1 **Synchronous activity patterns in the dentate gyrus during immobility**

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30   **Abstract**

31   The hippocampal dentate gyrus is an important relay conveying sensory information from the

32   entorhinal cortex to the hippocampus proper. During exploration, the dentate gyrus has been

33   proposed to act as a pattern separator. However, the dentate gyrus also shows structured

34   activity during immobility and sleep. The properties of these activity patterns at cellular

35   resolution, and their role in hippocampal-dependent memory processes have remained

36   unclear. Using dual-color in-vivo two-photon Ca^{2+} imaging, we show that in immobile mice

37   dentate granule cells generate sparse, synchronized activity patterns associated with

38   entorhinal cortex activation. These population events are structured and modified by changes

39   in the environment; and they incorporate place- and speed cells. Importantly, they are more

40   similar than expected by chance to population patterns evoked during self-motion. Using

41   optogenetic inhibition, we show that granule cell activity is not only required during

42   exploration, but also during immobility in order to form dentate gyrus-dependent spatial

43   memories.

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45   **Keywords:** Hippocampus, dentate gyrus, spatial learning, pattern separation, memory

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Introduction

The dentate gyrus receives polymodal sensory information from the entorhinal cortex, and relays it into the hippocampal network. The most prevalent view of the dentate gyrus input-output transformation in this circuit is that it acts as a pattern separator. This capability requires the animal to generate dissimilar neuronal representations from overlapping input states that represent similar but not identical environments (*Cayco-Gajic and Silver, 2019*). Such an operation, termed pattern separation, has been ascribed to the hippocampal dentate gyrus in species ranging from rodents to humans (*Berron et al., 2016; Leutgeb et al., 2007; Sakon and Suzuki, 2019*). In the dentate gyrus, polysensory inputs are mapped onto a large number of granule cells which exhibit extremely sparse firing patterns, resulting in a high probability of non-overlapping output patterns (*Danielson et al., 2016; GoodSmith et al., 2017; Hainmueller and Bartos, 2018; Pilz et al., 2016; Senzai and Buzsáki, 2017; van Dijk and Fenton, 2018*). This concept has been influential in understanding dentate gyrus function when processing multimodal, current or 'online' sensory information during mobility and exploration.

However, the dentate gyrus is far from silent during immobility. It displays prominent electrographic activity patterns such as dentate spikes and sharp waves, which occur primarily during immobility or sleep (*Bragin et al., 1995; Meier et al., 2020; Penttonen et al., 1997*). In downstream hippocampal regions such as CA1, neuronal activity during immobility incorporates the replay of behaviorally relevant sequences during sharp wave ripples, a process important in memory consolidation (*Davidson et al., 2009; Diba and Buzsáki, 2007; Dupret et al., 2010; Foster and Wilson, 2006; Girardeau et al., 2009; Malvache et al., 2016; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994*). In the dentate gyrus, little detail is known about how granule cells are active during immobility at the population level, and it is unknown whether activity during immobility reiterates behaviorally relevant information. Moreover, the role of dentate gyrus activity during immobility in memory formation is unclear.

76   Here, we have used dual-color two-photon in-vivo Ca^{2+} imaging to show that in immobile

77   mice, the dentate gyrus exhibits frequent, sparse and synchronous population events that at

78   the population level are similar to activity patterns during locomotion. Moreover, we have

79   tested the idea that dentate gyrus activity during immobility is relevant for dentate-gyrus

80   dependent spatial memory.

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Results

Sparse, structured dentate network events in immobile animals

We imaged the activity of large populations of hippocampal dentate granule cells (GCs) using a Thy1-GCaMP6s mouse line (GP4.12Dkim/J, (**Dana et al., 2014**)). In addition, we monitored the bulk activity of the major input system into the dentate gyrus, the medial perforant path (MPP). To this end, we expressed the red-shifted Ca^{2+} indicator jRGECO1a (**Dana et al., 2016**) in the medial entorhinal cortex using viral gene transfer (see Methods section, **Fig. 1A, Fig. 1-figure supplement 1A**). To allow efficient excitation of both genetically encoded Ca^{2+} indicators, we established excitation with two pulsed laser sources at 940 and 1070 nm (see **Fig. 1-figure supplement 1B-F**). The mice were placed under a two-photon microscope and ran on different variants of a linear track, equipped with different types of cues (see **Fig. 1-figure supplement 1G-J, Video 1**).

As previously described, the firing of GCs was generally sparse (**Danielson et al., 2016; Hainmueller and Bartos, 2018; Neunuebel and Knierim, 2012; Pilz et al., 2016**), both when animals were immobile and when they were running on a textured belt without additional cues (mean event frequency 1.38±0.19 events/min and 0.97±0.2 events/min, respectively, n=1415 granule cells in 9 mice, **Fig. 1B, Fig. 1-figure supplement 2C-E**). Despite the sparse activity of granule cells, we observed synchronized activity patterns (**Video 2**). To rigorously define such events, we used an algorithm that detects synchronized network events within a 200 ms time window, corresponding to 1±1 frame at our sampling rate (see Methods). Such synchronous network events could readily be observed in the dentate gyrus in all mice (**Fig. 1C, D**, network events depicted in different colors, see examples for ∆F/F traces of participating cells in **Fig. 1E**). Network events were sparse, incorporating on average only 5.7±0.09 % of the total active GC population. Shuffling analysis (see Methods) confirmed that network events do not arise by chance (**Fig 1F,** grey bars correspond to shuffled data, n=9 mice, 3 sessions/mouse). This was robust over three different types of shuffling analysis (**Fig. 1G, Fig. 1-figure supplement 3** and Methods for the description of the shuffling methods).

111   Notably, network events occurred mainly during immobility periods and were much less

112   prevalent during running (**Fig. 1D**, quantification in **Fig. 1G**). Accordingly, network event

113   frequencies were significantly higher during immobility (repeated measures ANOVA,

114   $F_{(1,8)}$=117, p=2x10^{-6}, n=9 mice, 3 sessions, **Fig. 1H**). During immobility periods (defined as

115   running speeds <4 cm/s), the vast majority of (99.6%) network events occurred when mice

116   were completely immobile. Network events were on average evenly distributed during the

117   20-minute imaging session (**Fig. 1I**), as well as during individual periods of immobility (**Fig.**

118   **1J**).

119

120   ***Dentate network events are correlated with MPP activation***

121   We then analyzed the activity in the MPP input fiber tract expressing jRGECO, and probed

122   the relation of this activity with GC activity patterns. As expected during exploratory states,

123   the bulk MPP activity was increased during locomotion, consistent with increased sensory

124   input (**Fig. 2A, B**, red channel, **Fig. 2C** for average value). During immobility in particular,

125   larger fluctuations of bulk MPP activity were observed. This phenomenon was reflected in a

126   larger variance of the bulk MPP signal during immobility (**Fig. 2D**). Cross-correlation revealed

127   that during immobility, the increases in bulk MPP activity were associated with peaks in

128   average GC activity levels (**Fig. 2E**). Both signals were significantly correlated in most

129   sessions for periods of immobility, (8/9 sessions, n=3 mice, 3 sessions per mouse, Granger

130   causality test p<0.05). This correlation was clearly visible during network events, because

131   aligning GC activity and MPP activity to the timepoint of network events revealed a strong

132   coactivation of GC and MPP during network events (**Fig. 2F**, n=3 mice). During running,

133   MPP signals did not correlate with average GC activity, which is not unexpected given the

134   asynchronous activation of GCs during running. (8/9 sessions, Granger causality test

135   p>0.05.

136   To explore in more detail how individual fluctuations in MPP bulk activity are associated with

137   GC activity, we used a deconvolution algorithm to identify synchronous activity of MPP axons

138   visible in the bulk MPP transients (see Methods). We then quantified amplitude and

139   frequency of these transients during locomotion and immobility. First, we found that bulk

140   MPP events detected during immobility are on average larger than those detected during

141   running (**Fig. 2G,** Kruskal-Wallis test, n=3 mice, 3 sessions per mouse, 8469 and 19106

142   events during running and resting, respectively, $p=2x10^{-44}$), in agreement with the larger

143   variance of the MPP signal during these periods. When we examined the frequencies of all

144   detected MPP bulk events during locomotion and immobility, we found that there were

145   significantly more events during running (repeated measures ANOVA, $F_{(1,2)}=255$, $p=3x10^{-6}$,

146   n=3 mice, 3 sessions, **Fig. 2H**). However, large events, defined as bulk MPP events with

147   amplitudes above two standard deviations of the mean, were significantly more frequent

148   during resting states (repeated measures ANOVA, $F_{(1,2)}=27$, $p=2x10^{-3}$, n=3 mice, 3 sessions,

149   **Fig. 2I**). Again, this is consistent with the larger variance of the MPP signal during immobility,

150   and likely reflects synchronized activation of MPP fibers. In line with the correlation of MPP

151   and GC signals, there was a short temporal delay between individual bulk MPP transients

152   and network events (**Fig. 2J**).

153

154   ***Dentate network events are correlated with pupil constriction***

155   Pupil diameter is an indicator of neuronal state and arousal (***Reimer et al., 2014; Reimer et***

156   ***al., 2016***), and can be used to track changes in neuronal states during quiet wakefulness

157   (***Reimer et al., 2014***). Of note, pupil changes have been shown to closely track the rate of

158   occurrence of hippocampal synchronous activity, namely sharp waves in the hippocampal

159   CA1 region (***McGinley et al., 2015***). We therefore asked if dentate network events are also

160   associated with specific changes in pupil diameter (**Fig. 3A** for example measurement of

161   pupil diameter over multiple resting and locomotor states). As previously described (***Reimer***

162   ***et al., 2014; Reimer et al., 2016***), we found pupil constriction during immobility with dilation

163   at locomotion onsets (**Fig. 3B, C,** n=6 mice, 3 sessions). Intriguingly, the average pupil

164   diameters during network events were significantly more constricted compared to the

165   average pupil diameters during entire periods of immobility (**Fig. 3D,** repeated measures

166   ANOVA for all three groups $F_{(2,28)}$=17.17, p=1x10^{-5}, n=6, data from 3 sessions each,

167   Bonferroni post-tests: pupil diameters during locomotion vs. immobility p=0.0068, locomotion

168   vs. network events p=0.0016, immobility vs. network events p=0.0017).

169   When looking at pupillary dynamics by assessing the rate of diameter change, locomotor

170   episodes were on average associated with pupil dilation, while network events were

171   specifically associated with pupil constriction (**Fig. 3E**, repeated measures ANOVA

172    $F_{(2,28)}$=34.18, p=3x10^{-8}, n=6 mice, data from 3 sessions each, Bonferroni post-tests: pupil

173   diameters during locomotion vs. immobility p=0.0016, locomotion vs. network events

174   p=2.53x10^{-5}, immobility vs. network events p=0.00053). The latter finding was clearly

175   illustrated by averaging pupil diameters aligned to NE times (**Fig. 3F**). Together, this

176   suggests that network events are associated with specific pupillary dynamics reflecting

177   substates of arousal and neuronal synchronization during immobility.

178

179   ***Network events are more orthogonal than expected by chance, but repetitively recruit***

180   ***GC sub-ensembles***

181   We then further characterized the participation of dentate granule cells in network events.

182   We first asked to what extent individual network events recruit orthogonal cell populations.

183   Indeed, while individual GCs can partake in multiple network events (see **Fig. 1C, D, Video**

184   **2**), we also observed network events that seemed completely distinct to others. To quantify

185   how similar network events are to one another, we computed population vectors for each

186   network event. We then computed the cosine similarity as a measure of similarity between

187   vectors representing individual network events (see Methods). With this measure, network

188   event pairs recruiting the same set of neurons have a cosine similarity of 1, and completely

189   orthogonal patterns exhibit a cosine similarity of 0. This analysis revealed that 38% of

190   network event pairs were completely orthogonal to one another (**Fig. 4A**, the fraction of

191   completely orthogonal patterns corresponds to the bar with a cosine similarity of zero).

192   Because in sparse activity patterns, orthogonality can and will arise by chance, we

193   additionally performed a shuffling analysis to ascertain if sparse activity per se can account

194   for the observed occurrence of orthogonal patterns. We found significantly more

195   orthogonality than expected by chance (38±4% vs. 29±4% in real vs. shuffled data,

196   respectively, see **Fig. 4A** inset, n=9 mice, 3 sessions, comparison to shuffled data: Wilcoxon

197   test, p=0.0039). This is consistent with the capability to represent separate sets of

198   information within network events.

199   Even though orthogonal network events were observed, we also observed a repeated

200   activation of granule cells in multiple network events (see i.e. **Video 2**). To examine if specific

201   sub-ensembles of granule cells are repeatedly recruited in network events, we performed a

202   pairwise Pearson's correlation of the activity of all cell pairs during all network events of a

203   recording session (correlation coefficients depicted in the correlation matrix in **Fig. 4B**). We

204   then re-arranged the cells by hierarchical clustering. Clusters were combined using a

205   standardized Euclidean distance metric and a weighted average linkage method (**Fig. 4C**,

206   more examples in **Fig. 4-figure supplement 1A-H**).

207   This visualization reveals the existence of subgroups of cells that are strongly correlated

208   within network event-related activity (**Fig. 4C**), as previously demonstrated for activity during

209   immobility in the CA1 region (**Malvache et al., 2016**). To more rigorously define what we

210   considered a cluster showing correlated activity, we used a comparison to a null distribution

211   generated by shuffling. Such approaches have been shown to outperform other approaches

212   to define how many clusters are present in complex data (**Tibshirani et al., 2001**). We

213   combined clusters until the mean of the cluster internal r-value reached a significance

214   threshold, which was defined by creating a null-distribution of r-values from shuffled datasets

215   (indicated with a vertical line in **Fig. 4C**). Thus, clusters were defined quantitatively as

216   exhibiting a mean correlation coefficient within the cluster above chance level. Using this

217   definition, the average cluster size was 6.7±0.4 cells per cluster (n=9 mice, 3 sessions). The

218   repetitive nature of GC cluster activation during an entire session becomes clearly apparent

219 when viewing cell activity during network events over an entire session, sorted by their

220 participation in clusters (example shown in **Fig. 4D**).

221

222 ***Participation of place- and speed-coding granule cells in network events***

223 To ask if network events carry specific spatial or locomotion-related information, we identified

224 GCs with position-related or speed-related activity. We first identified the group of GCs that

225 exhibited significant place coding (2.83% of n=1415 active cells imaged in 9 mice, **Fig. 5A** for

226 representative polar plots of three GCs). The place fields of place-coding GCs were

227 distributed over the linear track (**Fig. 5B**). If the fraction of place-coding cells was calculated

228 as a fraction of only those GCs active during running, the fraction of significantly place-

229 coding GCs was 6.09%. Secondly, we identified a fraction of GCs (0.85% of GCs, 1.83% of

230 running-active GCs, n=9 mice) displaying a significant correlation of activity with running

231 speed (**Fig. 5C-E**). This is in contrast to a previous study (***Danielson et al., 2016***), but

232 consistent with data obtained in freely moving mice (***Stefanini et al., 2020***). We also

233 examined recordings from sessions using two other linear track environments with sensory

234 cues placed on the textured belt. First, additional sensory cues were placed randomly on the

235 belt (cue-enriched condition). Under these conditions, the fraction of place cells observed

236 within the GC population increased (4.56% of GCs, 10.74% of running-active GCs), as did

237 the proportion of speed cells (1.54% of GCs, 3.64% of running-active GCs, n=1425 active

238 GCs imaged in 9 mice, **Fig. 5F**).

239 Second, we tested if there is a further increase in place cells with a commonly used linear

240 track divided into zones, each with very different spatial cues (see methods, **Fig. 1-figure**

241 **supplement 1G-J**). This was not the case. In these mice (n=3), we recorded 690 GCs, of

242 which 2.61% were place cells. As a fraction of those GCs active during running, we found

243 8.11% place cells. In all conditions, few cells exhibited both speed coding and place coding.

244 We then examined if place or speed cells are incorporated in network events, and if this

245 participation is altered when the environment changes. Specifically, we examined the

246 difference between the baseline linear track without additional cues and the cue-enriched

247 condition. We chose the cue-enriched condition for further experiments and analyses

248 because it provided sufficient spatial cues for a strong spatial representation, without

249 introducing edges between differently cued zones on the linear track. We found that in the

250 cue-enriched condition, dentate gyrus network events were again observed predominantly

251 during immobility (**Fig. 5-figure supplement 1D,** statistics of GC activity in **Fig. 5-figure**

252 **supplement 1A-C**) and were similarly related to MPP activity (**Fig. 5-figure supplement 1F-**

253 **J**). Increasing the cue density did not significantly alter the network event frequency (**Fig. 5-**

254 **figure supplement 1D**, 2.39±0.73 vs 3.63±0.90 events/minute, respectively, n=9 mice, 2-

255 way ANOVA, baseline vs. cue enriched: $F_{(1,30)}$=0.71, p=0.41, run vs. rest: $F_{(1,3)}$=59.13,

256 p=0.001). However, the average size of individual network events, measured as the number

257 of participating GCs, was significantly larger in the cue-enriched condition compared to the

258 baseline condition (**Fig. 6A**, Kruskal-Wallis test, n=9 mice, 1313 and 1493 network events in

259 baseline and cue-enriched condition, respectively, p=4x10^{-41}), with individual GCs

260 contributing more frequently to network events in the cue-rich condition (**Fig. 6B**, Kruskal-

261 Wallis test, p=1x10^{-40}). Fewer orthogonal networks were observed in the cue-rich condition,

262 but this was not significantly different to the baseline condition (not shown, Kruskal-Wallis

263 test, n.s. p=0.49).

264 We then examined if the participation of place and speed cells in network events is altered in

265 the cue-enriched compared to the baseline condition. As stated above, place cells are more

266 commonly observed in cue-enriched sessions. However, when we calculated the fraction of

267 all place cells that participated in network events, taking into account the total number of

268 place cells under each condition, the probability of being incorporated in network events was

269 increased significantly (**Fig. 6C**, 55.42 vs. 88.46% of place cells in baseline vs. cue-rich

270 conditions). This was not the case for speed cells (42.86 vs. 52.63% of speed cells in

271 baseline vs. cue-rich conditions, n=9 mice, chi^2 test regarding changes in the incorporation of

272 place and speed cells in network events p=3x10^{-4}, post-test: place cells baseline vs. cue-

273 enriched p=1x10^{-5}, indicated with asterisk in **Fig. 6C**, speed cells baseline vs. cue-enriched

274   p=0.22). Thus, irrespective of the increase in the number of place cells in cue-enriched

275   conditions, the probability of individual place cell to be integrated a network event is

276   significantly higher. Accordingly, the proportion of synchronous events that incorporated at

277   least one place cell increased (from 23±9 to 33±11%). The properties of correlated cell

278   clusters within network events did not change (cluster size comparison, Kruskal-Wallis test, n

279   = 9 mice, p = 0.13), but significantly more of the clusters contained place cells in the cue-rich

280   condition (**Fig. 6D**, n = 9 mice, Chi^2 test p = 0.004, post-test comparison baseline vs. cue-

281   enriched for place cells p = 0.004, speed cells p = 0.6), with the number of place or speed

282   cells per cluster remaining unchanged (**Fig. 6E**).

283   Thus, network events are responsive to changes in the environment, and incorporate more

284   place-coding neurons into correlated activity patterns.

285

286   ***Similarity of population activity patterns during locomotion to network events during***

287   ***immobility***

288   The incorporation of place and speed cells in network events, as well as the fact that

289   changing features of the environment modifies network event size and place cell participation

290   is consistent with the idea that animals, when immobile, represent information about the

291   environment in synchronous, sparse network events. Testing this idea is difficult, however,

292   given that place cells are less prevalent in the dentate gyrus compared to other hippocampal

293   sub-regions. It has been suggested that the dentate gyrus utilizes a population code

294   (***Stefanini et al., 2020***), meaning that even though only few cells can be rigorously classified

295   as place cells, many more neurons may encode relevant but partial information about the

296   environment. We used three different approaches to assess similarity between running and

297   resting activity in the dentate gyrus at the population level. All of these approaches are based

298   on analyzing population coding separately during either locomotion or network events using

299   Principal Component Analysis (PCA).

300 To obtain a first visual impression of population behavior during linear track locomotion, we

301 plotted the neuronal state captured by the first three components (**Fig. 7B, C**, **Fig. 7-figure**

302 **supplement 1B-C** for Independent Component Analysis, ICA, and Gaussian Process Factor

303 Analysis, GPFA). We observed smooth, large trajectories with high variability reflecting

304 movement along the linear track for some laps on the linear treadmill. Such large trajectories

305 did not occur for every lap. We examined this unexpected phenomenon in both the baseline

306 and cue-enriched condition (**Fig. 7-figure supplement 2A, B**), as well as in the belt with

307 three distinct zones (**Fig. 7-figure supplement 2C**). In all three types of linear tracks, we

308 found a similar, high lap to lap variability in the dentate gyrus population. To see how this

309 behavior compares to the CA1 region, which is known to exhibit a reliable place code over

310 these timeframes (**Rubin et al., 2019**), we examined CA1 neurons in mice running on a

311 linear track with zones (n=2 mice, 543 CA1 neurons, identical conditions to the zoned belt

312 used for GC measurements). Here, PCA trajectories showed a much lower lap-to-lap

313 variability and related smoothly to the position on the linear track (**Fig. 7C, Fig. 7-figure**

314 **supplement 1D-F,** for PCA, GPFA, ICA, see Methods, and **Fig. 7-figure supplement 2D**).

315 We have quantified this phenomenon across all laps in a session by plotting the weights of

316 the first 5 components of the PCA across laps. In this depiction for CA1, as well as the three

317 different versions of the linear track used for DG experiments, it is very clear that strong

318 periodicity for each round is observed in CA1, but much less so in all DG experiments (**Fig.**

319 **7-figure supplement 2E-H**).

320 To quantify the strength of lap-periodicity (i.e. population spatial stability throughout a

321 session) across animals, we performed an autocorrelation for all experiments in the four

322 conditions. The autocorrelation showed large magnitude peaks at integer multiples of 1 lap

323 for CA1, which were significantly larger than corresponding peaks for all linear track

324 conditions in DG (examples shown in **Fig. 7-figure supplement 2I-L**, averages across all

325 mice and sessions **Fig. 7-figure supplement 2M-P,** statistics **Fig. 7D** ANOVA, $F_{(1,3)}$=88.32,

326 p=2x10^{-30}, * Bonferroni post-test p<0.05, *** Bonferroni post-test p<0.001).

327   Thus, the population behavior in DG was similar across three different types of linear track,

328   with an episodic nature that was clearly distinct from the repetitive, stable population

329   dynamics in CA1.

330   After applying PCA to locomotor states in the dentate gyrus, we then also performed a PCA

331   analysis of population activity during network events, including the number of components

332   explaining 50% of the variance (see Methods, **Fig. 7E** for schematic description). In order to

333   compare the two sets of PCAs representing population activity during running states and

334   network events, respectively, we first used a vector-based similarity measure. Briefly, we

335   projected the traces recorded during locomotion into the PCA-space representing activity

336   during network events, and tested how much of their variance was captured by them. In this

337   analysis, similarity between both population measures would result in a large fraction of

338   explained variance (**Fig. 7F**).

339   To obtain the expected null distribution, we performed different types of shuffling analysis on

340   the resting activity (see Methods). In the first shuffling procedure, we shifted the entire ΔF/F

341   time series of each cell by random time values (compare **Fig. 7H, I**). Thus, all non-random

342   activity timing between cells is destroyed and cells will no longer be synchronously active at

343   NE timepoints. At the same time, individual cell event statistics will be maintained (i.e. inter-

344   event-intervals). This method thus preserves intra-neuronal correlations and event

345   frequencies, but destroys inter-neuronal correlations. The distributions from shuffled data

346   were clearly distinct from the real data (red vertical line in **Fig. 7G, Fig. 7-figure supplement**

347   **3** for comparisons to shuffled data for all sessions). The comparisons to shuffled data were

348   significant in all sessions, both for baseline and cue-enriched conditions (**Fig. 7I,** leftmost

349   bars in lower panel), indicating that synchronous activity is important for the similarity

350   between locomotor related activity and network events.

351   We used two further similarity measures that have been used so far to quantify similarity

352   between PCA bases. Firstly, we used a similarity factor S_{PCA} as described by Krzanowski

353   (***Krzanowski, 1979***) and the EROS similarity factor (***Yang, K., Shahabi, C., 2004***) (see

354   Methods for description), testing them against shuffled datasets in the same manner (**Fig. 7I,**

355   **Fig. 7-figure supplement 3** for comparisons to shuffled data for all sessions). With these

356   measures, significant comparisons to shuffled data were obtained with all (cosine similarity)

357   or a majority (EROS) of sessions (**Fig. 7I**, n=8 animals, last baseline session and cue-

358   enriched session).

359   This shuffling approach (**Fig. 7I**), however, does not specifically test if the composition of

360   NEs matters for the similarity between running and NE activity. We therefore implemented

361   two additional shuffling approaches that probe the importance of NE structure by shuffling

362   activity within NEs. In our second shuffling approach, we tested if the composition of

363   individual NEs is important. To this end, for each individual NE, we randomly reassigned a

364   given cells activity to a different cell. Thus, NEs have exactly the same number of active

365   cell's, but the identity of cells active within them has been randomly changed, and the

366   number of NEs that individual cells participate in will be altered (see schematic in **Fig. 7J,**

367   compare to panel H). This shuffling approach also revealed that NEs are significantly more

368   similar to locomotor related activity with all three similarity measures (**Fig. 7J**, lower panel).

369   If morpho-functional properties in the network simply confine activity during run and rest to

370   very specific populations of cells that are always very active, then a different type of shuffling

371   would be required to test if this phenomenon drives similarity. We therefore added a third

372   shuffling method, in which for each cell, we randomly reassigned its NE activity to other NEs

373   (see schematic in **Fig. 7K,** cf. panel H). Thus, how many NEs a given cell participates in is

374   maintained. At the same time, NE interactions between specific sets of cells will be altered,

375   although highly active cells that participate in multiple NEs will still be more likely to be co-

376   active in shuffled NEs. If the similarity were driven by such a population of always-active

377   cells, then this shuffling would not disrupt the similarity between running and shuffled NE

378   activity. However, also here NE activity was more similar to running activity than shuffled

379   data for all three similarity measures (**Fig. 7K**, lower panel).

380   Collectively, these data show that at the population level, NEs and locomotion related activity

381   are more similar than expected by chance. Moreover, the two shuffling procedures described

382   in Fig. 7J and K suggest that the cellular composition of network events matters for this

383   similarity.

384   In CA1, replay of place cell sequences has been described extensively. To ascertain the

385   robustness of our similarity measures, we have applied them to CA1 population activity, in

386   exactly the same manner as described in **Fig. 7F**. This approach showed significant

387   similarities between synchronous CA1 events during immobility, and activity during

388   locomotion in 100 % of the tested sessions for all three PCA-based measures (5 mice, 3

389   sessions per mouse, data not shown).

390

391   ***Inhibition of dentate granule cell activity during immobility disrupts pattern separation***

392   Collectively, these data suggest that during immobility, GCs engage in structured ensemble

393   activity that reiterates activity during running at the population level. This suggests that such

394   activity might be important for the formation of hippocampal dependent spatial memories.

395   The ideal test of this hypothesis would be to detect network events in freely moving animals

396   using two-photon imaging during a memory task, and then applying closed-loop inhibition of

397   granule cells during this task. The sparseness of granule cell activity, and the difficulties

398   inherent in triggering closed-loop inhibition to very sparse activity patterns renders this

399   experiment extraordinarily difficult. We therefore opted to use closed-loop inhibition of

400   granule cells during all periods of immobility during a dentate gyrus-dependent memory task

401   to test if dentate gyrus activity during immobility is necessary for memory formation. We used

402   an established memory task for spatial object pattern separation (OPS, (***van Goethem et al.,***

403   ***2018***)), in which DG-dependent spatial discrimination is assessed based on the differential

404   exploration of two objects. Briefly, animals are first exposed to two objects in defined

405   locations during an acquisition trial (5 min) and are then re-exposed following an intermediate

406 period, with one of the objects slightly displaced. Increased exploration of the displaced

407 object indicates that the animal has encoded the initial location and is able to discriminate the

408 displaced object. In preliminary experiments, we tested 4 degrees of object displacement

409 along a vertical axis (3-12 cm, **Fig. 8-figure supplement 1C**). We then determined the

410 extent to which spatial object pattern separation was dependent on the activity of the dentate

411 gyrus. We expressed either halorhodopsin (eNpHR, (***Gradinaru et al., 2008***)), or eYFP

412 (control group) selectively in dentate GCs using Prox1-Cre mice, which efficiently inhibited

413 GC firing (**Fig. 8-figure supplement 1F-J**), and bilaterally illuminated the dentate gyrus with

414 two implanted light fibers during the OPS task (**Fig. 8-figure supplement 1A, B, D**). We

415 found that GC activity was most important for an intermediate degree of displacement (9 cm),

416 while maximal displacement was no longer dependent on GC activity (**Fig. 8-figure**

417 **supplement 1E**).

418 We then used this intermediate degree of displacement for the further experiments. We first

419 inhibited GCs during locomotion only in the learning trial. As expected, inhibiting GC activity

420 when mice actively explored the environment to be memorized led to a loss of preference for

421 the displaced object in the subsequent recall trials (**Fig. 8-figure supplement 2**).

422 We then used the intermediate degree of displacement in the OPS task to see if dentate

423 gyrus activity during quiet immobility was equally required to establish a memory of object

424 location. We bilaterally inhibited GCs during periods of quiet immobility (running speed < 4

425 cm/s) only during the learning trial (**Fig. 8A, B**). This manipulation led to a complete loss of

426 preference for the displaced object in the subsequent recall trials (**Fig. 8H** for representative

427 sessions, analysis of discrimination index in **I**, unpaired T-test with Welch's correction, n = 6

428 and 9 for eNpHR and eYFP respectively, $t_{(12)}$ = 5.37, p = 0.0002) whereas control mice

429 displayed a clear preference for the displaced object (**Fig. 8G** for representative session).

430 Similar results were obtained in a separate cohort of animals, where the difference in

431 performance was measured in a paired experimental design (**Fig. 8-figure supplement 2,**

432 repeated measures ANOVA, $F_{(1,14)}$ = 54.58, p = 0.0003. Bonferroni post-tests: no illumination

433   vs. resting illumination, p = 0.0026; no illumination vs. illumination during locomotion, p =

434   0.0076; n = 5.).

435   Carrying out the OPS task in mice expressing eNpHR without illumination yielded

436   discrimination indices indistinguishable from the control group (not shown). For the three

437   groups, ANOVA revealed a significant effect ($F_{(2,18)}$ = 8.52, p = 0.003), with Bonferroni post-

438   tests showing that inhibition of GCs significantly reduces performance vs. the two control

439   groups (eYFP vs. eNpHR illuminated p = 0.006, eYFP vs. eNpHR without illumination p >

440   0.99, eNpHR with illumination vs. without illumination p = 0.006).

441   Because mice are also immobile while examining the objects, we also performed a set of

442   experiments in which light-stimulation was only carried out during immobility, but excluding a

443   4 cm zone surrounding the objects (**Fig. 8-figure supplement 3A**). This experiment yielded

444   similar results, with a virtually complete loss of object discrimination during the recall trial

445   (**Fig. 8-figure supplement 3B-I**). This effect was specific to acquisition. GC inhibition only

446   during immobility in the recall trial (**Fig. 8-figure supplement 3J**) elicited no significant

447   reduction in the recognition of the displaced object (**Fig. 8-figure supplement 3K-L**, n = 11

448   and 6 for eYFP and eNpHR groups, respectively, t-test with Welch correction n.s.). These

449   data suggest that activity of GCs during rest is important to form memories that require

450   discrimination of similar experiences.

451

Discussion

The dentate gyrus has been implicated in pattern separation of sensory-driven activity patterns during experience but is also active during immobility and sleep. The properties of these latter forms of activity at the cellular level and the role they play in behavior are largely unknown. The application of multiphoton in-vivo Ca^{2+} imaging allowed us to observe large-scale dentate gyrus dynamics at the cellular level and to detect a novel form of sparse, synchronized GC activity that occurs during immobility, termed dentate network events. These events were specifically modified by the environment, and showed higher similarity than expected by chance to population activity occurring during locomotion, indicating a sparse reiteration of locomotion-associated activity patterns.

Interestingly, network events were associated with pupil constriction and on average smaller pupil diameters when compared to entire periods of immobility, indicating that they may be associated with fluctuations in brain state during immobility, as described for visual cortex (*Reimer et al., 2014*). This finding is in agreement with the correlation of pupil constriction with the rate of hippocampal ripple oscillations during resting states (*McGinley et al., 2015*). The association of pupil constriction with synchronized activity is also very consistent with data from visual cortex, where brief episodes of pupil constriction during immobility are associated with synchronization and increased low-frequency oscillations (*Reimer et al., 2014*).

For neuronal activity during resting states to support learning or memory consolidation concerning a particular environment, one general requirement would be that there is reactivation of activity patterns induced by exploration of the relevant environment (*Davidson et al., 2009; Diba and Buzsáki, 2007; Dupret et al., 2010; Foster and Wilson, 2006; Girardeau et al., 2009; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994*). We have used three different similarity measures to show that this is the case in the dentate gyrus at the population level.

478   In addition to this general requirement, two specific features of resting activity are consistent

479   with the formation of precise memories that conserve the pattern separation capabilities of

480   the dentate gyrus. Firstly, the activity patterns, although sparse, should be capable of

481   generating orthogonal ensembles representing different features of the environment.

482   Secondly, the activity should repetitively recruit specific subsets of dentate GCs capable of

483   instructing the formation of CA3 attractors via Hebbian plasticity mechanisms. Indeed, we

484   found that activity during network events is sparse, recruiting just ~5-7% of the active GCs.

485   Given that only ~50% of GCs are active in head-fixed animals (*Danielson et al., 2016; Pilz*

486   *et al., 2016*), recruitment of dentate GCs during network events is much sparser than in other

487   forms of activity occurring during immobility or sleep. For instance, the fraction of CA1

488   neurons recruited during sharp wave ripple mediated replay of behaviorally relevant

489   sequences (*Davidson et al., 2009; Diba and Buzsáki, 2007; Dupret et al., 2010; Foster*

490   *and Wilson, 2006; Girardeau et al., 2009; Malvache et al., 2016; Skaggs and*

491   *McNaughton, 1996; Wilson and McNaughton, 1994*) is much higher than the recruitment

492   of GCs in network events. One consequence of the sparseness of network events is that

493   they are predicted to recruit highly constrained CA3 ensembles, both because of the sparse

494   excitatory connectivity of mossy fibers in CA3, and because of the properties of the powerful

495   inhibitory circuits in the CA3 region (*Acsady et al., 1998; Neubrandt et al., 2017;*

496   *Neubrandt et al., 2018*). This has been suggested to be important in the capability to store

497   information in CA3, while conserving the pattern separation benefits of the dentate gyrus

498   (*O'Reilly and McClelland, 1994*; *GoodSmith et al., 2019*).

499   We found that network events are structured, with subgroups of dentate GCs forming

500   correlated sub-ensembles that are repeatedly recruited (see **Fig. 4**). This finding is consistent

501   with the idea that dentate GC activity recruits plasticity mechanisms to form sparse attractor-

502   like representations in CA3 (*O'Reilly and McClelland, 1994*). A similar structure was also

503   observed for awake hippocampal reactivations in the hippocampal CA1 region, and may

504   serve similar plasticity mechanisms in downstream targets (*Malvache et al., 2016*). Thus,

505   dentate activity during immobility may be important to instruct downstream ensembles to

506   exhibit specific memory-related sequences. That the integrity of the dentate gyrus is

507   important in determining behaviorally relevant firing patterns in CA3 has also been

508   demonstrated by lesion experiments showing that activity of dentate GCs is necessary for

509   increased SWRs and prospective goal-directed firing of CA3 neurons (*Sasaki et al., 2018*).

510   One interesting feature of population activity in the dentate gyrus during locomotion became

511   apparent from our PCA analyses. We noted that population behavior in in the dentate gyrus

512   was very dissimilar during different laps, even though animals traversed the identical belt

513   sections. A qualitatively similar finding has been obtained in a recent publication, showing

514   that even after extensive training in the very same environment on successive days, different

515   sets of dentate granule cells were activated every day (Lamothe-Molina et al., 2020, bioRxiv,

516   Doi: https://doi.org/10.1101/2020.08.29.273391). The population dynamics that we observed

517   in DG were very different from CA1, which expectedly shows a very robust association with

518   space during repetitive laps. One interpretation of this finding is that the dentate gyrus

519   amplifies small difference between laps, and is able to represent successive laps in a

520   different way; this itself being a potential manifestation of the pattern separation capabilities

521   of this structure. We note that while this is conceptually compelling, these experiments do not

522   prove that this is the case.

523   If network events are important in memory processes, then inhibiting dentate gyrus activity

524   during the entire period the animal is resting should impede the formation of dentate gyrus-

525   dependent memories. It would be desirable to inhibit only network events to test this idea,

526   but, due to the sparseness and high synchrony of these events, a closed loop approach to

527   achieve this is not feasible. Thus, the behavioral experiments have to be interpreted with

528   caution, as all resting activity is being inhibited, regardless of whether they constitute network

529   events or not. Inhibiting only granule cells during immobility to test the effect this has on a

530   dentate-dependent memory tasks should therefore be considered a hypothesis testing

531   experiment, but does not provide definite proof of the relevance of network events. The OPS

532   task requires storage of the initial object location with a high degree of precision that can be

533   utilized later on for discriminating the translocated object. We show that optogenetically

534   inhibiting GCs, even if this was done only during immobility remote from the explored objects,

535   disrupted the capability to acquire such memories. This supports the idea that dentate

536   network events may rapidly and flexibly introduce information about the environment into the

537   hippocampal network, in the time intervals interspersed between episodes of exploration.

538   Consistent with this view of 'real-time updating', we observed increased incorporation of

539   spatial information via place cell integration into network events upon the first experience of a

540   cue-rich environment. Inhibition of the dentate gyrus during the recall phase did not

541   significantly inhibit task performance, consistent with the idea that recall of precise location

542   information is achieved via activation of memory-related attractors in downstream CA3

543   and/or CA1 regions. Indeed, behavioral analyses combined with selective lesions of dentate

544   gyrus and CA3 have also suggested an interaction between CA3 and DG in supporting

545   encoding but not retrieval processes in a spatial learning task (*Jerman et al., 2006*).

546   Moreover, disrupting dentate spikes via electrical stimulation has been shown to disrupt

547   acquisition of hippocampal-dependent trace eyeblink conditioning (Nokia et al. 2017).

548   We also performed inhibition of dentate granule cells only during locomotion in the OPS task.

549   This inhibition also prevented the formation of spatial memories. This may simply reflect that

550   mice are not able to store the initial object location if exploratory activity is disrupted. On the

551   other hand, it is possible that the dentate gyrus is encoding the experience of the initial

552   session in the OPS task as a single sequence spanning rest and running. In this case,

553   inhibition of population at any point during the entire experience could disrupt memory

554   formation, and would not reflect a specific role of DG activity during rest.

555   Two caveats have to be considered in these behavioral experiments. First, while it is very

556   likely that network events of a similar kind occur in freely moving mice during the OPS task,

557   we have not explicitly shown this. A second caveat when using optogenetics for behavioral

558   experiments are the known aberrant effects of some opsins. Because the design of our

559   experiment involves closed-loop stimulation and requires inhibition with relatively fast

560   kinetics, we had to select a fast inhibitory opsin for these experiments, with NpHR and ArchT

561   as the most established opsins in this category. While rebound excitation effects have been

562   described for NpHR, and not for the most prominent alternative ArchT following illumination

563   (*Raimondo et al., 2012*), ArchT has pH-dependent effects in synaptic terminals, which lead

564   to very powerful, action potential independent excitation of Arch-expressing terminals during

565   illumination (*Mahn et al., 2016*). Because this could lead to aberrant excitation of hilar

566   neurons during illumination, ArchT was not a viable alternative in our experiments. We

567   therefore used NpHR as the most appropriate strategy, and utilized pulsed stimulation to

568   minimize unwanted side effects. However, we acknowledge rebound excitation effects may

569   be a potential confounding factor.

570   How do network events correspond to the different types of activity that have been described

571   in the dentate gyrus during immobility or sleep, namely dentate spikes and sharp waves

572   (Bragin et al. 1995; Penttonen et al. 1997)? During dentate spikes, granule cells are

573   discharged anterogradely by entorhinal input, while they are activated retrogradely by the

574   CA3-mossy cell feedback pathway during sharp waves (Bragin et al. 1995; Penttonen et al.

575   1997). We show that dentate network events are associated with MPP activation, but would

576   be cautious in designating these events dentate spikes in the absence of parallel in-vivo

577   electrophysiology, especially given recent descriptions of other subclasses of DG sharp

578   waves (*Meier et al., 2020*).

579   Activity in the dentate gyrus may also be relevant for processes on more extended time

580   scales, such as maintenance of established memories. For instance, pharmacogenetic

581   inhibition of GCs induces loss of a hippocampal memory in trace eyeblink conditioning

582   (*Madroñal et al., 2016*). Such longer time-scale coding may be mediated by processes that

583   extend beyond local hippocampal computations. Along these lines, dentate gyrus activity

584   during dentate spikes is associated with wide-spread increases in single-cell activity, gamma

585   oscillations and intraregional gamma coherence (*Headley et al., 2017*). It is thus possible

586   that the precise activation patterns we observe in dentate gyrus here are part of a more

587   distributed, organized activity occurring in immobile animals.

588   In summary, we described a novel form of synchronized, sparse network activity during

589   immobility in DG that is potentially relevant to the formation of dentate gyrus-dependent

590   spatial memories.

591

Methods

Animals and procedures: All animal experiments were conducted in accordance with European (2010/63/EU) and federal law (TierSchG, TierSchVersV) on animal care and use and approved by the county of North-Rhine Westphalia (LANUV AZ 84-02.04.2015.A524, AZ 81-02.04.2019.A216). We used 9-12 weeks old Thy1-GCaMP6 mouse line (GP4.12Dkim/J) mice for imaging experiments, which express GCaMP6s in most hippocampal neurons (**Dana et al., 2014**). For optogenetic inhibition of the dentate gyrus granule cells, we used heterozygous Prox1-Cre animals (Tg(Prox1-cre)SJ39Gsat/Mmucd) obtained from MMRRC UC Davis as cryopreserved sperm and rederived in the local facility.

Virus injections and head fixation: Thy1-GCaMP6 mice were anesthetized with a combination of fentanyl/midazolam/medetomidine (0.05 / 5.0 / 0.5 mg/kg body weight i.p.) and head-fixed in a stereotactic frame. 30 min prior to induction of anesthesia, the animals were given a subcutaneous injection of ketoprofen (5 mg/kg body weight). Eyes were covered with eye-ointment (Bepanthen, Bayer) to prevent drying and body temperature was maintained at 37°C using a regulated heating plate (TCAT-2LV, Physitemp) and a rectal thermal probe. After removal of the head hair and superficial disinfection, the scalp was removed about 1 cm² around the middle of the skull. The surface was locally anesthetized with a drop of 10% lidocaine and after 3-5 min residual soft tissue was removed from the skull bones with a scraper and 3% H_2O_2/NaCl solution. After complete drying, the cranial sutures were clearly visible and served as orientation for the determination of the drilling and injection sites. For virus injection, a hole was carefully drilled through the skull with a dental drill, avoiding excessive heating and injury to the meninges. Any minor bleeding was stopped with a sterile pad. The target site was located as the joint of Parietal, Interparietal and Occipital skull plates. Subsequently, the tip of a precision syringe (cannula size 34 G) was navigated stereotactically through the burrhole (30° towards vertical sagittal plane, 1.5 mm depth from skull surface) to target the following coordinates: Anterioposterior [AP] measured from bregma ~4.6 mm; lateral [L] specified from midline ~3 mm; dorsoventral [DV] from surface of

619   the skull ~4.2 mm. Virus particles (rAAV2/1-CaMKIIa-NES-jRGECO1a (*Dana et al., 2016*))

620   were slowly injected (total volume 250 nl, 50 nl / min) in the medial entorhinal cortex. Correct

621   injection site in the medial entorhinal cortex was verified in all cases by confined expression

622   of jRGECO1a in the middle molecular layer of the dentate gyrus (**Fig. 1A**). To prevent reflux

623   of the injected fluid, the cannula was retained for 5 minutes at the injection site. Optibond

624   (Optibond^{TM} 3FL; two component, 48% filled dental adhesive, bottle kit; Kerr; FL, USA) was

625   then applied thinly to the skull to aid adhesion of dental cement. Subsequently, a flat custom-

626   made head post ring was applied with the aid of dental cement (Tetric Evoflow), the borehole

627   was closed and the surrounding skin adapted with tissue glue, also closing the borehole and

628   adapting the surrounding skin with tissue glue. At the end of the surgery, anesthesia was

629   terminated by i.p. injection of antagonists (naloxone/flumazenil/atipamezole, 1.2 / 0.5 / 2.5

630   mg / kg body weight). Postoperative analgesia was carried out over 3 days with 1 × daily

631   ketoprofen (5 mg/kg body weight, s.c.).

632   *Window implantation procedure:* Cranial window surgery was performed to allow imaging

633   from the hippocampal dentate gyrus. 30 minutes before induction of anesthesia, the

634   analgesis buprenorphine was administered for analgesia (0.05 mg/kg body weight) and

635   dexamethasone (0.1 mg/20 g body weight) was given to inhibit inflammation. Mice were

636   anesthetized with 3-4% isoflurane in an oxygen/air mixture (25/75%) and then placed in a

637   stereotactic frame. Eyes were covered with eye-ointment (Bepanthen, Bayer) to prevent

638   drying and body temperature was maintained at 37°C using a regulated heating plate (TCAT-

639   2LV, Physitemp) and a rectal thermal probe. The further anesthesia was carried out via a

640   mask with a reduced isoflurane dose of 1-2% at a gas flow of about 0.5 l / minute. A circular

641   craniotomy (Ø 3 mm) was opened above the right hemisphere hippocampus using a dental

642   drill. Cortical and CA1 tissue was aspirated using a blunted 27-gauge needle until the blood

643   vessels above the dentate gyrus became visible. A custom-made cone-shaped silicon inset

644   (Upper diameter 3 mm, lower diameter 1.5 mm, length 2.3 mm, RTV 615, Movimentive)

645   attached to by a cover glass (Ø 5 mm, thickness 0.17 mm) was inserted and fixed with dental

646   cement. This special window design allowed easy implantation and maintenance and

647 minimized the amount of aspirated tissue. Further the geometry was optimal for conserving

648 the numerical aperture of the objective (see **Fig. 1-figure supplement 1D-F**). Postoperative

649 care included analgesia by administering buprenorphine twice daily (0.05 mg/kg body weight)

650 and ketoprofen once daily (5 mg/kg body weight s.c.) on the three consecutive days after

651 surgery. Animals were carefully monitored twice daily on the following 3 days, and recovered

652 from surgery within 24-48 hours, showing normal activity and no signs of pain. The

653 preparation of CA1 imaging windows followed mainly the same protocol. Here only the cortex

654 was aspirated until the alveus fibers above CA1 became visible. The silicon inset was shorter

655 version (length 1.5 mm) of the one used for DG experiments.

656 *Two-photon calcium imaging:* We used a commercially available two photon microscope (A1

657 MP, Nikon) equipped with a 25x long-working-distance, water-immersion objective (N.A.=1,

658 WD=4 mm, XLPLN25XSVMP2, Olympus) controlled by NIS-Elements software (Nikon).

659 GCaMP6s was excited at 940 nm using a Ti:Sapphire laser system (~60 fs laser pulse width;

660 Chameleon Vision-S, Coherent) and a fiber laser system at 1070 nm (55 fs laser pulse width,

661 Fidelity-2, Coherent) to excite jRGECO1a (see **Fig. 1-figure supplement 1B**). Emitted

662 photons were collected using gated GaAsP photomultipliers (H11706-40, Hamamatsu).

663 Movies were recorded using a resonant scanning system at a frame rate of 15 Hz and

664 duration of 20 minutes per movie.

665 *Habituation and behavior on the linear track:* Experiments were performed in head fixed

666 awake mice running on a linear track. Two weeks before the measurements, mice were

667 habituated to the head fixation. Initially mice were placed on the treadmill without fixation for

668 5 minutes at a time. Subsequently, mice were head-fixed, but immediately removed if signs

669 of fear or anxiety were observed. These habituation sessions lasted 5 minutes each and

670 were carried out three times per day, flanked by 5 minutes of handling. During the following

671 3-5 days, sessions were extended to 10 minutes each. The duration of sessions used for

672 experiments was always 20 minutes each. After habituation, mice ran well on the treadmill

673 for average distances between 30 and 40 meters per session (see **Fig. 1-figure supplement**

674 **1H**). The treadmill we implemented was a self-constructed linear horizontal treadmill, similar

675 to (*Royer et al., 2012*)). Three different belt configurations were used. In the first, no spatial

676 cues were added to the belt beyond the texture of the belt itself (baseline condition). Three

677 twenty-minute sessions were carried out for each mouse on consecutive days. In the second,

678 cue enriched condition, the belt surface was equipped with tactile cues (see **Fig. 1-figure**

679 **supplement 1G**). In the zone condition the belt was divided in three zones where each zone

680 contained unique tactile ques. Belt position and running speed were measured by modified

681 optical computer-mouse sensors. All stimulation and acquisition processes were controlled

682 by custom-made software written in LabView (**Source Code File 1**).

683 *Pupil diameter measurement and analysis:* On the linear track, the pupil diameter was

684 measured using a high-speed camera (Basler Pilot, Basler, Germany) at a framerate of 100

685 Hz. To estimate pupil diameter, a circular shape was fitted to the pupil using the LabView NI

686 Vision toolbox (National Instruments), providing a real-time readout. Post-hoc, the pupil-

687 diameter trace was normalized to its mean. As in a published study (*Reimer et al., 2014*),

688 frames in which pupil diameters could not be obtained due to blinking or saccades were

689 removed from the trace. The pupil diameter trace was filtered using a Butterworth low-pass

690 filter at a cutoff frequency of 4 Hz. To match the time resolution of the imaging data, the

691 pupil-trace was down-sampled to 15 Hz. Average pupil diameters were calculated for entire

692 episodes of locomotion, entire periods of immobility, and for the single frame coincident with

693 the peak of granule cell activity during network events.

694 *Data analysis, 2-photon imaging:* All analysis on imaging data and treadmill behavior data

695 were conducted in MATLAB using standard toolboxes, open access toolboxes and custom

696 written code. To remove motion artifacts, recorded movies were registered using a Lucas–

697 Kanade model (*Greenberg and Kerr, 2009*). Individual cell locations and fluorescence

698 traces were identified using a constrained nonnegative matrix factorization based algorithm

699 and afterwards Ca^{2+} events were identified with a constrained deconvolution algorithm

700 (*Pnevmatikakis et al., 2016*). All components were manually inspected and only those that

701 showed shape and size of a granular cell and at least one Ca^{2+}-event amplitude three

702 standard deviations above noise level in their extracted fluorescence trace were kept. We

703 binarized individual cell fluorescence traces by converting the onsets of detected Ca^{2+} events

704 to binary activity events. We did not observe any indication of epileptiform activity in Thy1-

705 GCaMP6 (GP4.12Dkim/J) mice, in line with previous work (**Steinmetz et al., 2017**). On

706 average 5-6% of the GC population was active during synchronized network events (Mean:

707 5,71 %, Median: 5.03%, n = 1312 NEs, 9 mice, 3 sessions).

708 *Analysis of MPP input signals:* MPP input bulk signal was analyzed by setting a region of

709 interest in the molecular layer. For that, a threshold of 50 % maximum fluorescence was

710 used within the field of view on the average projection of the movie. The bulk fluorescence

711 signal trace was calculated as the average signal of the defined region of interest in each

712 frame. The baseline for the bulk signal was defined as the low pass filtered signal of the raw

713 trace with a cutoff frequency of 0.01 Hz using a Butterworth filter model. We used a

714 constrained deconvolution algorithm (**Pnevmatikakis et al., 2016**) to create a proxy for the

715 underlying activity of the bulk signal. This allowed for identification of precise onset times and

716 normalized amplitude values of Ca^{2+} events in MPP input data.

717 *Network activity:* To define events of synchronized activity we used binarized data that

718 marked the onset of each significant Ca^{2+}-event. First, we searched for events occurring

719 simultaneously in several GCs within a moving time window of 200ms which corresponds to

720 1±1 frames in our recordings, where multiple events in one cell were counted as one. We

721 then defined the distribution of synchronous events that could arise by chance in each

722 individual session using three different shuffling approaches. Firstly, for every individual cell

723 the event onset times were redistributed to random times, thereby conserving the mean

724 event frequency per cell but destroying temporal correlations. This was done for every cell

725 and repeated a thousand times to create a null-distribution of population behavior. To

726 ascertain how robustly the data were different from the null distribution, we identified the

727 number of synchronous events for different network event size thresholds (See **Fig. 1-figure**

supplement 3A for average values, green line real data, grey line shuffled data, see **Fig. 1-figure supplement 3D-I** for six individual representative examples). The second shuffling approach shifted complete traces of onset times with respect to each other. This maintains within-cell correlations of firing (i.e. episodes of higher frequency firing), but reduces between-cell correlations. This is shown in **Fig. 1-figure supplement 3B** (averages, green line real data, red line shuffled data). In the representative examples in **Fig. 1-figure supplement 3D-I**, this shuffling approach is shown as red lines in the rightmost panels (note that the three shuffling curves are closely superimposed). The third shuffling approach considers potential differences of individual cell activity levels during locomotion and immobility. To account for this, event onset times were randomly re-distributed only within these activity states (**Fig. 1-figure supplement 3C** for averages green line real data, purple line shuffled data, **Fig. 1-figure supplement 3D-I**, this shuffling method shown as purple line in rightmost panels). All three shuffling methods reveal that significantly more synchrony is observed than expected by chance. We then set the minimal threshold for network events in each individual session at that number of synchronously active granule cells where less than 0.1% of events ($p<0.001$) could be explained by chance.

Orthogonality between pairs of network events was assessed using cosine-similarity measures. To this end, population vectors of all network events derived from binarized data were multiplied using the normalized vector-product in a pair-wise manner. To test which fraction of orthogonal pairs could be explained by chance, we generated a null distribution by randomly reassigning the cell participations to different population vectors a 1000 times. We then tested the real fraction of orthogonal pairs against the fraction derived from the shuffled data (**Source Code File 2, 3**).

Spatial tuning: To assess spatial tuning of activity in sparsely coding GCs we used spatial tuning vector analysis (***Danielson et al., 2016***). We restricted analysis to running epochs, where a running epoch was defined as an episode of movement with a minimal duration of 2.5 s above a threshold of 4 cm/s in a forward direction. The threshold of 4cm/s was chosen

755   in line with both literature using head-fixed mice (i.e. (***Danielson et al., 2016***)), as well as a

756   very extensive literature in freely moving animals (i.e. (***Kay et al., 2016***)). Only cells with 4 or

757   more event onsets during running epochs were included in the analysis. We used binarized

758   data to calculate the mean of the vectors pointing in the mouse position at the times of

759   transient onsets, weighted by the time spent in that bin. We addressed statistical significance

760   by creating the null distribution for every spatially tuned cell. This was achieved by randomly

761   shuffling the onset times and recalculating the spatial tuning vector 1000 times. The p value

762   was calculated as the percentage of vector lengths arising from the shuffled distribution that

763   was larger than the actual vector length.

764   *Velocity tuning:* To analyze speed modulated activity of GCs, velocity values were divided in

765   20 evenly sized bins between 0 and the maximum velocity of the animal. We calculated the

766   mean $\Delta F/F$ at all times the animal was running at velocities within each specific velocity bin.

767   Putative speed cells were those granule cells that showed a Pearson's r of at least 0.9. To

768   further exclude the possibility that correlations arise by chance we shifted the individual $\Delta F/F$

769   traces with respect to the behavior randomly in the time domain 1000 times. The cell was

770   considered a significant speed coder if the shuffle-data r-values were below the original one

771   in at least 95 % of the cases.

772   *Hierarchical cluster analysis*: To find ensembles of correlated activity within network activity,

773   we focused only on those granule cell Ca^{2+} events that occurred within network events. We

774   calculated the correlation matrix from binarized data using Pearson's r for all cell

775   combinations. To identify clusters of correlated cells we used agglomerative hierarchical

776   cluster trees. Clusters were combined using a standardized Euclidean distance metric and a

777   weighted average linkage method. Clusters were combined until the mean of the cluster

778   internal r-value reached a significance threshold. To define the significance threshold, we

779   created a null-distribution of r-values from randomized data sets. Data was shuffled by

780   randomly reassigning all network related events to different network events for every cell.

781   This process was repeated 1000 times and the 95 percentiles of the created r-value null-

782   distribution was used as the threshold for the clustering procedure. Only clusters in which the

783   mean intra-cluster r-value exceeded the threshold obtained from the null distribution were

784   considered for further analysis (see **Source Code File 4**).

785   *Principal Component Analysis*: To perform Principal Component Analysis (PCA) and

786   Independent Component Analysis (ICA) we used standard MATLAB procedures and

787   calculated the maximal number of components. Gaussian Process Factor Analysis (GPFA)

788   was conducted using a formerly described procedure and toolbox (***Yu et al., 2009***), that we

789   adapted for Ca^{2+}-imaging data. Principals were calculated using singular value

790   decomposition (SVD) of the data $\mathbf{X}$ of size N by T, where N is the number of cells, T the

791   number of recorded frames and the rows of $\mathbf{X}$ are the z-scored $\Delta F/F$ traces, decomposing

792   the data-matrix as

$$\mathbf{X} = \mathbf{VW}$$

793   where $\mathbf{V}$ is an orthogonal matrix whose columns are the principal components, and $\mathbf{W}$ is a

794   matrix of associated weights. For an analysis of population activity patterns relative to spatial

795   location, we projected the animal position onto PCA trajectories, allowing us to identify loops

796   in component space reflecting complete laps on the belt. Further we projected all individual

797   component amplitudes onto the position of the mouse to detect repetitive patterns. This

798   analysis had comparable results for PCA, as well as ICA and GPFA (**Fig. 7-figure**

799   **supplement 1A-C** for dentate gyrus, **D-F** for CA1).

800   *Analysis of spatial representation using PCA weights:* After performing PCA we quantified

801   spatial representation within our data using the weights $\mathbf{W}_{\mathrm{run}}$. To that end we projected the

802   amplitudes of $\mathbf{W}_{\mathrm{run}}$ of the five first components onto the linear space defined by the 150 cm

803   linear track. Spatial tuning leads to a harmonic behavior of amplitudes with respect to mouse

804   position (see **Fig. 7-figure supplement 2E-H**) and the periodicity was quantified using the

805   normalized autocorrelation of each weight. In the individual examples (see **Fig. 7-figure**

806   **supplement 2I-L**) as well as averaged over animals (**see Fig. 7-figure supplement 2M-P**),

807   peaks in the autocorrelation were observed at integer multiples of rounds, in particular in

808   CA1. To compare the strength of spatial tuning in different DG-experimental conditions as

809   well as CA1 data we quantified and averaged the peak values at a delay of one round (see

810   **Fig. 7D**).

811   *PCA-based comparison of population activity during running and immobility epochs:* For

812   further analysis, we restricted the number of components so that 50 % of variance in each

813   individual data set was explained. To compare running and network related epochs we

814   calculated principal components $\mathbf{V}_{\text{run}}$ and $\mathbf{V}_{\text{net}}$ independently from each other so that

$$\mathbf{X}_{\text{run}} \approx \mathbf{V}_{\text{run}} \; \mathbf{W}_{\text{run}}, \quad \mathbf{X}_{\text{net}} \approx \mathbf{V}_{\text{net}} \; \mathbf{W}_{\text{net}}$$

815   , where $\mathbf{X}_{\text{run}}$ contains all the data from epochs of running and $\mathbf{X}_{\text{net}}$ the data from 2 s

816   windows around all network events. To calculate the similarity between these two bases, the

817   covariance of $\mathbf{X}_{\text{run}}$ was projected into the principal space of the network activity

$$\mathbf{S}_{\text{net} \times \text{run}} \approx \mathbf{V}_{\text{net}}^{T} \; \text{cov}(\mathbf{X}_{\text{run}}) \, \mathbf{V}_{\text{net}}$$

818   , where $\mathbf{S}_{\text{net} \times \text{run}}$ is the matrix of projected co-variances and $\text{trace}(\mathbf{S}_{\text{net} \times \text{run}})$ quantifies the

819   amount of projected co-variance. This number was normalized to the total amount of

820   covariance of locomotion activity in the locomotion principal space $\text{trace}(\mathbf{S}_{\text{run} \times \text{run}})$.

821   To compare our results against chance level, we used three different shuffling approaches to

822   exclude possible mechanisms for similarities that could arise by chance. In the first

823   procedure we used the entire traces recorded during immobility, randomly shift those with

824   respect to each other in the time domain. This approach conserved individual activity levels

825   and intra-neuronal correlations while creating randomized inter-neuronal correlations. We

826   applied the original network times to these time shifted traces to create a random principal

827   direction space $\mathbf{V}_{\text{rand}}$ and calculated the projected co-variances of $\mathbf{X}_{\text{run}}$ into the random-

828   network space as $\text{trace}(\mathbf{S}_{\text{rand} \times \text{run}})$. For shuffling approaches two and three, we shuffled within

829   the matrix of concatenated NEs – in other words, only the 2 seconds around NEs rather than

830   the entire time-series used in shuffle one. For the second approach we tested if the

831 composition of individual NEs is important. To this end, for each individual NE, we randomly

832 reassigned activity of a given cells activity to a different cell. Thus, NEs have exactly the

833 same number of active cells, but the identity of cells active within them has been randomly

834 changed, and the number of NEs that individual cells participate in will be altered. In the third

835 shuffling method we tested whether similarity could be driven by the activity level of individual

836 cells within NEs. Therefore, we randomly reassigned each cells' NE activity to other NEs.

837 This approach maintains the number of NEs a given cell participates and randomizes the

838 interactions between specific sets of cells. All procedures were repeated 1000 times and the

839 p-value was calculated as the percentage of random projections that exceeded the initial

840 value.

841 Additionally, we used two alternative approaches to quantify similarity between the PCA

842 bases. First a similarity factor $\mathbf{S}_{\mathrm{PCA}}$ as described by Krzanowski (**Krzanowski, 1979**).

$$S_{\mathrm{PCA}} = \mathrm{trace}\left(\mathbf{V}_{\mathrm{net}}^{T}\,\mathbf{V}_{\mathrm{run}}\,\mathbf{V}_{\mathrm{run}}^{T}\,\mathbf{V}_{\mathrm{net}}\right) = \sum_{i=1}^{k} \cos^{2}\theta_{i}$$

843 , where θ_{i} is the angle between the i'th principal directions of $\mathbf{V}_{\mathrm{run}}$ and $\mathbf{V}_{\mathrm{net}}$. Further the

844 Eros similarity factor as described in (**Yang, K., Shahabi, C., 2004**) was used:

$$\mathrm{Eros} = \sum_{i=1}^{k} w_{i}\,|\cos\theta_{i}|$$

845 where w_{i} is a weighting factor. All measures delivered comparable results as compared to

846 shuffled data. All different procedures of similarity calculation and shuffling available with this

847 paper (see **Source Code File 5**).

848 *In-vitro patch-clamp experiments:* Acute slices were prepared from mice expressing NpHR-

849 eYFP in GCs. NpHR expression was induced by rAAV mediated gene transfer (rAAV2/1-

850 DOI-eNpHR3-eYFP) into Prox1-Cre mice (see below for virus injection protocols). >2 weeks

851 after virus injection, animals were deeply anesthetized with Isoflurane (Abbott Laboratories,

852 Abbot Park, USA) and decapitated. The head was instantaneously submerged in ice-cold

carbogen saturated artificial cerebrospinal fluid (containing in mM: NaCl, 60; sucrose, 100; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $CaCl_2$, 1; $MgCl_2$, 5; glucose, 20) and the brain removed. Horizontal 350 μm thick sections were cut with a vibratome (VT1200 S, Leica, Wetzlar, Germany). Slices were incubated at 35 °C for 20 to 40 minutes and then stored in normal ACSF (containing in mM: NaCl, 125; KCl, 3.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $CaCl_2$, 2.0; $MgCl_2$, 2.0; glucose, 15) at room temperature. Recordings were performed in a submerged recording chamber at 33- 35 °C under constant superfusion with carbogen saturated ACSF (3 ml/min). Visually identified GCs were recorded in current clamp using a low chloride intracellular solution (containing in mM: K-gluconate, 140; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES-acid), 5; ethylene glycol tetraacetic acid (EGTA), 0.16; $MgCl_2$, 0.5; sodium phosphocreatine, 5) and a Multiclamp 700B and Digidata 1322A (Molecular Devices). Illumination (~560nm, ~1mW) was achieved through the Objective. Action potential frequencies were calculated using the smallest current injection yielding at least 4 action potentials.

Light fiber implantation for behavioral experiments: Mice were injected with buprenorphine (0.05 mg/kg BW) 30 minutes before inducing anesthesia using 3.5 % isoflurane for induction and 1-1.5 % for maintenance. Mice were placed in a stereotactic frame (Kopf Instruments) and the scalp opened with surgical scissors after disinfecting it with iodine solution and applying local anesthetic (10% lidocaine). The skull was thoroughly cleaned using 2% H_2O_2, covered with a thin layer of two-component dental adhesive (Optibond) and the surrounding wound sealed with tissue glue (Vetbond). Small craniotomies were performed above the target sites and 500 nl virus suspension (rAAV2/1-Ef1a-DOI-NLS-eYFP for controls, rAAV2/1-EF1a-DOI-eNpHR3-eYFP for experimental group) was bilaterally injected using a 34 G syringe (Nanofill Syringe, World Precision Instruments, Inc.) at a speed of 50 nl/min. After each injection, the syringe was kept in place for 5 minutes to ensure permeation of the virus into the parenchyma. Coordinates for viral injections into dorsal dentate gyrus were: (from bregma): AP: -2.3; ML: -/+ 1.6 and DV: 2.5 mm). Afterwards, fiber optic cannulae of 200 μm diameter (NA: 0.39, CFMLC12, Thorlabs) were bilaterally implanted at (from

881   bregma): AP: -1.7; ML: -/+ 1.35 and DV: 1.7 and fixed to the skull with a layer of flowable

882   opaque composite (Tetric Evoflow) topped by multiple layers of dental cement (Paladur,

883   Heraeus). Finally, antibiotic cream (Refobacin, Almirall) was applied to the wound and the

884   animals received ketoprofen (5 mg/kg BW) s. c. Analgesia was applied post-surgery by

885   injecting ketoprofen (5 mg/kg BW) s. c. after 24, 48, and 72 hours. All mice recovered for at

886   least 3 weeks after surgery before the start of behavioral experiments.

887   *Behavioral experiments.* To test the effect of optogenetic inhibition of granule cells, 23

888   heterozygous Prox1-Cre animals (5 male, 18 female) between the age of 4 and 9 months

889   were used. Males were single caged and females were group-caged (2-4 individuals per

890   cage) in standard mouse cages under an inverted light-dark cycle with lights on at 8 pm and

891   ad libitum access to food and water. Prior to experiments, animals were randomly assigned

892   to the control or experimental group. All experiments were conducted during the dark phase

893   of the animals. Prior to experiments, mice were handled by the experimenter for at least 5

894   days (at least 5 min/day). On experimental days, animals were transported in their home

895   cages from the holding facility to the experimental room and left to habituate for at least 45

896   minutes. All experiments were performed under dim light conditions of around 20 Lux.

897   Animals were also habituated to the procedure of photostimulation by attaching a dual patch

898   cord for around 10 minutes to the bilaterally implanted light fibers and letting them run in their

899   home cage on multiple days.

900   For optogenetic stimulation, 561 nm laser light (OBIS/LS FP, Coherent, Santa Clara, CA)

901   was delivered bilaterally into the implanted optical fibers using a dual patch cord (NA: 0.37,

902   Doric lenses, Quebec, Canada) and a rotary joint (FRJ, Doric lenses, Quebec, Canada)

903   located above the behavioral test arena. The laser power was set to 5 mW at the tip of the

904   fiber probes.

905   We used a pulsed laser light at 20 Hz with a 50% duty cycle instead of continuous

906   illumination to keep the light-induced heat effect minimal in the brain. Previous studies have

907   shown that continuous light delivery to brain tissues can cause a temperature increase of up

908   to 2°C (*Owen et al., 2019*). It has been reported that changes in temperature can alter

909   neuronal physiology of rodents (*Stujenske et al., 2015*) and birds (*Long and Fee, 2008*).

910   We simulated the light-induced heat effect using the model developed by (*Stujenske et al.,*

911   *2015*). We first examined the continuous light photostimulation of 561 nm light with 5 mW

912   output power. The temperature increase reached a steady-state and was found to be 0.7°C

913   in 60 seconds. For the pulsed light stimulation used in this study, the temperature change did

914   not exceed 0.3°C in 60 seconds (see **Fig. 8-figure supplement 1K-M**). Both pulsed and

915   continuous stimulation resulted in efficient silencing of granule cell activity (**Fig. 8-figure**

916   **supplement 1F-J**). The average illumination times were not significantly different between

917   eNpHR and eYFP control groups in any of the experiments. To apply photostimulation only

918   during immobility, we used a closed loop system employing EthoVision 8.5 that opened an

919   optical shutter (SH05, Thorlabs) in the light path of the laser via a TTL pulse only when the

920   tracking software detected that the body center of the animal was moving less than 5 cm/s

921   and closed the shutter if the speed exceeded 5 cm/s over a period of 0.5 seconds. Behavior

922   was recorded using EthoVision 8.5 software (Noldus, Netherlands) and an IR camera with a

923   frame rate of 24 Hz. Videos were stored on a computer for offline analysis. To apply

924   photostimulation only during mobility, we reversed the parameters and let the shutter open

925   when the speed of the animal exceeded 5 cm/s and closed the shutter at speeds of below 5

926   cm/s for more than 0.5 seconds.

927   *Object pattern separation task:* We used the object location memory test as a test for spatial

928   learning ability. We used a circular arena (diameter: 45 cm) made of red Plexiglas with 40 cm

929   high walls as described in (*van Goethem et al., 2018*). Two pairs of almost identical building

930   blocks (4 x 3 x 7 cm) made of either plastic or metal were used as objects. All building blocks

931   were topped with a little plastic cone to prevent animals from climbing onto the objects. To

932   habituate animals to the arena and the experimental procedure, they were taken out of their

933   home cage and placed into an empty cage only with bedding for five minutes in order to

934   increase their exploration time. After connecting the mice to the photostimulation apparatus

935   via the light fiber, they were placed into the empty arena for 10 minutes with no laser light.

936   On test days, mice were again first placed into a new empty cage for 5 minutes, then

937   connected to the photostimulation apparatus and placed into the arena for 5 minutes. The

938   arena contained two identical objects placed in the middle of the arena with an inter-object

939   distance of 18 cm. The animals were allowed to freely explore the arena and the objects.

940   After 5 minutes, the animals were transferred into their home cage for 85 minutes. For the

941   recall trial, everything was done identically to the previous acquisition trial, but one of the two

942   previously encountered objects was displaced. Experiments for individual displacement

943   configuration were repeated in some cases up to three times with an interval of two days and

944   the discrimination indices averaged. The following variants of this task regarding object

945   displacement were performed using a first batch of up to 18 mice (3 males, 15 females). In a

946   first set of experiments, variable displacements (3, 6, 9 and 12 cm) were used, with inhibition

947   of granule cells carried out during the entire acquisition and recall trial (schematic in **Fig. 8-**

948   **figure supplement 1A-C**). In a second set of experiments a fixed, intermediate degree of

949   displacement (9 cm, position 3 in **Fig. 8-figure supplement 1C**) was used, and inhibition of

950   granule cells was carried out only during quiet immobility using a closed-loop system (see

951   above) in either only the acquisition trials (**Fig. 8**) or only the recall trials (**Fig. 8-figure**

952   **supplement 3**). In a final set of experiments with those mice, we applied photostimulation in

953   the acquisition trials only when the mouse was in quiet immobility and the nose point of the

954   mouse at least 4 cm away from the objects (**Fig. 8-figure supplement 3**). Lastly, with a

955   separate batch of mice consisting of 2 males and 3 females, all bilaterally injected into the

956   dentate gyrus with eNpHR-eYFP, we performed the pattern separation task with an object

957   displacement of 9 cm either without illumination, with illumination at speeds below 5 cm/s or

958   at speeds above 5 cm/s, both times in the acquisition trials (**Fig. 8-figure supplement 2**).

959   After each mouse, the arena and the objects were cleaned with 70% ethanol. An

960   experienced observer blinded to the experimental group of the animals manually scored the

961   time the animals explored the displaced and the non-displaced object by sniffing with the

962   snout in very close proximity to the objects and their head oriented towards them. We

963   calculated the discrimination index based on the following formula: (time exploring displaced

object – time exploring the non-displaced object) / (time exploring displaced object + time exploring the non-displaced object). Trials in which the total exploration time in an acquisition or recall trial was lower than 4 seconds were excluded from further analysis. Trajectory maps and occupancy plots were generated using custom-written MATLAB Scripts.

Histology and microscopy: To verify successful viral transduction and window position, animals were deeply anesthetized with ketamine (80 mg/kg body weight) and xylazine (15 mg/kg body weight). After confirming a sufficient depth of anesthesia, mice were heart-perfused with cold phosphate buffered saline (PBS) followed by 4% formalin in PBS. Animals were decapitated and the brain removed and stored in 4% formalin in PBS solution. Fifty to 70 µm thick coronal slices of the hippocampus were cut on a vibratome (Leica). For nuclear staining, brain slices were kept for 10 min in a 1:1000 DAPI solution at room temperature. Brain slices were mounted and the red, green and blue channel successively imaged under an epi fluorescence or spinning disc microscope (Visitron VisiScope). In optogenetic inhibition experiments, post-hoc microscopy was used to confirm successful expression of NpHR-eYFP. Of the animals used, 3 control animals showed no eYFP expression and one experimental animal lacked NpHR-eYFP expression. The animal lacking NpHR expression was excluded from the study. Control animals lacking eYFP expression were assumed to lack modulation of granule cell activity by illumination and were pooled with eYFP expressing control animals.

Expression of NpHR was highly selective to the dentate gyrus, as described previously (**Braganza et al., 2020; Truman et al., 2012**), and as reported in the Gensat project (http://www.gensat.org/ShowMMRRCStock.jsp?mmrrc_id=MMRRC:036632). Functional evidence has excluded recombination in hilar interneurons in this mouse line (**Braganza et al., 2020**). Crossing the Prox1-Cre mouse line used in the present study with a mouse leading to Cre-dependent expression of ChR2 showed a lack of monosynaptic inhibitory responses, as would be expected if ChR2 were also present in hilar interneurons.

1001   **Competing interests:** The authors declare no competing interests.

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References

Berron D, Schütze H, Maass A, Cardenas-Blanco A, Kuijf HJ, Kumaran D, Düzel E. 2016. Strong Evidence for Pattern Separation in Human Dentate Gyrus **36**:7569–7579. doi: 10.1523/JNEUROSCI.0518-16.2016.

Braganza O, Mueller-Komorowska D, Kelly T, Beck H. 2020. Quantitative properties of a feedback circuit predict frequency-dependent pattern separation. *eLife* **9**. doi: 10.7554/eLife.53148.

Bragin A, Jandó G, Nádasdy Z, van Landeghem M, Buzsáki G. 1995. Dentate EEG spikes and associated interneuronal population bursts in the hippocampal hilar region of the rat. *Journal of Neurophysiology* **73**:1691–1705. doi: 10.1152/jn.1995.73.4.1691.

Cayco-Gajic NA, Silver RA. 2019. Re-evaluating Circuit Mechanisms Underlying Pattern Separation. *Neuron* **101**:584–602. doi: 10.1016/j.neuron.2019.01.044.

Dana H, Chen TW, Hu A, Shields BC, Guo C, Looger LL, Kim DS, Svoboda K. 2014. Thy1-GCaMP6 transgenic mice for neuronal population imaging in vivo **9**:e108697.

Dana H, Mohar B, Sun Y, Narayan S, Gordus A, Hasseman JP, Tsegaye G, Holt GT, Hu A, Walpita D, Patel R, Macklin JJ, Bargmann CI, Ahrens MB, Schreiter ER, Jayaraman V, Looger LL, Svoboda K, Kim DS. 2016. Sensitive red protein calcium indicators for imaging neural activity. *eLife* **5**. doi: 10.7554/eLife.12727.

Danielson NB, Kaifosh P, Zaremba JD, Lovett-Barron M, Tsai J, Denny CA, Balough EM, Goldberg AR, Drew LJ, Hen R, Losonczy A, Kheirbek MA. 2016. Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. *Neuron* **90**:101–112. doi: 10.1016/j.neuron.2016.02.019.

Davidson TJ, Kloosterman F, Wilson MA. 2009. Hippocampal replay of extended experience. *Neuron* **63**:497–507. doi: 10.1016/j.neuron.2009.07.027.

Diba K, Buzsáki G. 2007. Forward and reverse hippocampal place-cell sequences during ripples **10**:1241–1242. doi: 10.1038/nn1961.

Dupret D, O'Neill J, Pleydell-Bouverie B, Csicsvari J. 2010. The reorganization and reactivation of hippocampal maps predict spatial memory performance **13**:995–1002.

Foster DJ, Wilson MA. 2006. Reverse replay of behavioural sequences in hippocampal place cells during the awake state **440**:680–683. doi: 10.1038/nature04587.

Girardeau G, Benchenane K, Wiener SI, Buzsáki G, Zugaro MB. 2009. Selective suppression of hippocampal ripples impairs spatial memory **12**:1222–1223. doi: 10.1038/nn.2384.

GoodSmith D, Chen X, Wang C, Kim SH, Song H, Burgalossi A, Christian KM, Knierim JJ. 2017. Spatial Representations of Granule Cells and Mossy Cells of the Dentate Gyrus. *Neuron* **93**:677-690.e5. doi: 10.1016/j.neuron.2016.12.026.

GoodSmith D, Lee H, Neunuebel JP, Song H, Knierim JJ. 2019. Dentate gyrus mossy cells share a role in pattern separation with dentate granule cells and proximal CA3 pyramidal cells. doi: 10.1523/JNEUROSCI.0940-19.2019.

Gradinaru V, Thompson KR, Deisseroth K. 2008. eNpHR: A Natronomonas halorhodopsin enhanced for optogenetic applications **36**:129–139.

Greenberg DS, Kerr JND. 2009. Automated correction of fast motion artifacts for two-photon imaging of awake animals **176**:1–15. doi: 10.1016/j.jneumeth.2008.08.020.

Hainmueller T, Bartos M. 2018. Parallel emergence of stable and dynamic memory engrams in the hippocampus **558**:292–296. doi: 10.1038/s41586-018-0191-2.

Headley DB, Kanta V, Paré D. 2017. Intra- and interregional cortical interactions related to sharp-wave ripples and dentate spikes **117**:556–565. doi: 10.1152/jn.00644.2016.

1047   **Jerman T**, Kesner RP, Hunsaker MR. 2006. Disconnection analysis of CA3 and DG in mediating
1048   encoding but not retrieval in a spatial maze learning task **13**:458–464. doi: 10.1101/lm.246906.

1049   **Kay K**, Sosa M, Chung JE, Karlsson MP, Larkin MC, Frank LM. 2016. A hippocampal network for spatial
1050   coding during immobility and sleep **531**:185–190. doi: 10.1038/nature17144.

1051   **Krzanowski WJ**. 1979. Between-Groups Comparison of Principal Components. *Journal of the*
1052   *American Statistical Association* **74**:703–707. doi: 10.2307/2286995.

1053   **Leutgeb JK**, Leutgeb S, Moser MB, Moser EI. 2007. Pattern separation in the dentate gyrus and CA3
1054   of the hippocampus **315**:961–966.

1055   **Long MA**, Fee MS. 2008. Using temperature to analyse temporal dynamics in the songbird motor
1056   pathway **456**:189–194. doi: 10.1038/nature07448.

1057   **Madroñal N**, Delgado-García JM, Fernández-Guizán A, Chatterjee J, Köhn M, Mattucci C, Jain A,
1058   Tsetsenis T, Illarionova A, Grinevich V, Gross CT, Gruart A. 2016. Rapid erasure of hippocampal
1059   memory following inhibition of dentate gyrus granule cells. *Nature communications* **7**:10923.
1060   doi: 10.1038/ncomms10923.

1061   **Mahn M**, Prigge M, Ron S, Levy R, Yizhar O. 2016. Biophysical constraints of optogenetic inhibition at
1062   presynaptic terminals **19**:554–556. doi: 10.1038/nn.4266.

1063   **Malvache A**, Reichinnek S, Villette V, Haimerl C, Cossart R. 2016. Awake hippocampal reactivations
1064   project onto orthogonal neuronal assemblies **353**:1280–1283. doi: 10.1126/science.aaf3319.

1065   **McGinley MJ**, David SV, McCormick DA. 2015. Cortical Membrane Potential Signature of Optimal
1066   States for Sensory Signal Detection. *Neuron* **87**:179–192. doi: 10.1016/j.neuron.2015.05.038.

1067   **Meier K**, Merseburg A, Isbrandt D, Marguet SL, Morellini F. 2020. Dentate Gyrus Sharp Waves, a
1068   Local Field Potential Correlate of Learning in the Dentate Gyrus of Mice **40**:7105–7118.
1069   doi: 10.1523/JNEUROSCI.2275-19.2020.

1070   **Neunuebel JP**, Knierim JJ. 2012. Spatial firing correlates of physiologically distinct cell types of the rat
1071   dentate gyrus **32**:3848–3858. doi: 10.1523/JNEUROSCI.6038-11.2012.

1072   **O'Reilly RC**, McClelland JL. 1994. Hippocampal conjunctive encoding, storage, and recall: Avoiding a
1073   trade-off **4**:661–682. doi: 10.1002/hipo.450040605.

1074   **Owen SF**, Liu MH, Kreitzer AC. 2019. Thermal constraints on in vivo optogenetic manipulations
1075   **22**:1061–1065. doi: 10.1038/s41593-019-0422-3.

1076   **Penttonen M**, Kamondi A, Sik A, Acsády L, Buzsáki G. 1997. Feed-forward and feed-back activation of
1077   the dentate gyrus in vivo during dentate spikes and sharp wave bursts **7**:437–450.
1078   doi: 10.1002/(SICI)1098-1063(1997)7:4<437::AID-HIPO9>3.0.CO;2-F.

1079   **Pilz GA**, Carta S, Stauble A, Ayaz A, Jessberger S, Helmchen F. 2016. Functional Imaging of Dentate
1080   Granule Cells in the Adult Mouse Hippocampus. *Journal of Neuroscience* **36**:7407–7414.

1081   **Pnevmatikakis EA**, Soudry D, Gao Y, Machado TA, Merel J, Pfau D, Reardon T, Mu Y, Lacefield C, Yang
1082   W, Ahrens M, Bruno R, Jessell TM, Peterka DS, Yuste R, Paninski L. 2016. Simultaneous Denoising,
1083   Deconvolution, and Demixing of Calcium Imaging Data. *Neuron* **89**:285–299.
1084   doi: 10.1016/j.neuron.2015.11.037.

1085   **Raimondo JV**, Kay L, Ellender TJ, Akerman CJ. 2012. Optogenetic silencing strategies differ in their
1086   effects on inhibitory synaptic transmission **15**:1102–1104. doi: 10.1038/nn.3143.

1087   **Reimer J**, Froudarakis E, Cadwell CR, Yatsenko D, Denfield GH, Tolias AS. 2014. Pupil fluctuations
1088   track fast switching of cortical states during quiet wakefulness. *Neuron* **84**:355–362.
1089   doi: 10.1016/j.neuron.2014.09.033.

1090   **Reimer J**, McGinley MJ, Liu Y, Rodenkirch C, Wang Q, McCormick DA, Tolias AS. 2016. Pupil
1091   fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. *Nature*
1092   *communications* **7**:13289. doi: 10.1038/ncomms13289.

1093 **Royer S**, Zemelman BV, Losonczy A, Kim J, Chance F, Magee JC, Buzsaki G. 2012. Control of timing,
1094   rate and bursts of hippocampal place cells by dendritic and somatic inhibition **15**:769–775.

1095 **Rubin A**, Sheintuch L, Brande-Eilat N, Pinchasof O, Rechavi Y, Geva N, Ziv Y. 2019. Revealing neural
1096   correlates of behavior without behavioral measurements **10**:4745. doi: 10.1038/s41467-019-
1097   12724-2.

1098 **Sakon JJ**, Suzuki WA. 2019. A neural signature of pattern separation in the monkey hippocampus.
1099   *Proceedings of the National Academy of Sciences of the United States of America* **116**:9634–9643.
1100   doi: 10.1073/pnas.1900804116.

1101 **Sasaki T**, Piatti VC, Hwaun E, Ahmadi S, Lisman JE, Leutgeb S, Leutgeb JK. 2018. Dentate network
1102   activity is necessary for spatial working memory by supporting CA3 sharp-wave ripple generation
1103   and prospective firing of CA3 neurons **21**:258–269. doi: 10.1038/s41593-017-0061-5.

1104 **Senzai Y**, Buzsáki G. 2017. Physiological Properties and Behavioral Correlates of Hippocampal
1105   Granule Cells and Mossy Cells. *Neuron* **93**:691-704.e5. doi: 10.1016/j.neuron.2016.12.011.

1106 **Skaggs WE**, McNaughton BL. 1996. Replay of neuronal firing sequences in rat hippocampus during
1107   sleep following spatial experience **271**:1870–1873. doi: 10.1126/science.271.5257.1870.

1108 **Stefanini F**, Kushnir L, Jimenez JC, Jennings JH, Woods NI, Stuber GD, Kheirbek MA, Hen R, Fusi S.
1109   2020. A Distributed Neural Code in the Dentate Gyrus and in CA1. *Neuron* **107**:703-716.e4.
1110   doi: 10.1016/j.neuron.2020.05.022.

1111 **Steinmetz NA**, Buetfering C, Lecoq J, Lee CR, Peters AJ, Jacobs EAK, Coen P, Ollerenshaw DR, Valley
1112   MT, Vries SEJ de, Garrett M, Zhuang J, Groblewski PA, Manavi S, Miles J, White C, Lee E, Griffin F,
1113   Larkin JD, Roll K, Cross S, Nguyen TV, Larsen R, Pendergraft J, Daigle T, Tasic B, Thompson CL,
1114   Waters J, Olsen S, Margolis DJ, Zeng H, Hausser M, Carandini M, Harris KD. 2017. Aberrant Cortical
1115   Activity in Multiple GCaMP6-Expressing Transgenic Mouse Lines. *eNeuro* **4**.
1116   doi: 10.1523/ENEURO.0207-17.2017.

1117 **Stujenske JM**, Spellman T, Gordon JA. 2015. Modeling the Spatiotemporal Dynamics of Light and
1118   Heat Propagation for In Vivo Optogenetics. *Cell reports* **12**:525–534.
1119   doi: 10.1016/j.celrep.2015.06.036.

1120 **Tibshirani R**, Walther G, Hastie T. 2001. Estimating the number of clusters in a data set via the gap
1121   statistic. *J. R. Statist. Soc. B* **63 Part 2**:411–423.

1122 **Truman LA**, Bentley KL, Smith EC, Massaro SA, Gonzalez DG, Haberman AM, Hill M, Jones D, Min W,
1123   Krause DS, Ruddle NH. 2012. ProxTom lymphatic vessel reporter mice reveal Prox1 expression in
1124   the adrenal medulla, megakaryocytes, and platelets. *The American journal of pathology* **180**:1715–
1125   1725. doi: 10.1016/j.ajpath.2011.12.026.

1126 **van Dijk MT**, Fenton AA. 2018. On How the Dentate Gyrus Contributes to Memory Discrimination.
1127   *Neuron* **98**:832-845.e5. doi: 10.1016/j.neuron.2018.04.018.

1128 **van Goethem NP**, van Hagen BTJ, Prickaerts J. 2018. Assessing spatial pattern separation in rodents
1129   using the object pattern separation task. *Nature protocols* **13**:1763–1792. doi: 10.1038/s41596-
1130   018-0013-x.

1131 **Wilson MA**, McNaughton BL. 1994. Reactivation of hippocampal ensemble memories during sleep
1132   **265**:676–679. doi: 10.1126/science.8036517.

1133 **Yang, K., Shahabi, C.** 2004. A PCA-based similarity measure for multivariate time series. *Proceedings*
1134   *of The 2nd ACM International Workshop on Multimedia Databases (MMDB)* 65–74.

1135 **Yu BM**, Cunningham JP, Santhanam G, Ryu SI, Shenoy KV, Sahani M. 2009. Gaussian-process factor
1136   analysis for low-dimensional single-trial analysis of neural population activity. *Journal of*
1137   *Neurophysiology* **102**:614–635. doi: 10.1152/jn.90941.2008.

1138

Figure captions:

1140

1141 **Fig. 1: Synchronous dentate granule cell activations, 'network events', occur**
1142 **preferentially during immobility. A,** Expression of GCaMP6s in granule cells (Thy1-
1143 GCaMP mouse line, GP4.12Dkim/J). jRGECO was expressed in medial entorhinal cortex
1144 neurons using rAAV mediated gene transfer, and is visible in the middle molecular layer
1145 (MML) corresponding to the medial perforant path (MPP). Upper panel: Post hoc analysis in
1146 70 µm fixed slice. Nuclei stained with DAPI (blue). Lower panel: Imaging plane for
1147 simultaneous recording of MPP bulk and individual GC activity. Scale bar 100 µm. **B,** Data of
1148 representative recording session. Bulk fluorescence signal of MPP fibers (red), extracted
1149 fluorescence signals from a subset of individual GCs (black), mouse position on linear track
1150 (blue) and diameter of mouse pupil (black). **C,** Participation of granule cells in synchronous
1151 network events. Representative field of view with highlighted simultaneously active GCs.
1152 Cells active during an individual network event are depicted in the same color. A subset of
1153 neurons is active in multiple network events, recognizable as white color. **D,** Raster plot of
1154 network events. Dashed lines mark network events, corresponding to simultaneous activity of
1155 > four cells. Participating cells are highlighted according to color scheme from panel C.
1156 Running speed is depicted (blue) to distinguish running and resting periods. **E,** Fluorescence
1157 transients of participating granule cells from NEs in panel D. Color scheme corresponds to
1158 panel C and D. Each column shows all respective transients of the respective synchronous
1159 ensemble. Shown is a time window of ±1s around each NE. Vertical scale bars correspond to
1160 100% ∆F/F. **F,** Mean number of network events per twenty-minute recording session during
1161 running and resting. Network events occurred mainly during immobility (repeated measures
1162 ANOVA, $F_{(1,8)}$=71.80, p=2x10^{-7}, n=9 animals, 3 sessions). Grey bars depict shuffled data for
1163 each condition (n=9 animals). **G,** Average number of identified network events plotted
1164 against different thresholds for the size of network events in terms of numbers of
1165 synchronously active cells (green line, shaded area depicts SEM, n=3 mice, 3 sessions).
1166 Shuffled data null-distribution is created by randomly shuffling event times for every
1167 individual cell (grey line, shaded area depicts SEM). **H,** Frequencies of network events
1168 calculated from equal time intervals for locomotion and immobility (Repeated measures
1169 ANOVA, $F_{(1,8)}$ = 117.28 , p = 2x10^{-6}). **I,** Cumulative probability distribution of network event
1170 occurrence during the entire twenty-minute session for individual sessions (gray lines) and
1171 the pooled sessions (Green line, n=9 mice, 3 sessions). **J,** Pooled cumulative probability
1172 distribution of network event occurrence during resting periods. All resting periods of one
1173 session that were longer than 5 s were normalized to their length (n=9 mice, 3 sessions).

1174

1175 **Fig. 2: Granule cell and MPP activity during locomotion and immobility. A,** Mean MPP
1176 activity (red) and the sum of all granule cell activities (green) for a representative section of a
1177 recording session in an individual mouse. Dashed lines mark transition between resting and
1178 running periods (see blue line indicating running speed). **B,** Average MPP fluorescence and
1179 running speeds, both aligned to running onsets (dashed line). Shaded areas indicate
1180 standard error (n=4 mice, one mouse had only jRGECO expression in MPP, but no granule
1181 cell signal, 3 sessions per mouse). **C,** Mean fluorescence averaged during resting (dark red)
1182 and running (light red). Asterisk indicates 5 % significance threshold (repeated measures
1183 ANOVA, $F_{(1,3)}$ = 7,86, p = 0.032, n = 4 mice, 3 sessions). **D,** Variance of MMP bulk signal
1184 during resting (dark red) and running (light red, n=4). Asterisk indicates results of repeated
1185 measures ANOVA, $F_{(1,3)}$ = 7,07, p = 0.037, n = 4 mice, 3 sessions. **E,** Cross correlation of
1186 MPP bulk signal and summed GC signal during resting. Shaded grey area indicates standard
1187 error. **F,** Average MPP activity (red) and probability of granule cell activity across all mice, 3
1188 baseline sessions each, both aligned to the time point of network events (n=3 mice, 2008
1189 network events, shaded red area depicts SEM). **G,** Amplitudes of deconvolved events in

MPP bulk data during locomotion (light red) und resting (dark red). **H**, Frequencies of all deconvolved MPP bulk events during locomotion and immobility (repeated measures ANOVA, $F_{(1,2)}$ = 255, p = 3×10^{-6}, n = 3 mice, 3 sessions). **I**, Frequencies of deconvolved events with amplitudes above two standard deviations (repeated measures ANOVA, $F_{(1,2)}$ = 27, p = 2×10^{-3}, n = 3 mice, 3 sessions). **J**, Delay of NEs to the closest identified MPP event.

Fig. 3: Pupil dynamics during network events. A, Representative example of pupil size measurements during different locomotor states. Green dots indicate timepoints of network events. **B, C,** Average pupil diameters (grey lines) aligned to locomotion onsets (B) or offsets (C, blue lines) reveals pupil dilation at locomotion onsets, and constriction during locomotion offset. Shaded areas indicate standard error. GC activity stays on baseline value during change of behavioral state (green). **D,** Average pupil diameters during locomotion, during resting periods, and during network events. Asterisks indicate significant Bonferroni post-test at 5% level. **E,** Average rate of pupil diameter change during locomotion, during resting periods and during network events. Asterisks indicate significant Bonferroni post-test at 5% level. **F,** Averaging pupil diameters aligned to NE times (green bars) reveals pupil constriction during NEs. Shaded areas indicate standard error. (n=6 mice, 3 sessions). (n=6 mice, 3 sessions).

Fig. 4: *Network events are orthogonal, but repetitively recruit GC sub-ensembles.* A, Similarity between network events. Similarity of population vectors computed for individual network events. Comparisons were carried out between all possible pairwise combinations of vectors and quantified using cosine similarity. Inset: Mean number of orthogonal NEs for baseline sessions (Black bar) compared to shuffled data (gray bar) with SEM. Grey lines depict individual sessions. **B,** Graphical representation of the correlation matrix using Pearson's r for all cell combinations, with values for r being color coded. Data from one representative session in an individual mouse. **C,** Identification of clusters of correlated cells using agglomerative hierarchical clustering. Clusters were combined using a standardized Euclidean distance metric and a weighted average linkage method, until the mean of the cluster internal r-value reached a significance threshold. The 5% significance threshold was defined by creating a null-distribution of r values from randomized data sets, and is indicated for this particular experiment with a vertical line. Right panel in C depicts the reordered correlation matrix showing clusters of highly correlated cells. Only clusters whose mean intra-correlation exceeded the threshold were included in further analysis (significant clusters indicated with grey frames) **D,** Raster plot showing the reactivation of clusters identified in panel C during multiple episodes of running and immobility. Individual dots indicate participation of individual cells. Clusters are color-coded according to the agglomerative tree. Network events are indicated by vertical dashed lines. Running episodes are indicated at the lower border with the running speed (blue).

Fig. 5: Characterization of dentate gyrus place and speed neurons. A, B, Place cells in the dentate gyrus. **A,** Representative polar plots of two significantly place-coding granule cells (left, middle), and one without significant place preference (right). Place coding is depicted as spiral plot, where each 360° turn of the spiral represents a transition through the 1.5 m linear track without additional cues (baseline condition). Detected events are shown as black dots. The red line represents the place vector. The corresponding heatmap of normalized fluorescence is shown in the inset. In lower panels, the distributions for place vector lengths generated from shuffled data (see Methods) are shown (grey histograms), the place vector for the individual cell is indicated by the red line. **B,** Place field heatmaps of cells showing significant place preference. **C,** Representative examples of three significantly speed-modulated neurons (black traces, running speed depicted in cyan). **D,** Speed-

1238 modulated mean fluorescence signal of a representative example cell. Grey area indicates
1239 standard error. **E,** Mean fluorescence signals of all significantly speed-modulated cells.
1240 Normalized fluorescence is color coded and running speed is normalized to every individual
1241 mouse maximum running speed. **F**, Fractions of place and speed coding cells (cyan and
1242 green bar, respectively) normalized to all active cells (left panel) and only running active cells
1243 (right panel). Only a very small number of cells carried encoded both features (dark green
1244 bar).

1245 **Fig. 6: Increasing sensory cues is associated with enlargement of network events and**
1246 **increased incorporation of place cells. A,** Network events comprise more granule cells in
1247 cue-enriched environments. Cumulative probability of network event size (number of cells
1248 per network event) for baseline and cue enriched condition (dark and light grey lines,
1249 respectively). **B,** Cumulative probability of participation in multiple network events per cell for
1250 baseline and cue enriched condition (dark and light grey lines, respectively). **C,** Fraction of
1251 place and speed cells that participate in network events (total number of place/speed cells
1252 equals 100%). **D,** Relation of place and speed cells to correlated cell clusters (c.f. Fig. 4).
1253 Fraction of the total number of clusters containing place cells (cyan), speed cells (light
1254 green), or both (dark green). Gray indicates clusters containing neither place nor speed cells.
1255 **E,** Mean number of place and speed cells per cluster, in baseline and cue enriched
1256 conditions, n = 9 mice.**Fig. 7: Similarity of activity patterns during network events to**
1257 **population patterns during locomotion. A,** Color code for position on the linear track used
1258 in panels B. **B,** Trajectories during an individual representative session plotted in a three-
1259 dimensional coordinate system corresponding to the first three PCA components (Comp 1-
1260 3).   **C,** Trajectories calculated from CA1 Ca^{2+}-imaging data during an individual
1261 representative session for comparison. **D,** average peak value of weight-autocorrelations at
1262 Δlap = 1 (ANOVA, $F_{(1,3)}$ = 88.32, p = 2×10^{-30}, * Bonferroni post-test at p<0.05, *** Bonferroni
1263 post-test at p<0.001). **E,** Schematic of the procedure for comparing population activity during
1264 network events (NE) and locomotion. Population activity is represented by 3 cells (upper
1265 traces), recorded during running and quiet immobility (blue trace indicates speed). Time point
1266 of three network events is indicated schematically by red lines. Activity during network events
1267 (NE) was used to perform PCA, computing the transformation matrix V_{net}. Similarly, PCA was
1268 performed on the neuronal population activity only from running periods (speed indicated in
1269 blue, bordered by vertical grey dashed lines), to generate the transformation matrix V_{run}
1270 representing the covarying activity during locomotion. **F,** Schematic description of the
1271 procedure for projecting co-variances of running activity into the PCA basis of network events
1272 (or shuffled data). Grey dots show covarying activity of two representative cells during
1273 running. The blue graph denotes the projection into the locomotion PCA-space using V_{run}
1274 and the width of the distribution shows the projected variance. The red graph shows the
1275 same information for the network space using V_{net}. **G,** Individual example of shuffle analysis
1276 for a representative session. The vertical red line indicates the observed projected variance
1277 explained normalized to variance explained in the original space (50 % of the overall
1278 variance). The observed variance explained is larger than the shuffled distribution (blue
1279 bars), indicating that the population activity during locomotion and network events is more
1280 similar than expected by chance (i.e. for network activity without correlations). **H,** Cartoon
1281 illustrating NE structure for four cells and three synchronous events. **I,** Upper panel: Cartoon
1282 illustrating the first shuffling procedure where each cells time series is shifted by a
1283 randomized time interval. Lower panel: Fraction of sessions in which comparisons of
1284 population activity were significant vs. chance level for the two cue conditions and all
1285 similarity measures (n = 8 mice, 1 session per condition, see Fig. 7-figure supplement 3 for
1286 comparisons to shuffled datasets for all sessions). **J,** Upper panel: Cartoon illustrating the

1287 second shuffling procedure where cell IDs within each NE are randomly shuffled. This
1288 approach randomizes NE-composition while maintaining the number of cells per NE. Lower
1289 panel: Analogous to I. **K,** Upper panel: Cartoon illustrating the third shuffling procedure
1290 where the NE participation is randomly shuffled for each cell. This approach randomizes NE-
1291 participation while maintaining the activity level for each cell. Lower panel: Analogous to I.

1292 **Fig. 8: Inhibition of dentate granule cell activity during immobility prevents memory**
1293 **acquisition. A,** Schematic of the bilateral optogenetic inhibition of the dentate gyrus granule
1294 cells expressing eNpHR. **B,** Schematic of the experimental procedure. In the acquisition
1295 phase, mice were familiarized with an arena containing two objects. Following an
1296 intermediate period of 90 minutes, the mice were placed in the same arena in which one
1297 object was moved slightly. **C, D,** Representative sessions from acquisition trials in control
1298 (eYFP) mice and mice expressing eNpHR in granule cells showing the tracking of the mouse
1299 center of mass (dashed white lines), as well as normalized occupancy within the arena. **E,**
1300 Discrimination index from the acquisition trial quantifying the specific exploration activity of
1301 the objects relative to one another (see Methods), with 0 values indicating equal exploration
1302 (see Methods). **F,** Total time spent exploring the objects in the eYFP and eNpHR groups
1303 during the acquisition trial. **G, H,** Representative sessions from recall trials depicted as
1304 shown in B, C. **I,** Discrimination index for recall trials, showing strong preference for the
1305 displaced object in the eYFP group, but not the eNpHR group if granule cell activity was
1306 inhibited during acquisition trials only during immobility. **J,** Total time spent exploring the
1307 objects in the eYFP and eNpHR groups during the recall trial (n=6 animals for eNpHR group,
1308 n=9 animals for eYFP group).

1309

1310 **Figures supplement captions:**

1311 **Figure 1-figure supplement 1: Dual color two-photon imaging in the dentate gyrus. A,**
1312 Representative hippocampal sections from three different mice with expression of jRGECO
1313 in the MPP (red), GCaMP6s in granule cells (green), and DAPI as nuclear staining. **B,** Setup
1314 of the 2-photon microscope for dual-color two-photon imaging. To allow efficient excitation of
1315 both genetically encoded Ca^{2+} indicators, we established excitation with two pulsed laser
1316 sources at 940 and 1070 nm. **C, D,** Dimensions of flat, custom head fixation ring. **F,** conical
1317 transparent inset used to maximize NA in deep imaging. Linked to Methods. **D,** Properties of
1318 the linear track designs for baseline, cue-enriched and zone configuration. **H, I, J,**
1319 Locomotion on the linear track. Neither the total distance run on the linear track (D), nor the
1320 average running speed (E), nor the fraction of time spent running differed significantly
1321 between the baseline and cue-enriched and zone conditions (n.s., ANOVA, p = 0.31, 0.58
1322 and 0.67 respectively).

1323 **Figure 1-figure supplement 2: Granule cell activity during locomotion on empty**
1324 **textured belt. A,** Image plane for granule cell recording in the dentate gyrus. **B,** One
1325 representative component of non-negative matrix factorization. Upper panel: spatial
1326 component as extracted from image stack. Middle panel: Corresponding ΔF/F trace
1327 generated from a ROI drawn around the corresponding somatic region. Lower panel:
1328 Extracted ΔF/F trace (gray) with deconvolved trace (black). Identified event onsets are
1329 depicted with vertical green lines. **C, D,** Frequencies of Ca^{2+} events (C) and magnitude of
1330 Ca^{2+} transients during each recording session (D) recorded during quiet immobility (rest, dark
1331 bars) and locomotion (run, light bars) on an empty textured belt (data from 3 sessions per
1332 animal, n=9 mice, comparisons between running and resting states ANOVA, n.s.). **E,**
1333 Distribution of event frequencies for all cells. Black bars represent events during resting

1334 periods, grey bars represent frequencies during locomotion (data from 3 sessions per animal,
1335 n=9 mice).

Figure 1-figure supplement 3: Shuffling analyses demonstrating that network events
1336
do not arise by chance. A, Average number of identified network events plotted against
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different used thresholds for the size of network events in terms of numbers of synchronously
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active cells (green line, shaded area depicts SEM, n = 3 mice, 3 sessions). Shuffled data
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null-distribution is created by randomly shuffling event times for every individual cell (grey
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line, shaded area depicts SEM). **B**, Same as A, but using a shuffling approach where traces
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are randomly shifted with respect to each other (red line, shaded area depicts SEM). **C**,
1342
Same as A and B, but using a shuffling method were event times are randomly shuffled for
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locomotion and immobility periods separately (Purple line, shaded area depicts SEM). **D-I**,
1344
Six representative example data sets. Left panels: Raster plot of event onsets (grey dots)
1345
and identified network events (Colored dots) during episodes of locomotion and immobility
1346
(blue line). Right panels: Number of network events identified for different threshold (green
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lines). All three different shuffling approaches are shown in each panel (grey, red, purple
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lines for the shuffling approaches depicted in panels A-C, respectively. Error bars depict
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standard deviation. All shuffling approaches yield uniform results.
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Figure 4-figure supplement 1: Clustering of cells active during network events into
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correlated sub-ensembles via a correlation matrix. A_H, Examples of correlation
1352
matrices after hierarchical clustering from four of the nine mice investigated. Those clusters
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with an intra-cluster correlation above threshold that were considered for further analysis are
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indicated with a grey frame. Upper row (A-D) shows baseline condition and lower row (E-H)
1355
cue-enriched condition.
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Figure 5-figure supplement 1: Activity of granule cells and MPP inputs in cue-enriched
1357
conditions. A, Average frequencies of Ca^{2+} events (A) for baseline and cue-enriched
1358
conditions recorded during quiet immobility (rest, dark bars) and running (run, light bars).
1359
Data from n=9 mice, last baseline session and cue-enriched session for panels A and B (2-
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way ANOVA, run vs. rest: $F_{(1,32)}$ = 1.71, p = 0.20, baseline vs cue-enriched $F_{(1,32)}$ = 1.80, p =
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0.19) . **B**, Same as A for the magnitude of Ca^{2+} transients (2-way ANOVA, run vs. rest: $F_{(1,32)}$
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= 1.67, p = 0.21, baseline vs cue-enriched $F_{(1,32)}$ < 0.01, p = 0.99) **C**, Distribution of event
1363
frequencies for all cells for the cue-enriched condition. **D,** Under cue-enriched conditions,
1364
network events also occurred mainly during immobility. Mean number of network events per
1365
recording session during running (light green) and resting (dark green). Grey bars depict
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shuffled data for each condition. Section sign indicates ANOVA $F_{(3,28)}$ = 8.6, p = 0.0003,
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asterisk indicates Bonferroni post-test resting vs. shuffled p = 0.0019). **E,** Cos-similarity
1368
between network events under cue-enriched conditions. Similarity of population vectors
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computed for individual network events. Comparisons were carried out between all possible
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pairwise combinations of vectors and quantified using cosine similarity. The number of
1371
orthogonal network events were significantly higher than expected by chance (Comparison
1372
shuffled vs. real data, Wilcoxon signed rank test p = 0.031). **F-J,** Activity of MPP inputs in the
1373
cue-enriched condition. Data from n = 4 mice, one mouse included additionally to Fig. 1F that
1374
had only jRGECO expression in MPP, but no granule cell signal for panels G-J. Shaded
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areas indicate standard errors. **F,** Mean fluorescence averaged during resting (dark red) and
1376
running (light red, n = 4) in cue-enriched trials. ANOVA for running vs. immobile states $F_{(1,3)}$ =
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9.64, p = 0.02. **G,** Variance of MPP bulk signal during resting (dark red) and running (light
1378
red, n = 4) in cue-enriched trials. ANOVA for running vs. Immobile states n.s. **H,** Cross
1379
correlation of MPP bulk signal and summed GC signal during resting. Grey shaded area
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1381   indicates standard error. **I,** In cue-enriched conditions, MPP activity also increases at
1382   transitions from immobility to running (red line, MPP activity, blue line indicates running
1383   speed, n = 4). **J,** Average MPP activity (red) and probability of granule cells being active in
1384   cue-enriched trials, both aligned to the time point of network events. Red shaded areas
1385   indicate standard error.

1386   **Figure 7-figure supplement 1**: **Analysis of population activity in dentate gyrus and the**
1387   **CA1 subfield of the hippocampus using PCA, ICA and GPFA. A-C,** Upper panels depict
1388   the first three components from representative sessions (A: PCA, B: ICA, C: GPFA) plotted in
1389   a coordinate system. The color code refers to the place on the linear track, with the same
1390   locations represented in the same color. **D-F,** As in A-C, but for CA1 neurons. Note the
1391   smooth and repetitive trajectories. Linked to Fig. 7.

1392   **Figure 7-figure supplement 2**: **PCA-based analysis of spatial representation in DG and**
1393   **CA1. A-D,** Trajectories of first three components for DG example recordings in different
1394   environments (A-C for baseline, cue-enriched and zones, respectively) and CA1 data (D).
1395   The animals' position is color coded analogous to Fig. 7-figure supplement 1. **E-H,** Weights of
1396   five first principal components plotted against the animal position during the first 6 laps of the
1397   recording. **I-L,** normalized autocorrelation of five first PCA-weights from example data
1398   calculated with respect to different laps. Place information in weights leads to peaks at
1399   integer multiples of Δlap. **M-P,** autocorrelations from five first components averaged for all
1400   animals (n = 9 animals for baseline and cue-enriched, n = 3 animals for DG zones, n = 5
1401   animals for CA1).

1402   **Figure 7-figure supplement 3: Similarity of individual network events to population**
1403   **activity during running.** Data from all sessions under baseline and cue-enriched conditions
1404   are depicted (as indicated on the leftmost border of the figure) for all three measures. PCA
1405   similarity using the vector projection method introduced in this paper (see Methods), cosine
1406   similarity measures (***Krzanowski, 1979***) and EROS (***Yang, K., Shahabi, C., 2004***) are
1407   depicted in the leftmost, middle and rightmost columns, respectively. In all graphs, shuffled
1408   data distributions generated with shuffle method 1 are shown in light blue, a vertical red line
1409   indicates the observed similarity value between network and locomotion related population
1410   activity in the particular session. P-values are indicated above each graph. In the variance
1411   projection method, values were normalized to the locomotion related variances projected into
1412   the locomotor states. As expected, this results in a high proportion of explained variance.
1413   Linked to Fig. 7.

1414   **Figure 8-figure supplement 1: Establishing a dentate gyrus-dependent variant of the**
1415   **object pattern separation task. A,** Schematic of the bilateral optogenetic inhibition of the
1416   dentate gyrus granule cells expressing eNpHR. **B,** Description of the task. Following
1417   familiarization with the object location, one of the objects is moved in a subsequent recall
1418   session, and the extent to which mice explore the moved vs. stationary object is examined.
1419   These trials can be repeated allowing to explore the effects of variable movement of the
1420   objects. **C,** Schematic of possible object locations for the displaced object. Displacement was
1421   randomized for each animal, such that either the left or the right object was displaced, in
1422   either a forward or back direction (LB i.e. corresponding to left, back, and RF to right,
1423   forward). The experiment used four possible new locations along a vertical axis, increasing
1424   from minor displacement (3 cm) to maximal displacement, indicated by numbers 1-4. **D,**
1425   Stitched wide-field image of a hippocampal slice showing expression of NpHR-eYFP in both
1426   DGs. **E,** Results of light-based inhibition of granule cells during acquisition and recall trials for

1427 different degrees of object separation indicate on the x-axis (eNpHR group, n=4, green bars)
1428 vs. an eYFP expressing control group (n=3, black bars). The effect of granule cell inhibition is
1429 most pronounced for intermediate degrees of object movement. **F,** Representative, typical
1430 discharge behavior of a granule cell. **G,** inhibition of granule cell firing evoked with long
1431 current injections by light-based activation of NpHR (green vertical bars) with continuous
1432 stimulation. **H,** quantification of firing rates before, during and after illumination for continuous
1433 stimulation (ANOVA, $F_{(2,5)}$ = 28.30 , p = 0.001, Dunnett's multiple comparison test, asterisks
1434 indicate 5% significance level). **I,** like F with pulsed stimulation at 50% duty cycle and 20 Hz
1435 (d). **J,** like G with pulsed stimulation (ANOVA, $F_{(2,5)}$ = 61.00 , p = 0.001, Dunnett's multiple
1436 comparison test, asterisks indicate 5% significance level). **K, L,** Estimation of light-induced
1437 warming within brain tissue for continuous illumination (K) or pulsed illumination at 50% duty
1438 cycle and 20 Hz (L) at intensities used for the behavioral experiments. Predicted temperature
1439 changes are plotted as a function of time and depth. **M,** Analysis of warming over time,
1440 showing that the warming effects of pulsed light stimulation are asymptotic, and remain
1441 below 0.4 °C at a distance of 300 µm from the fiber front end. Linked to Fig. 8.

1442 **Figure 8-figure supplement 2: Inhibition of dentate granule cell activity during**
1443 **locomotion only in the acquisition trial impairs memory formation in the OPS task. A,**
1444 Description of the experimental protocol. In the acquisition phase, mice were familiarized with
1445 an arena containing two objects. Following an intermediate period of 90 minutes, the mice
1446 were placed in the same arena in which one object was moved slightly. Inhibition of granule
1447 cells was carried out not at all, during periods of quiet immobility, or during periods of
1448 locomotion in the acquisition trial in three consecutive experiments. **B,** Discrimination indices
1449 for animals performing the OPS task during acquisition. Experiments were performed in three
1450 conditions. First, granule cells were not inhibited. In a second run GC activity was
1451 optogenetically inhibited during resting periods. In the third run GC activity was inhibited
1452 exclusively during running periods (Repeated measures ANOVA, $F_{(1,14)}$ = 1.48, p = 0.28, n =
1453 5). **C,** Animals showed comparable exploration times during the acquisition trial in all three
1454 conditions (Friedmann test, p = 0.69). **D,** During the recall trial animals were only able to
1455 discriminate the displaced object, when no GC inhibition had happened during the acquisition
1456 trial. No difference was found for the animals depending on whether inhibition had happened
1457 during immobility or locomotion (Repeated measures ANOVA, $F_{(1,14)}$ = 54.58, p = 0.0003.
1458 Bonferroni post-tests: none vs. rest, p = 0.0026; none vs. run, p = 0.0076; rest vs. run p =
1459 0.14, n = 5). **E,** Exploration times did not differ with regard to the experimental condition
1460 (Friedmann test, p = 0.78). **Figure 8-figure supplement 3: Inhibition of dentate granule**
1461 **cell activity during immobility in the acquisition trial only in non-object locations**
1462 **impairs memory formation in the OPS task. A,** Description of the experimental protocol.
1463 In the acquisition phase, mice were familiarized with an arena containing two objects.
1464 Following an intermediate period of 90 minutes, the mice were placed in the same arena in
1465 which one object was moved slightly. Inhibition of granule cells was carried out only during
1466 periods of quiet immobility in the acquisition trial, and only if the periods of immobility were
1467 not adjacent to the objects. **B, C,** Representative sessions from acquisition trials in control
1468 (eYFP) mice (b, n=10) and mice expressing eNpHR in granule cells (c, n=4) showing the
1469 tracking data (dashed white lines), as well as occupancy within the open field as a heat map.
1470 **D,** Discrimination index quantifying relative exploration times of the two objects, with 0 values
1471 indicating equal exploration (see Methods). Comparison between groups n.s., t-test with
1472 Welch correction p=0.3537. **E,** Total time spent exploring the objects in the eYFP and
1473 eNpHR groups during the acquisition trial. Comparison between groups n.s., t-test with
1474 Welch correction p=0.6126. **F, G,** Representative sessions from recall sessions. **H,**

Discrimination index for recall trials, showing a significant reduction in the recognition of the displaced object in the eNpHR group. t-test with Welch correction p=0.0018. **I,** Total time spent exploring the objects in the eYFP and eNpHR groups during the recall trial. Comparison between groups n.s., t-test with Welch correction p=0.4285. **J,** Inhibition of dentate granule cell activity during immobility only during recall trials does not significantly impair performance in the OPS task. In the acquisition phase, mice were familiarized with an arena containing two objects. Following an intermediate period of 90 minutes, the mice were placed in the same arena in which one object was moved slightly. Inhibition of granule cells was carried out only during periods of quiet immobility in the recall trial. **K, L,** Representative sessions from acquisition trials in control (eYFP) mice (b, n=11) and mice expressing eNpHR in granule cells (c, n=6) showing the tracking data (dashed white lines), as well as occupancy within the open field as a heat map. **M,** Discrimination index quantifying relative exploration times of the two objects, with 0 values indicating equal exploration (see Methods). Comparison between groups n.s., t-test with Welch correction p = 0.8613. **N,** Total time spent exploring the objects in the eYFP and eNpHR groups during the acquisition trial. Comparison between groups n.s., t-test with Welch correction p = 0.6097. **O, P,** Representative sessions from recall sessions. **Q,** Discrimination index for recall trials, showing no significant reduction in the recognition of the displaced object in the eNpHR group. Comparison between groups n.s., t-test with Welch correction p = 0.2802. **R,** Total time spent exploring the objects in the eYFP and eNpHR groups during the recall trial. Comparison between groups n.s., t-test with Welch correction p = 0.8236.

Supplementary file captions:

Supplementary File 1: Spread Sheet containing statistical test results for present data with respective figure numbers.

Supplementary video captions:

Video 1: Video showing activity of granule cells and MPP, corresponding to Fig. 1A, B.

Video 2: Video showing network events, corresponding to Fig. 1C

Supplementary References

1. Krzanowski, W.J. Between-Groups Comparison of Principal Components. *Journal of the American Statistical Association* **74**, 703–707 (1979).
2. Yang, K., Shahabi, C. A PCA-based similarity measure for multivariate time series. *Proceedings of The 2nd ACM International Workshop on Multimedia Databases (MMDB)*, 65–74 (2004).

A (top) Confocal image of hippocampus with fluorescent labeling. Scale bar. (bottom) Higher magnification showing MML, IML, and GCL layers. Scale bar.

B ΔF/F traces (red, black), position trace (blue, 1 m), and pupil/velocity trace (gray, 20%). Scale bars: 100% ΔF/F, 1 min.

C Field of view with color-coded synchronous ensembles. Scale bar.

D Raster plot of cell ID (0–500) versus time (s) with cyan running epochs and velocity trace (10 cm/s) below.

synchronous ensemble #
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

E Individual ΔF/F traces for each synchronous ensemble (100% ΔF/F, 1 s).

F NEs per session during Rest versus Run. * indicates significance.

G NE count mean versus NE thresh.

H NEs per minute during rest versus run. * indicates significance.

I cumulative NE prob. versus time (min).

J cumulative NE prob. versus Normalized resting period.

A

B beam size attenuator
beam combiner
Ti:Sa laser 940 nm, 75 fs
fiber laser 1070nm, 55 fs
xy scanner
GaAsP detector
GaAsP detector

D

E
8.00
16.80
14.00

F cover slip (Ø 5 mm)
l = 2.3 mm
RTV 615
Ø 1.5 mm

G
baseline
cue-enriched
zones
linear treadmill
1.5 m
66 % hot glue droplets
tactile cues
10 mm
33 % tube caps
soft tubes
velcro stripes
glues spines

H
total distance (m)
baseline
cue-enriched
Zones

I
mean speed (cm s^{-1})
baseline
cue-enriched
Zones

J
fraction of locomotion time (%)
baseline
cue-enriched
Zones

A

B

Direct readout

100 % ΔF/F

NNMF and deconvolution

100 % ΔF/F

1 min

C

Events min^{-1}

*

rest run

D

ΔF/F

*

rest run

E

Probability

rest
run

Events per min

A 20 %   10 cm/s   1 min

B Run onset   1 %   5 cm/s   0   2 % prob   1 s

C Run offset   1 %   5 cm/s   0   2 % prob   1 s

D pupil diameter (/mean)   *   *   *   run   rest   NE

E d/dt pupil diameter   ×10⁻³   *   *   *   run   rest   NE

F NE   1 %   5 cm/s   0   2 % prob   1 s

A probability / cos-similarity; inset: orthogonal pairs % (real, shuffle), *

B r values of network related events

C minimized distance after hierachical clustering

D cluster ID vs time/s; 10 cm/s speed trace

A Baseline M103 **B** M155 **C** M194 **D** M226

E Cue-enriched **F** **G** **H**

r

A

position on belt (cm)

probability

place vector length

$p < e^{-6}$ $p < e^{-6}$ p = 0.39

B

cell ID

position on belt (cm)

normlized F

C

10 cm/s   50 % F/F_max

30 sec

D

$\Delta F/F$

speed (cm / s)

E

cell ID

speed / max

normlized F

F

Fraction of cells (%)

baseline   cue-enriched

Fraction of run-act cells (%)

baseline   cue-enriched

speed cells
both
place cells

A cumulative prob vs cells per network event
B cumulative prob vs network event per cell
C Fraction of cells %
D baseline / cue-enriched, Cluster contains: place cells, speed cells, both
E Cells per cluster

A running belt 150 cm

B PCA on DG

C PCA on CA1

D

Comp 1, Comp 2, Comp 3

Peak value at Δlap 1

DG baseline, DG cue-rich, DG zones, CA1 zones

E

Cell 1
Cell 2
Cell 3

100 % ΔF/F

Speed

5 cm/s

1 min

NE $\mathbf{X}_{net}$

Locomotion $\mathbf{X}_{run}$

PCA

PCA

Principal directions $\mathbf{V}_{net}$

Principal directions $\mathbf{V}_{run}$

F

$\mathbf{V}_{net}\,cov(\mathbf{X}_{run})\,|$

$cov(\mathbf{X}_{run})$

$|\,\mathbf{V}_{run}\,cov(\mathbf{X}_{run})\,|$

activity cell 1

activity cell 2

G

probability

projected variance

H NE structure

NE$_1$ NE$_2$ NE$_3$

cell$_1$
cell$_2$
cell$_3$
cell$_4$

I Shuffle 1

NE$_1$ NE$_2$ NE$_3$

cell$_1$ ΔT
cell$_2$ ΔT
cell$_3$ ΔT
cell$_4$ ΔT

shift time series per cell

Significant comparisons %

baseline, cue-enriched

proj. var.   cos^2 Eros

J Shuffle 2

NE$_1$ NE$_2$ NE$_3$

cell$_1$
cell$_2$
cell$_3$
cell$_4$

shuffle cell composition per NE

Significant comparisons %

baseline, cue-enriched

proj. var.   cos^2 Eros

K Shuffle 3

NE$_1$ NE$_2$ NE$_3$

cell$_1$
cell$_2$
cell$_3$
cell$_4$

shuffle NE participation per cell

Significant comparisons %

baseline, cue-enriched
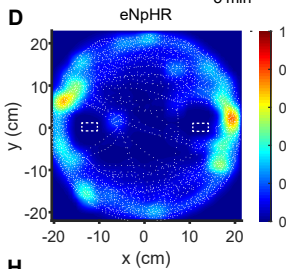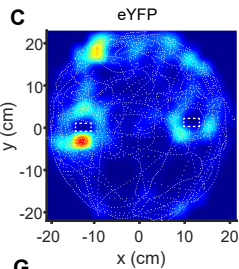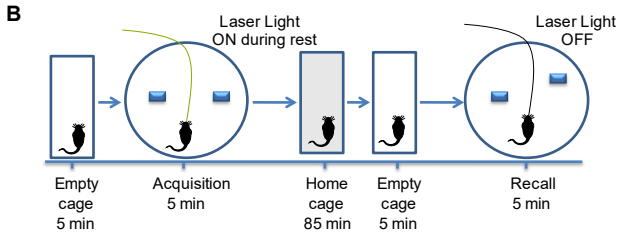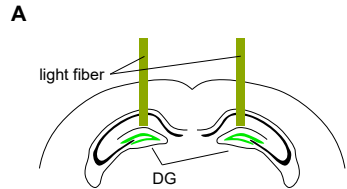
proj. var.   cos^2 Eros

A PCA on DG   **B** ICA on DG   **C** GPFA on DG

D PCA on CA1   **E** ICA on CA1   **F** GPFA on CA1

running belt
150 cm

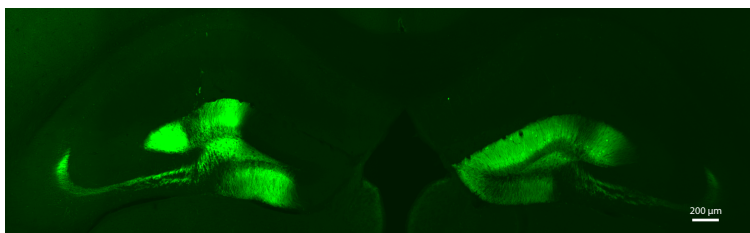|			
A PCA on DG, baseline	**B** PCA on DG, cue-enriched	**C** PCA on DG, zones	**D** PCA on CA1, zones
E	**F**	**G**	**H**
I	**J**	**K**	**L**
M	**N**	**O**	**P**

A light fiber — DG

B Laser Light ON during rest — Laser Light OFF

Empty cage 5 min → Acquisition 5 min → Home cage 85 min → Empty cage 5 min → Recall 5 min

C eYFP

D eNpHR

E DI

F total exploration time (s)

G

H

I DI — ***

J total exploration time (s)

A light fiber, DG

B Empty cage 5 min, Laser Light ON, Aquisition 5 min, Home cage 85 min, Empty cage 5 min, Laser Light ON, Recall 5 min

C 4 LB, 3 LB, 2 LB, 1 LB, L, 1 LF, 2 LF, 3 LF, 4 LF, 4 RB, 3 RB, 2 RB, 1 RB, R, 1 RF, 2 RF, 3 RF, 4 RF

D 200 μm

E DI vs Displacement (cm): 3, 6, 9, 12

F 20 mV, 0.2 s

G ~1mW continuous, Vr = -68 mV, 0.2 s, 20 mV

H AP freq. [Hz]: pre, light, post; *

I ~1mW pulsed (20Hz), Vr = -67 mV, 0.2 s, 20 mV

J AP freq. [Hz]: pre, light, post; **

K depth (mm), time (s), ΔT (°C)

L depth (mm), time (s), ΔT (°C)

M ΔT /°C, time (s), pulsed light, cw light

A Laser Light ON: none/during rest/during run ... Laser Light OFF

Empty cage 5 min → Acquisition 5 min → Home cage 85 min → Empty cage 5 min → Recall 5 min

B Acquisition — DI vs illumination group (none, rest, run)

C exploration time (s) vs illumination group (none, rest, run)

D Recall — DI vs illumination group (none, rest, run), ** , **

E exploration time (s) vs illumination group (none, rest, run)

A

Empty cage 5 min → Acquisition 5 min (Laser Light ON during rest) → Home cage 85 min → Empty cage 5 min → Recall 5 min (Laser Light OFF)

B eYFP

C eNpHR

D

E

F

G

H

I

J

Empty cage 5 min → Acquisition 5 min (Laser Light OFF) → Home cage 85 min → Empty cage 5 min → Recall 5 min (Laser Light ON during rest)

K eYFP

L eNpHR

M

N

O

P

Q

R