conducted first, followed by a week of recovery and then training in the maze.
607 B) Another group of animals was used for studying the role of DSs in pattern separation
608 (context-object discrimination, COD) and in temporal interval learning (fixed interval task).
609 C) In the 8-arm radial maze task, the same three reward locations were always used. Animals
610 were trained for 15 days, 4 trials per day. vHC stimulation was applied during a 2-hr rest
611 period after each session (excluding sessions 8 and 15). More specifically, during this
612 supposed memory consolidation period, local-field potentials from the hilus were monitored
613 and recorded and vHC stimulation was either withheld (normal control condition),
614 administered at random (yoked control condition) or administered when DSs were detected
615 (experimental condition). D) During COD training, animals were exposed to two contexts,
616 each containing a pair of identical objects, for 5 min each, in random order. A 20-minute
617 break was held in between exposures, for which the animals were placed in home cage. After
618 the latter exposure, vHC stimulation was conducted for 2 hours, as for the maze task.
619 Training was conducted on two consecutive days. On the third day, animals were placed in
620 either of the two contexts but now containing two different objects, one from each context.
621 The animal was assumed to indicate memory of the context-object entities by preferentially
622 exploring the out-of-context –object. Note that COD training was conducted thrice so that
623 each animal experienced the normal control condition, experimental condition and a yoked
624 control condition. E) For the fixed interval task, animals were placed in the chamber for
625 training for 1 hour, during which they received a reward when they poked the nose port when
626 at least 113 s had elapsed since the last reward. Again, vHC stimulation was carried out after
627 the training session, but this time for only 1 hour.

628

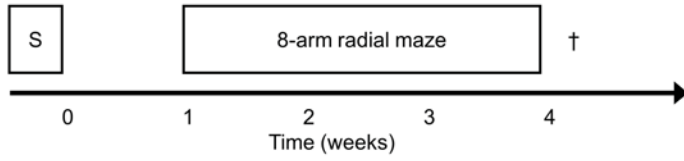
629 **Figure 2. Recording electrodes were placed in the hilus (h) to detect dentate spikes and**
630 **stimulation electrodes in the ventral hippocampal commissure (vHC) to stimulate the**
631 **hippocampus.** Electrode placement was verified from histological samples. The coordinates
632 for implantation were based on the atlas of Paxinos and Watson (1998). The stimulation
633 electrodes were aimed at 1.3 mm posterior to, 1.0 mm lateral to and 4.0 mm below bregma
634 whereas the recording electrodes were aimed at 3.6 to 4.5 mm posterior to, 1.5 to 2.2. mm
635 lateral to and 3.6 to 4.0 mm below bregma. A) Light grey shading indicates the range of
636 locations for stimulation electrodes in the vHC (left panel) and recording electrodes in the
637 hippocampus, aimed at the hilus (h) (right panel). B) Representative examples of stimulation
638 electrode placement in the right vHC (left) and of a recording electrode in the hippocampus
639 (right). Arrows point to electrode tips. Hilus is indicated by the letter “h”. C) Local-field
640 potentials from the hilus of one rat illustrating a representative dentate spike recorded after
641 training in the context-object discrimination task in the normal control (NC) condition (top), a
642 response to vHC stimulation recorded in the yoked control (YC) condition (middle) and a
643 dentate spikes followed by a response to vHC stimulation recorded in the experimental (EXP)
644 condition (bottom).

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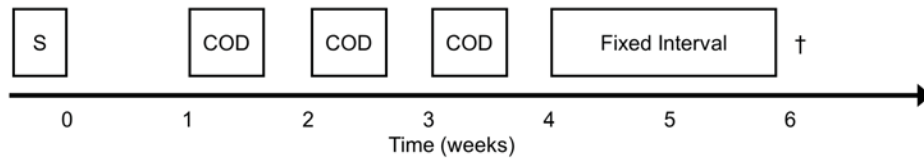
646 **Figure 3. Stimulation of the hippocampus, especially contingent on dentate spikes**
647 **improved pattern separation performance in a context-object discrimination task**
648 **(COD).** A) Stimulation of the hippocampus via the vHC either contingent on dentate spikes
649 (experimental group, EXP) or randomly, during immobility (yoked control, YC) after each
650 conditioning session had no effect on learning a hippocampus-dependent spatial reference
651 memory task in the 8-arm radial maze. Animals learned the fixed locations of the rewards
652 equally well as those in the normal control (NC) group. Note that for statistical analysis

653 (repeated measures ANOVA, ANOVA-RM), performance indexes from three consecutive
654 sessions (days) were averaged to produce five blocks. Vertical lines indicate standard error of
655 mean. Asterisks refer to statistical significance ($p < 0.001$). B) Pattern separation in the COD
656 task was improved by hippocampal stimulation during the memory consolidation period,
657 especially when the stimulation was triggered by dentate spikes. Note that for this
658 experiment, the same nine animals were trained three times, once in each condition. The
659 animals indicated memory for the objects in context by preferring to explore the out-of-
660 context object only when the hippocampal stimulation had been delivered contingent on
661 dentate spikes (EXP). This is indicated by the statistically significant difference (one sample
662 t-test, $p < 0.05$) from chance (dashed horizontal line at 50 %). There was also a statistically
663 significant difference between performance in the non-stimulated control condition (NC =
664 normal control) and that in the EXP condition (paired sample t-test, $p < 0.05$). C) Stimulation
665 of the hippocampus via the vHC during the memory consolidation period had no effect on
666 learning a fixed interval (FI) task. Animals received a reward when they poked their nose to a
667 hole in the wall of the chamber when at least 113 s had elapsed since the last reward delivery.
668 D-F) Rate per minute for DSs and vHC stimulations in each task.

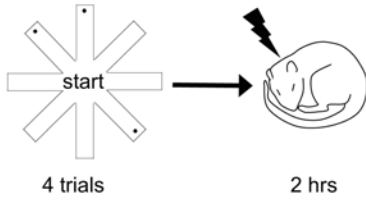
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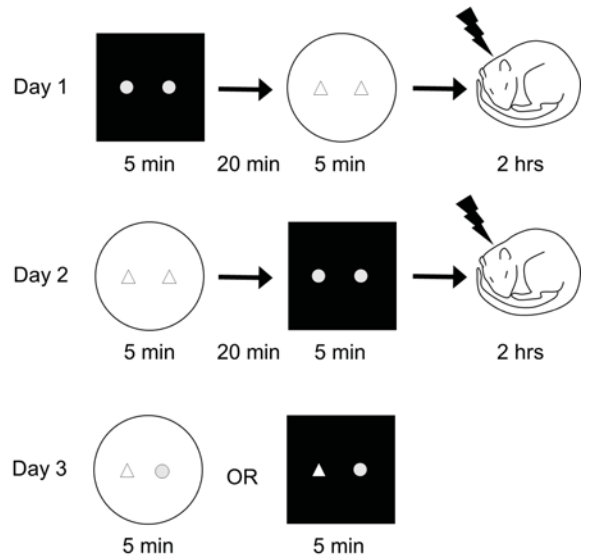
B



C



D



E

