Long-term Transverse Imaging of the Hippocampus with Glass Microperiscopes

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ABSTRACT

The hippocampus consists of a stereotyped neuronal circuit repeated along the septal-temporal axis. This transverse circuit contains distinct subfields with stereotyped connectivity that support crucial cognitive processes, including episodic and spatial memory. However, comprehensive measurements across the transverse hippocampal circuit *in vivo* are intractable with existing techniques. Here, we developed an approach for two-photon imaging of the transverse hippocampal plane in awake mice via implanted glass microperiscopes, allowing optical access to the major hippocampal subfields and to the dendritic arbor of pyramidal neurons. Using this approach, we tracked dendritic morphological dynamics on CA1 apical dendrites and characterized spine turnover. We then used calcium imaging to quantify the prevalence of place and speed cells across subfields. Finally, we measured the anatomical distribution of spatial information, finding a non-uniform distribution of spatial selectivity along the DG-to-CA1 axis. This approach extends the existing toolbox for structural and functional measurements of hippocampal circuitry.

INTRODUCTION

The hippocampus is critical for episodic and spatial memories[1–3], but the neural computations underlying these functions are not well understood. The trisynaptic circuit linking entorhinal cortex (EC) to dentate gyrus (DG), DG to CA3, and CA3 to CA1 is believed to endow the hippocampus with its functional capabilities. Since the circuit was first described in the anatomical studies of Ramon y Cajal over a century ago[4], considerable work has focused on each of the major hippocampal subfields (CA1-3 and DG) to identify their roles in hippocampal processing. The resulting body of literature has indicated that the subfields have related, but distinct roles in pattern separation and completion[5–14], response to novelty[15–19], and the encoding of social variables[20–22]. Additionally, there appear to be differences between the subfields in place field stability[19,23,24]. Although this work has increased our understanding of each of the subfields individually, it is not clear how neuronal activity is coordinated across the hippocampus.

This lack of knowledge comes, in part, from the technological limitations that prevent the recording of neuronal populations across hippocampal subfields in the same animal. Historically, electrophysiology has been the principal tool used to study the hippocampus. Electrophysiological recordings have the advantage of high temporal resolution and can directly measure spiking, but they are typically limited to small numbers of neurons in particular subfields. Additionally, localization of recorded neurons within the hippocampus is approximate,

requiring post-hoc histological analysis to estimate the position of the electrode tracks, and the distances between the recorded neurons and the electrode sites are poorly defined. In recent years, calcium imaging approaches (i.e. single photon mini-endoscopes and two-photon microscopy) have been used to record hippocampal activity, allowing for the simultaneous measurement of large numbers of neurons with known spatial relationships[24–29]. However, these approaches require aspiration of the overlying neocortex and are generally restricted to a single subfield for each animal.

Taken together, current experimental techniques are limited in their ability to: (1) record response dynamics and coordination across the hippocampus, (2) identify and distinguish between different neural subtypes, (3) allow for the chronic recording of cells across subfields, and (4) resolve key cellular structures, such as apical dendritic spines.

To address these challenges, we have developed a procedure for transverse imaging of the trisynaptic hippocampal circuit using chronically implanted glass microperiscopes. As has been found with previous studies using implanted microprisms in cortex[30–33], the neural tissue remained intact and healthy for prolonged periods of time (up to 10 months), and both dendritic structure and calcium activity could be repeatedly measured in behaving mice. Optical modeling and point spread function measurements showed that axial resolution is decreased compared to traditional cranial windows, but is sufficient to image individual apical dendritic spines in hippocampal neurons several millimeters below the pial surface. Using this approach, we quantified spine turnover in CA1 apical dendrites across days. We then measured functional responses from CA1, CA3, and DG in head-fixed mice as they explored a floating carbon fiber arena[34,35]. We found neurons in all regions whose activity met criteria to be considered place cells (PC) and speed cells (SC). Further, we found non-uniform distributions of spatial information across the extent of the DG-to-CA1 axis, in agreement with earlier electrophysiological studies[36–38]. Taken together, this approach adds to the existing neurophysiological toolkit by enabling chronic structural and functional measurements across the entire transverse hippocampal circuit.

RESULTS

Optical access to the transverse hippocampus using implanted microperiscopes

In order to image the transverse hippocampal circuit using two-photon (2P) imaging, we developed a surgical procedure for chronically implanting a glass microperiscope into the septal (dorsomedial) end of the mouse hippocampus (Fig. 1A; see Methods). For imaging CA1 only, we used a 1 mm x 1 mm x 2 mm microperiscope ($v1_{CA1}$; Fig 1B, left), and for imaging the entire

transverse hippocampus (CA1-CA3, DG), we used a 1.5 mm x 1.5 mm x 2.5 mm microperiscope ($v2_{HPC}$; Fig 1B, right). The microperiscope hypotenuse was coated with enhanced aluminum in order to reflect the imaging plane orthogonally onto the transverse plane of the hippocampus (Fig. 1C, D). To insert the microperiscope, we made a single incision through the dura and tissue, then lowered the tip of the microperiscope into the incision, pushing the cortical tissue medially. Although this approach eliminated the need for the aspiration of cortical tissue typically performed prior to hippocampal imaging[25], it nonetheless results in severed connections and compressed tissue medial to the implant. Since the septal end of the hippocampus is affected by the implant, we used immunohistochemistry to quantify the effect of microperiscope implantation on microglia and astrocyte proliferation as a function of distance from the prism face (Fig. 1E). Similar to previous research using microprism implants[31], we found an increase in the prevalence of astrocytes and microglia <200 μm from the microperiscope face, but the prevalence decreased past this distance and was indistinguishable from the control hemisphere 300-400 μm from the microperiscope face (Fig. 1F).

Use of the microperiscope requires imaging through several millimeters of glass, which could cause beam clipping or optical aberrations, resulting in decreased optical resolution. To determine the extent to which this occurred in our experiments, we modeled the expected point spread function and compared it to the experimentally-determined point spread function measurements using fluorescent microspheres (Fig. S1). Compared to a standard cranial window, we found that the lateral resolution of the microperiscope, measured as the full width at half maximum (FWHM) of the fluorescent microsphere profile, was similar to a standard cover slip (coverslip: 0.7 μm; $v1_{CA1}$ microperiscope: 1.0 μm; 2.5 mm microperiscope: 0.7; Fig. S1), while the axial resolution of the craniotomy window with the microperiscope was significantly lower (coverslip: 3.0 μm; 2 mm microperiscope: 9.0 μm; 2.5 mm microperiscope: 7.0 μm; Fig. S1). Optical modeling indicates that the decrease in axial resolution is predominantly due to clipping of the excitation beam, resulting in a reduction of the functional numerical aperture of the imaging system (theoretical point spread function of $v1_{CA1}$ microperiscope: 10.9 μm; $v2_{HPC}$ microperiscope: 7.7 μm; see Methods) rather than optical aberrations. As a result, use of adaptive optics did not significantly improve the axial resolution, though adaptive optics did improve the signal intensity by 40-80% (data not shown). Despite the decrease in axial resolution resulting from the microperiscopes, the resolution is still sufficient to clearly image individual HPC neurons (Fig. 1C) and sub-micron morphological structures (Fig. 2).

Resolving spines on the apical dendrites of CA1

Dendritic spines are highly dynamic and motile structures that serve as the postsynaptic sites of excitatory synapses in the hippocampus[39,40]. Previous *in vitro* studies suggest a role for dendritic spines in structural and functional plasticity, but the transient and dynamic nature of these structures make them ideally suited to being studied *in vivo*[41–43]. Although existing techniques allow imaging of spines on the basal CA1 dendrites near the surface of the hippocampus[43,44], imaging the apical dendritic spines of hippocampal neurons has not previously been possible. Using the microperiscope, we were able to track dendritic spine dynamics throughout the somato-dendritic axis of both CA1 and CA3 neurons in intact mice.

In order to visualize apical dendrites in CA1 neurons, we implanted a cohort of Thy1-GFP-M mice, sparsely expressing GFP in a subset of pyramidal neurons[45], with $v1_{CA1}$ microperiscopes (Fig. 2A; n = 7 mice). We focused on CA1 apical dendrites, but both apical and basal dendrites could be imaged in CA1-3 neurons. Although it is theoretically possible to image DG dendritic structures using the microperiscope, the Thy1-GFP-M mouse line has dense expression throughout DG (Fig. 1C), which prevented clear identification of distinct processes. To resolve individual spines along the dendrite, high-resolution images were taken from several axial planes spanning the segment, and a composite image was generated using a weighted average of individual planes (Fig. 2B, C; see Methods). We reduced noise by filtering and binarizing and isolated dendrites of interest for tracking across days (Fig. 2B-D; Fig. S2A; see Methods).

Previous studies have shown that dendritic spines fall into four major morphological subtypes: filopodium, thin, mushroom, and stubby[46–48]. We found that our resolution was sufficient to classify dendritic spines into their relative subtypes and evaluate density and turnover based on these parameters (Fig. 2E, F; Fig. S2B). Consistent with previous studies, we found a non-uniform distribution of dendritic spines: 30.4% thin, 41.0% stubby, 26.2% mushroom, and 2.3% filopodium ($F(3,100) = 51.47$, $p < 0.0001$, one-way ANOVA; Fig. 2E). The low proportion of filopodium found in this and previous 2P imaging and histological studies[49,50] (2-3%), as compared to electron microscopy studies[51,52] (~7%), may result from the narrow width of these structures causing them to fall below the detection threshold. Consistent with previous work[53–55], we found that particular classes, such as filopodium, had a high turnover rate, while other classes were more stable across sessions ($F(3,100) = 7.17$, $p < 0.001$, one-way ANOVA; Fig. 2F).

We found that total turnover dynamics reflect 15.0% $\pm$ 2.0% spine addition and 13.0% $\pm$ 1.9% spine subtraction across consecutive days (Fig. 2H, I). We computed the survival fraction,

137 a measure indicating the fraction of spines still present from day one[42–44]. Although daily spine
138 addition and subtraction was 15.0% in our original analysis (Fig. 2H, I), the survival fraction
139 curve yields a more conservative estimate of turnover dynamics. Across 10 days, we found a
140 23.5% net loss in original spines (Fig. 2G), indicating that most spine turnover takes place within
141 an isolated population of transient spines. Both our cumulative turnover and survival fraction
142 results were similar to previous findings from basal dendrites in CA1[43,44], indicating that apical
143 and basal dendrites exhibit similar spine dynamics. Although we only tracked spine turnover for
144 up to 10 days, we found that we could identify the same dendritic processes over long time
145 periods (up to 150 days; Fig. S3), allowing for long-term longitudinal experiments tracking
146 isolated dendritic structures.
147

148 **Recording place and speed cells in CA1, CA3, and DG**

149 Much of the experimental work testing the hypothesized roles of CA1, CA3, and DG neurons
150 has come from place cell (PC) recordings[7–9,13,56,57]. While the results of these studies have been
151 instrumental, it has not been possible to measure activity throughout the transverse
152 hippocampal circuit in the same animal . We therefore investigated the ability of our
153 microperiscope to record from PCs in each of the hippocampal subfields during exploration of a
154 spatial environment.
155 To measure functional responses, we implanted $v2_{HPC}$ microperiscopes in transgenic
156 mice expressing GCaMP6s in glutamatergic neurons[58,59] (see Methods). As with the Thy1-GFP-
157 M mice, we were able to image neurons from CA1-CA3, and DG in the same animal (Fig. 3A, B;
158 Video S1). In some cases, depending on microperiscope placement, we were able to record
159 from all three areas simultaneously (Fig. S4). In all HPC subfields, we found normal calcium
160 dynamics with clear transients (Fig. 3C, D; Videos S2, S3). The imaging fields remained stable
161 and the same field could be imaged over 100 days later (Fig. 3E). In addition, microperiscope
162 implantation allowed measurement of neural responses from mossy cells in the dentate gyrus
163 (Figs. S4, S5A) and, depending on prism placement, simultaneous imaging of deep-layer
164 cortical neurons in parietal cortex (Fig. S5B).
165 As 2P microscopy generally requires the animal to be head-fixed, the behavioral assays
166 used to probe PC activity are limited. Previous work has made use of virtual reality (VR)[24,25],
167 however it remains unclear how similar rodent hippocampal activity in real world environments
168 is to that in VR[60,61]. To study PC activity in a physical environment, our head-fixed mice explored
169 a carbon fiber arena that was lifted via an air table[34] (Fig. 4A; see Methods). The mice were thus
170 able to navigate the physical chamber by controlling their movement relative to the floor.

171 Although this approach lacks the vestibular information present in real world navigation, it
172 captures somatosensory and proprioceptive information missing from virtual environments.
173 Moreover, recent work has found that place field width and single cell spatial information using
174 this approach is comparable to the responses of free foraging animals[35]. For measurement of
175 place fields, we allowed mice to navigate a curvilinear track over the course of 20-40 minutes
176 (Video S4). As found previously[35], using a curvilinear track allowed for robust sampling of the
177 spatial environment and improved place field localization, though they could also be measured
178 in the open field. In order to measure spatial properties of the hippocampal neurons
179 independent of reward, we relied on exploration rather than active reward administration for
180 sampling of the environment.
181 To characterize place fields, we recorded from neurons in CA1, CA3, and DG (CA1: n =
182 1026; CA3: n = 832; and DG: n = 463) in transgenic mice with panexcitatory expression of
183 GCaMP6s (Fig. 4B; n = 8 mice). We found PCs in all three subfields, with a distribution that was
184 in general agreement with previous 2P imaging experiments[19,24] (Fig. 4C, F; CA1: 31.7%; CA3:
185 24.5%; DG: 17.7%; see Methods) and with fields that spanned the entirety of the track (Fig. 4E).
186 The spatial information and place field width of the CA1 place cells were similar to those found
187 in a previous study using the same floating chamber design[35]. We found that spatial information
188 was highest in CA1 (Fig. 4H; CA1: 0.86 $\pm$ 0.03 bits/inferred spike; CA3: 0.74 $\pm$ 0.04
189 bits/inferred spike; DG: 0.62 $\pm$ 0.05 bits/inferred spike; mean $\pm$ s.e.m.) and that place field
190 widths were comparable across the three regions (Fig. 4G; CA1: 18.3 $\pm$ 0.4 cm; CA3: 18.1 $\pm$
191 0.5 cm; DG: 18.7 $\pm$ 0.7 cm; mean $\pm$ s.e.m.). As cells responsive to speed have recently been
192 found in the medial EC[62,63] and CA1[63,64], we also identified neurons as speed cells (SCs) if their
193 activity was significantly related to running speed[62] (see Methods). We found SCs in CA1, CA3,
194 and DG, with all areas having cells that showed both increased and decreased activity with
195 higher running speeds (Fig. 4D, F). Speed cells were most abundant in DG (Fig. 4F; CA1: 9.1%;
196 CA3: 13.5%; DG: 30.2%), consistent with past work that found it was possible to decode the
197 speed of freely moving animals from the activity of DG, but not CA1[57].
198

199 **Distribution of PC properties along the DG-to-CA1 axis**

200 Recent work has suggested that PC properties are heterogeneously distributed along the extent
201 of the DG-to-CA1 axis. In particular, place field width and spatial information have been found to
202 vary among different subregions of CA3, with dorsal CA3 (dCA3)/CA2 having lower spatial
203 information and larger place fields than medial CA3 (mCA3)[36–38], and proximal CA3 having
204 values that were most similar to DG[13]. Such distributions could be supported by known

205 anatomical gradients in connectivity of CA3[65–69]. However, given that these studies required
206 separate animals for the recording of each location along the DG-to-CA1 axis, and that
207 electrophysiology has limited spatial resolution along the transverse axis, we used the
208 microperiscopes to measure these properties throughout the DG-to-CA1 axis in the same mice.
209 Using the $v2_{HPC}$ microperiscope, we simultaneously imaged from several hundred cells
210 (range: 270-292 neurons) extending from pCA3 to pCA1 (Fig. 5A). Recordings from distinct
211 imaging planes in different mice (n = 3) were compared by calculating the distance of individual
212 cells from the inflection point of the DG-to-CA1 transverse axis (Fig; 5A; see Methods). In
213 agreement with previous studies[36–38], we found a non-uniform distribution of spatial information
214 along this axis (Fig. 5B, C; $F(6, 801) = 2.67$, $p = 0.01$, General Linear F-test against a flat
215 distribution with the same mean). In particular, we found that pCA3 cells had spatial information
216 that was closer in value to those in DG (Fig. 5C; Fig. 4H), and that mCA3 had spatial
217 information that was greater than dCA3/CA2 (Fig. 5B, C). We found that place field widths were
218 smallest in mCA3, and largest in dCA3/CA2, although we failed to find a statistically significant
219 non-uniform distribution with respect to place field width across the extent of the DG-to-CA1 axis
220 (Fig. S6; $F(4, 221) = 1.61$, $p = 0.17$, General Linear F-test against a flat distribution with the
221 same mean).
222
223 **DISCUSSION**
224 The microperiscope hippocampal imaging procedure we developed allows researchers, for the
225 first time, to chronically image neuronal structure and functional activity throughout the
226 transverse hippocampal circuit in awake, behaving mice. This approach builds on microprism
227 procedures developed for imaging cortex[30–32], allowing multiple hippocampal subfields in the
228 same animal to be accessed optically. Using the microperiscope, we were able to resolve
229 spines on the apical dendrites of CA1 pyramidal cells and track them across time. Additionally,
230 we were able to characterize place cells (PCs) and speed cells (SCs) in all three hippocampal
231 subfields, and investigate their anatomical distribution across subfields.
232
233 **Comparison to other methods**
234 Historically, electrophysiology has been the principal tool used to study the hippocampus.
235 Electrophysiological recordings have much higher temporal resolution than calcium imaging and
236 can directly measure spikes, but have limited spatial resolution. Our approach allows large scale
237 imaging of neurons across multiple hippocampal subfields, with known spatial and
238 morphological relationships. In addition, our approach allows genetically-controlled labeling of

239 particular cell types, imaging of cellular structures such as dendrites and spines, and
240 unequivocal tracking of functional and structural properties of the same cells across time, none
241 of which are possible with existing electrophysiological approaches.
242 Several approaches making use of optical imaging have been developed for use in the
243 hippocampus. These include gradient index (GRIN) lenses[70,71], and cannulas that can be
244 combined with both one-photon (1P) head-mounted microendoscopes[26–28] and 2P
245 imaging[24,25,29]. These methods have been limited to horizontal imaging planes, making it difficult
246 to image CA3 and DG, and intractable to image all three subfields in the same animal. While all
247 of these methods cause damage to the brain, and some require the aspiration of the overlying
248 cortex, the damage is largely restricted to superficial hippocampus. This contrasts with the
249 implantation of our microperiscope, which is inserted into the septal end of the hippocampus
250 and necessarily causes some damage to the structure. Despite this, we find normal response
251 properties, including selectivity for location and speed, in CA1-CA3 and DG (Fig. 4). Damage in
252 the direction orthogonal to the transverse axis caused by the implantation of the microperiscope
253 is similar to that caused by microprisms in cortex[31], as glial markers were found to decay to
254 baseline levels 300-400 μm away from the face of the microperiscope. We showed that,
255 following successful implantation, the imaging fields were stable and the same cells could be
256 imaged up to 3 months later (Fig. 3C). However, we emphasize that tissue damage caused by
257 the microperiscope assembly to the hippocampus should be taken into consideration when
258 planning experiments and interpreting results.
259
260 **Structural and functional properties along the transverse hippocampal circuit**
261 We utilized the microperiscope in two experiments that would not have been possible with
262 existing methods: (1) we tracked the spines on apical CA1 dendrites *in vivo* (Fig. 2); (2) we
263 simultaneously recorded from PCs along the extent of the DG-to-CA1 transverse axis (Fig. 5).
264 Several studies have tracked the spines of basal CA1 dendrites *in vivo* by imaging the
265 dorsal surface of the hippocampus[43,44]. However, spines on the apical dendrites have not been
266 tracked *in vivo*. Given that these spines make up the majority of the input to CA1 pyramidal
267 neurons, there is a significant need for understanding their dynamics. Using the microperiscope,
268 we tracked isolated apical dendrites for up to 10 consecutive days (Fig. 2D). We found
269 moderate addition and subtraction across days, indicating dynamic turnover in apical dendrites
270 (Fig 2H, I). However, consistent with previous studies in basal spines[43,44], we found the majority
271 of spines (76.5%) survived throughout the imaging period (Fig. 2G). This high survival fraction
272 suggests that the cumulative turnover rates we observe are reflective of a distinct pool of

273 transient spines, while the majority of spines remain stable across days over a longer timescale.
274 These results add to the growing notion that, even in the absence of salient learning and reward
275 signals, dendritic spines are dynamic structures[43,72,73]. Understanding the nature and timescales
276 of these dynamics has significant implications for the reported instability of PCs[23,24,27,74–76].
277 Previous studies have found gradients of connectivity in CA3[65–69], suggesting that there
278 may be functional gradients as well. Electrophysiological recordings have indeed found that
279 spatial information and place field width vary as a function of distance along the DG-to-CA1
280 axis[36–38], and that the most proximal part of CA3 is functionally more similar to DG than to the
281 rest of CA3[13]. This has led to a more nuanced understanding of the hippocampal circuit, with
282 coarse anatomical subdivisions having finer functional subdivisions. However, given that these
283 previous studies relied on recordings in different animals for each location along the DG-to-CA1
284 axis, and given electrodes' poor spatial resolution in the transverse axis, the results have had to
285 be cautiously interpreted. Using the microperiscope, we imaged several hundred neurons in
286 multiple mice along the extent of the DG-to-CA1 axis (Fig. 5A). The location of each neuron
287 relative to the CA3 inflection point could be easily identified, allowing for unequivocal
288 characterization of spatial information and place field width along the DG-to-CA1 axis across
289 several mice (Fig. 5B, C; Fig. S6). Similar to the previous studies[36–38], we found a non-uniform
290 distribution of spatial information (Fig. 5B, C). Place field width also appeared to vary along the
291 DG-to-CA1 axis, though we failed to find statistically significant non-uniformity with respect to
292 place field width (Fig. S6B). Indeed, we found that spatial information, but not place field width,
293 differed significantly between CA1, CA3, and DG (Fig. 4H). Given the high spatial resolution and
294 ability to simultaneously record from cells across the DG-to-CA1 axis using this approach, our
295 results strengthen the hypothesis of distributed spatial coding across hippocampal subfields.
296

297 **Future applications**

298 This paper explored a few possible uses for the microperiscope in interrogating the hippocampal
299 circuit. However, there are a number of candidate applications we did not pursue in this
300 manuscript that could reveal novel insight into hippocampal function. Here, we describe several
301 potential applications: (1) The microperiscope allows for the recording from multiple
302 hippocampal subfields simultaneously (Fig. S4), allowing investigation of interactions between
303 neurons in different subfields during behavior. This could also be useful for determining the
304 effect of neuron- or subfield-specific optogenetic manipulations on downstream subfields. (2)
305 The microperiscope enables the investigation of local circuits by allowing morphological and
306 genetic identification of different cell types. This includes identifying genetically-distinct

307 hippocampal neurons (e.g. CA2 neurons, specific interneuron subtypes) as well was identifying
308 particular cell types by position or morphological characteristics (e.g. mossy cells in DG; Fig.
309 S5A). As these distinct cell types play important roles in hippocampal function[20,77–81], having
310 access to them will enable a greater understanding of the hippocampal circuit. (3) The
311 microperiscope can be combined with retrograde-transported viruses to allow projection-based
312 cell identification, making it possible to identify neurons that project to specific downstream
313 targets. Since hippocampal neurons that project to different brain regions have been found to
314 exhibit distinct functional properties (e.g. neurons in ventral CA1 that project to the nucleus
315 accumbens shell have been implicated in social memory[21]), the union of these tools will be a
316 powerful means for understanding hippocampal outputs. (4) Finally, the microperiscope
317 provides access to the entire dendritic tree of pyramidal neurons (Figs. 1C, 2A, 4B; Video S2),
318 giving optical access to dendritic signaling over a much larger spatial extent than has been
319 previously possible[82–84]. By sparsely expressing calcium or glutamate sensors in hippocampal
320 pyramidal neurons, spines throughout the somatodendritic axis could be imaged, allowing
321 determination of how place field responses arise from the responses of individual spines, similar
322 to experiments in visual cortex investigating the cellular origin of orientation tuning from synaptic
323 inputs[85,86].
324 Combined with electrophysiological and traditional imaging approaches, imaging of the
325 transverse hippocampal circuit with microperiscopes will be a powerful tool for investigating
326 hippocampal circuitry, structural dynamics, and function.

FIGURES

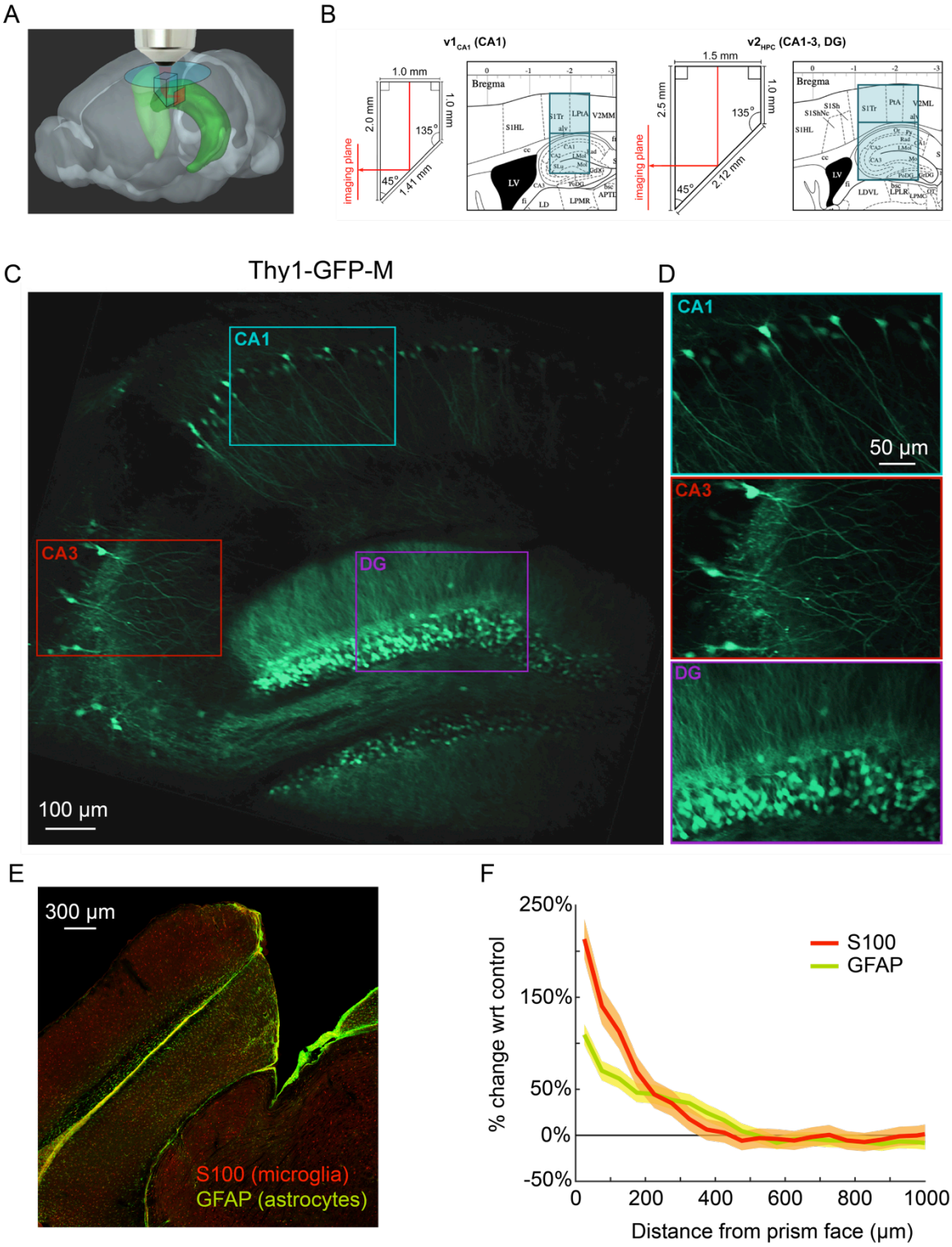
A
B
v1CA1 (CA1)
v2HPC (CA1-3, DG)
1.0 mm
2.0 mm
1.0 mm
135°
45°
1.41 mm
imaging plane
Bregma
1.5 mm
2.5 mm
1.0 mm
135°
45°
2.12 mm
imaging plane
Bregma
C
Thy1-GFP-M
CA1
CA3
DG
100 µm
D
CA1
50 µm
CA3
DG
E
300 µm
S100 (microglia)
GFAP (astrocytes)
F
250%
150%
50%
0%
-50%
% change wrt control
S100
GFAP
0
200
400
600
800
1000
Distance from prism face (µm)

Figure 1. Implanted microperiscopes allow imaging of the hippocampal transverse plane.

(A) Three-dimensional schematic[87] illustrating microperiscope implantation and light path for hippocampal imaging.

(B) Schematics[88] showing the imaging plane location of $v1_{CA1}$ (1 mm imaging plane, 2 mm total length) and $v2_{HPC}$ (1.5 mm imaging plane, 2.5 mm total length) microperiscopes.

(C) Tiled average projection of the transverse imaging plane using the $v2_{HPC}$ microperiscope implant in a Thy1-GFP-M transgenic mouse. Scale bar = 100 μm.

(D) Enlarged images of hippocampal subfields (CA1, CA3, DG) corresponding to the rectangles in (C). Scale bar = 50 μm.

(E) Example histological section stained for microglia (S100 - red) and astrocytes (GFAP - green). Scale bar = 300 μm.

(F) Quantification of microglia and astrocyte density as a function of distance from the prism face, normalized to the density in the unimplanted contralateral hemisphere (n = 2 mice; mean $\pm$ bootstrapped s.e.m.).

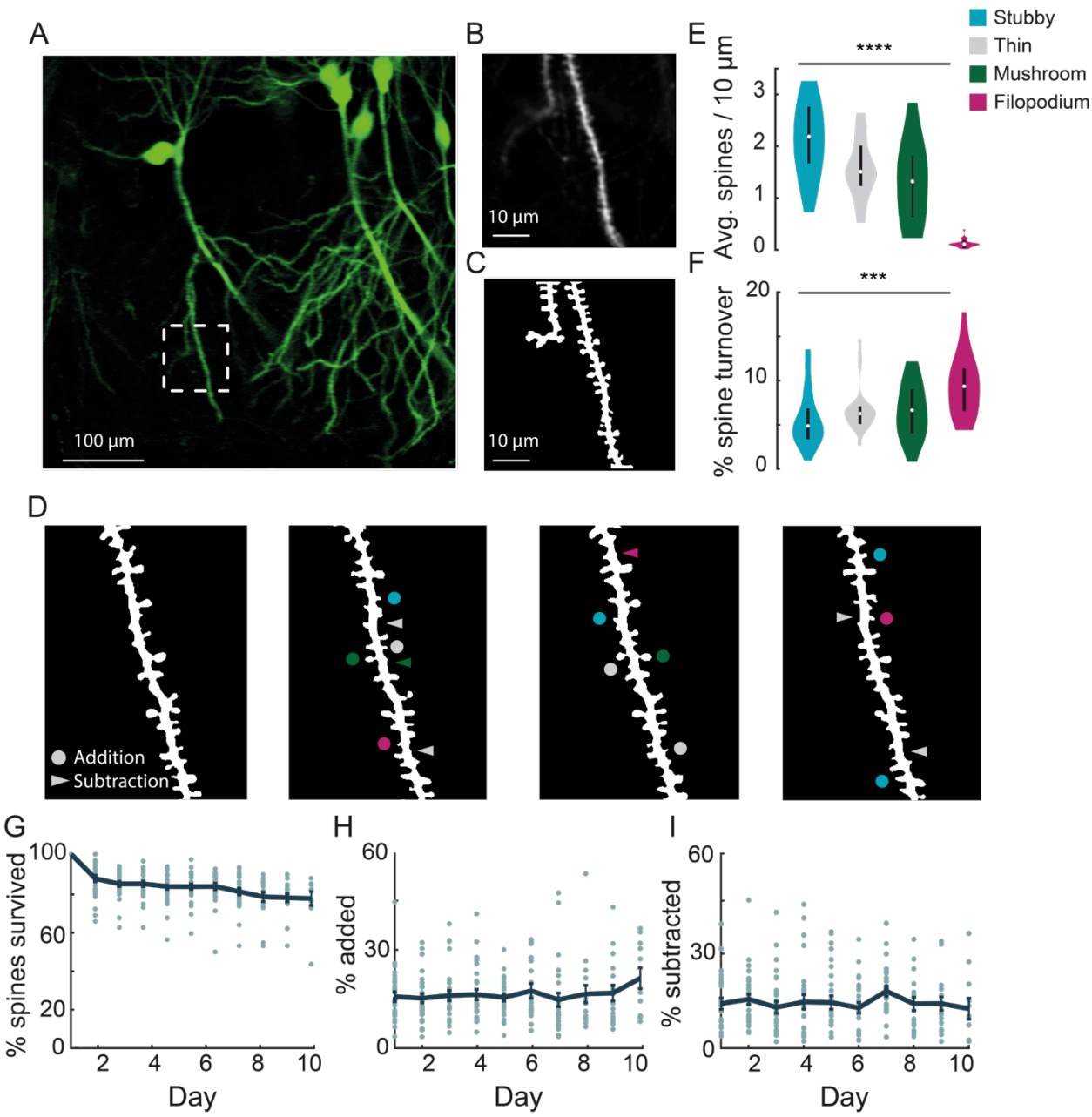

Figure 2. Chronic imaging of spine morphology in CA1 apical dendrites.

(A) Average projection of CA1 neurons sparsely expressing a GFP reporter (Thy1-GFP-M) imaged through the $v1_{CA1}$ microperiscope. Scale bar = 100 μm.

(B) Weighted projection (see Methods) of the apical dendrites shown in the dashed box of (A). Scale bar = 10 μm.

(C) Filtered and binarized image (Figure S2A; see Methods) of the dendrites in (B) to allow identification and classification of individual dendritic spines. Scale bar = 10 μm.

(D) Tracking CA1 dendritic spines over consecutive days on a single apical dendrite. Arrowheads indicate subtracted spines and circles indicate added spines. Colors indicate spine type of added and subtracted spines: filopodium (magenta), thin (grey), stubby (blue), and mushroom (green).

(E) Average number of spines per 10 μm section of dendrite, for each of the four classes of spine (n = 26 dendrites from 7 mice). one-way ANOVA, $F(3,100) = 51.47$, ****$p < 0.0001$

(F) Percent spine turnover across days in each spine type (n = 26 dendrites from 7 mice). one-way ANOVA, $F(3,100) = 7.17$, ***$p < 0.001$.

(G) Spine survival fraction across processes (n = 26 dendrites from 7 mice) recorded over 10 consecutive days.

(H) Percent of spines added between days over 10 days of consecutive imaging.

(I) Percent of spines subtracted between days over 10 days of consecutive imaging.

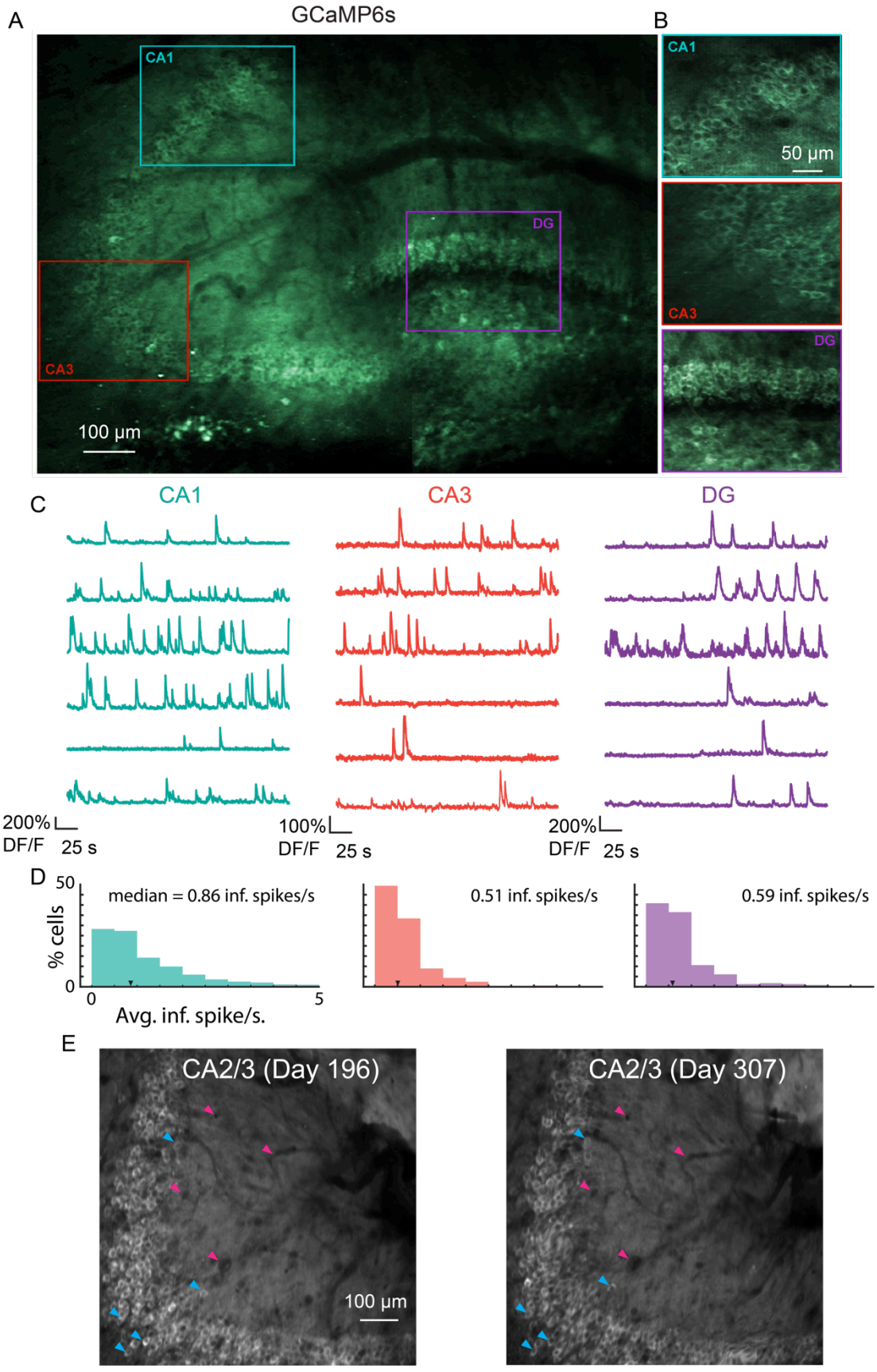
A
GCaMP6s
CA1
DG
CA3
100 µm
B
CA1
50 µm
CA3
DG
C
CA1
CA3
DG
200%
DF/F
25 s
100%
DF/F
25 s
200%
DF/F
25 s
D
50
% cells
0
median = 0.86 inf. spikes/s
0
5
Avg. inf. spike/s.
0.51 inf. spikes/s
0.59 inf. spikes/s
E
CA2/3 (Day 196)
100 µm
CA2/3 (Day 307)

Figure 3. Microperiscope imaging of calcium dynamics across subfields in awake mice.

(A) Example tiled image (as in Fig. 1C) for a single panexcitatory transgenic GCaMP6s mouse (Slc17a7-GCaMP6s). Scale bar = 100 μm.

(B) Enlarged images of each hippocampal subfield (CA1, CA3, DG) corresponding to the rectangles in (A). Scale bar = 50 μm.

(C) Example GCaMP6s normalized fluorescence time courses (% DF/F) for identified cells in each subfield.

(D) Distribution of average inferred spiking rate during running for each hippocampal subfield (CA1, CA3, DG). Median is marked by black arrowhead.

(E) Example average projection of CA2/3 imaging plane 196 days post implantation (left) and 111 days later. Image was aligned using non-rigid registration (see Methods) to account for small tissue movements. Magenta arrowheads mark example vasculature and blue arrowheads mark example neurons that are visible in both images. Scale bar = 100 μm.

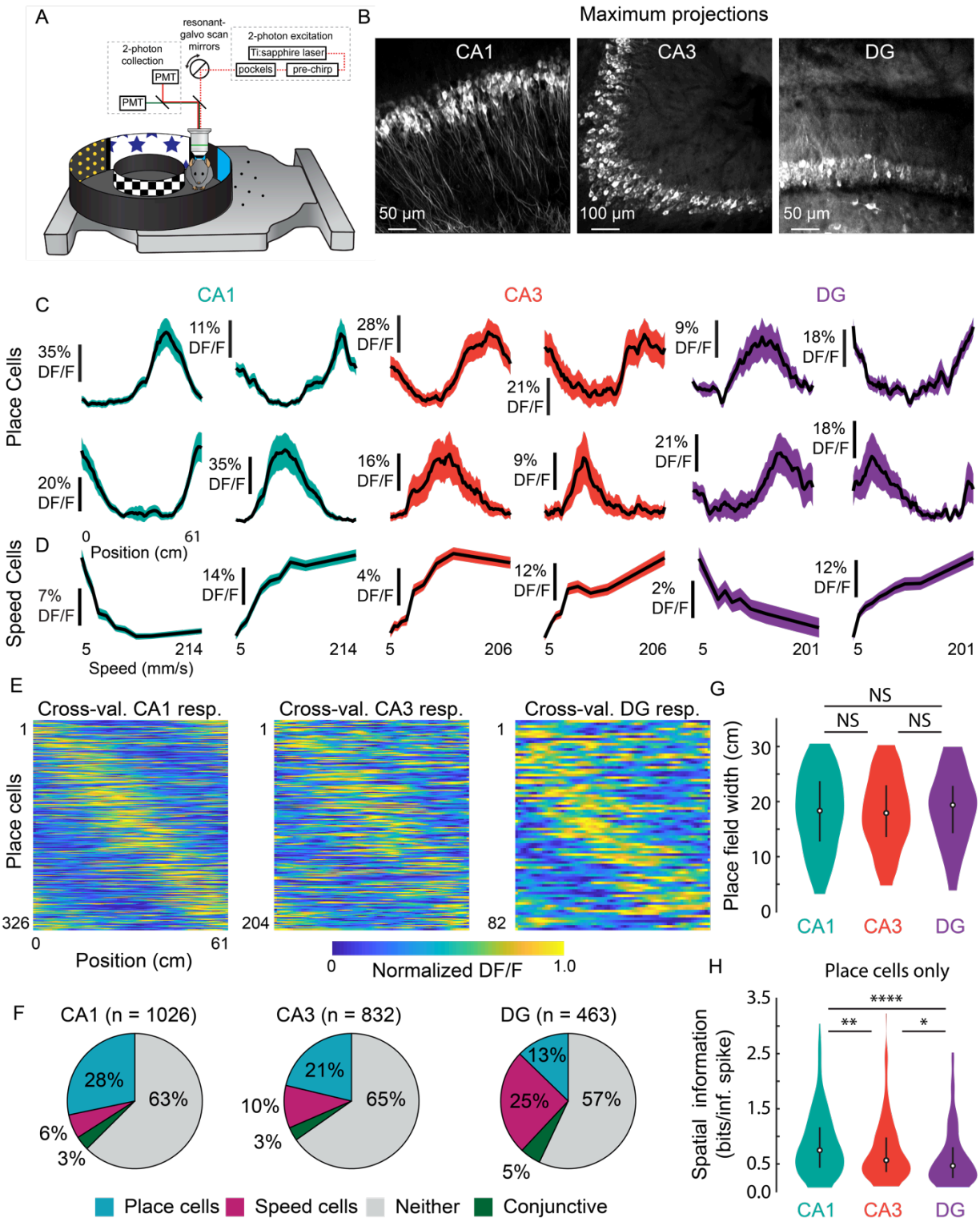
A
resonant-galvo scan mirrors
2-photon collection
2-photon excitation
Ti:sapphire laser
pockels
pre-chirp
PMT
PMT
B
Maximum projections
CA1
CA3
DG
50 µm
100 µm
50 µm
C
CA1
CA3
DG
Place Cells
35% DF/F
11% DF/F
28% DF/F
21% DF/F
9% DF/F
18% DF/F
20% DF/F
35% DF/F
16% DF/F
9% DF/F
21% DF/F
18% DF/F
D
Speed Cells
0
61
Position (cm)
7% DF/F
14% DF/F
4% DF/F
12% DF/F
2% DF/F
12% DF/F
5
214
5
214
5
206
5
206
5
201
5
201
Speed (mm/s)
E
Cross-val. CA1 resp.
Cross-val. CA3 resp.
Cross-val. DG resp.
Place cells
1
326
204
82
0
61
Position (cm)
0
Normalized DF/F
1.0
G
NS
NS
NS
Place field width (cm)
30
20
10
0
CA1
CA3
DG
H
Place cells only
Spatial information (bits/inf. spike)
3.5
2.5
1.5
0.5
0.0
****
**
*
CA1
CA3
DG
F
CA1 (n = 1026)
28%
63%
6%
3%
CA3 (n = 832)
21%
65%
10%
3%
DG (n = 463)
13%
57%
25%
5%
Place cells
Speed cells
Neither
Conjunctive

Figure 4. Prevalence of place and speed cells across hippocampal subfields.

(A) Schematic of air-lifted carbon fiber circular track (250 mm outer diameter) that the mice explored during imaging (Video S4). Four sections of matched visual cues lined the inner and outer walls.

(B) Example maximum projections of GCaMP6s-expressing neurons in each subfield. Scale bar = 50, 100, 50 μm respectively.

(C) Plots of mean normalized calcium response (% DF/F) versus position along the circular track for four identified place cells (PCs) in each subfield. Shaded area is s.e.m.

(D) Plots of mean calcium response (% DF/F) versus speed along the circular track for two identified speed cells (SCs) in each subfield. Shaded area is s.e.m.

(E) Cross-validated average responses (normalized DF/F) of all PCs found in each subfield, sorted by the location of their maximum activity. Responses are plotted for even trials based on peak position determined on odd trials to avoid spurious alignment.

(F) Distribution of cells that were identified as PCs, SCs, conjunctive PC+SCs, and non-coding in each subfield.

(G) Distribution of place field width for all place cells in each subfield. Error bars indicate the interquartile range (75th percentile minus 25th percentile). Two-sample Kolmogorov-Smirnov test: CA1-CA3, $p = 0.47$; CA3-DG, $p = 0.21$; CA1-DG, $p = 0.58$. NS, not significant ($p > 0.05$).

(H) Distribution of spatial information (bits per event) for all place cells in each subfield. Error bars are the same as in (G). Two-sample Kolmogorov-Smirnov test: CA1-CA3, $p = 0.0087$; CA3-DG, $p = 0.04$; CA1-DG, $p = 7.6\times10^{-5}$. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

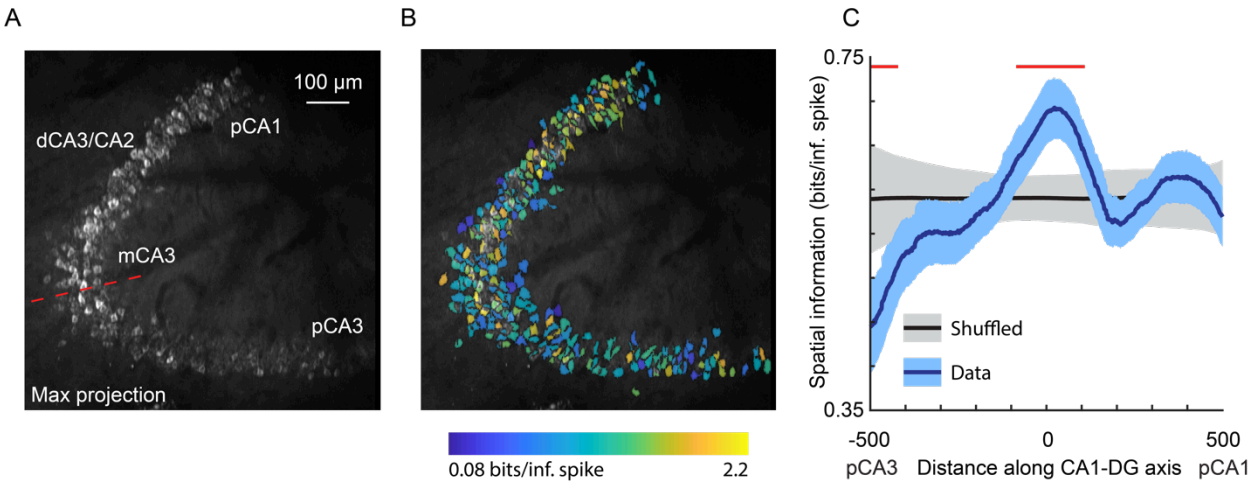

Figure 5. Spatial selectivity of neurons varies along the DG-to-CA1 axis.

(A) Maximum projection of an example DG-to-CA1 axis recording. Approximate locations of CA3 and CA1 subfields are labeled. Inflection point labeled with red line. Scale bar = 100 μm.

(B) Spatial information (bits/inferred spike) pseudo-colored on a logarithmic scale, for each neuron overlaid on the maximum projection in (A).

(C) Spatial information, as a function of distance along the DG-to-CA1 axis (pCA3 to dCA1), of real data (blue) vs. shuffled control (black). Shaded area is bootstrapped s.e.m. Red lines indicate values that are outside the shuffled distribution ($p < 0.05$). A general linear F-test revealed significant non-uniformity (n = 3 fields from 3 mice; $F(6, 801) = 2.67$, $p = 0.01$).

SUPPLEMENTAL FIGURES

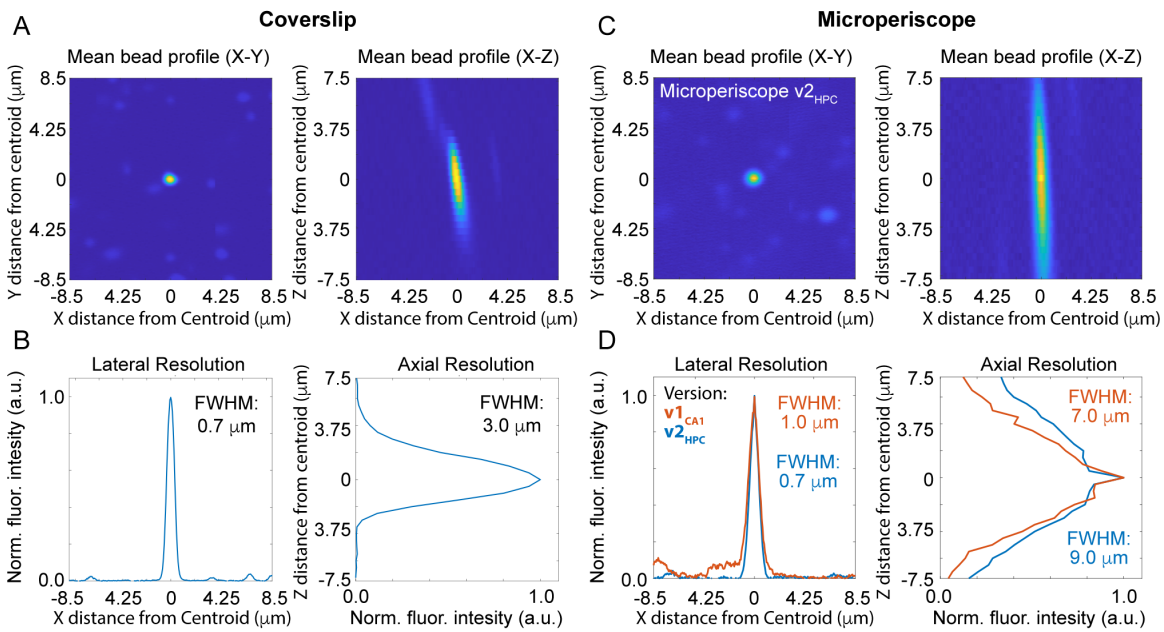

Figure S1: Lateral and axial resolution of cranial window and microperiscopes.

(A) Average X-Y profile (left) and X-Z profile (right) of 0.1 μm fluorescent microspheres (n = 17 microspheres) embedded in agar under a single glass coverslip (0.15 mm thickness), imaged with a 16x/0.8NA Nikon objective.

(B) Plot of normalized fluorescence intensity profile of X dimension (lateral resolution; FWHM = 0.7 μm) and Z dimension (axial resolution; FWHM = 3.0 μm) through the centroid of the microsphere (n = 17 microspheres).

(C) Average X-Y profile (left) and X-Z profile (right) of 0.1 μm fluorescent microspheres (n = 20 microspheres) imaged through the $v2_{HPC}$ microprism (2.5 mm path length through glass) with a 16x/0.8NA Nikon objective.

(D) Plot of normalized fluorescence intensity profile of X dimension (lateral resolution; $v1_{CA1}$ FWHM = 1.0 μm; $v2_{HPC}$ FWHM = 0.7 μm) and Z dimension (axial resolution; $v1_{CA1}$ FWHM = 7.0 μm; $v2_{HPC}$ FWHM = 9.0 μm) through the centroid of the microsphere (n = 10 microspheres for $v1_{CA1}$, n = 20 microspheres for $v2_{HPC}$).

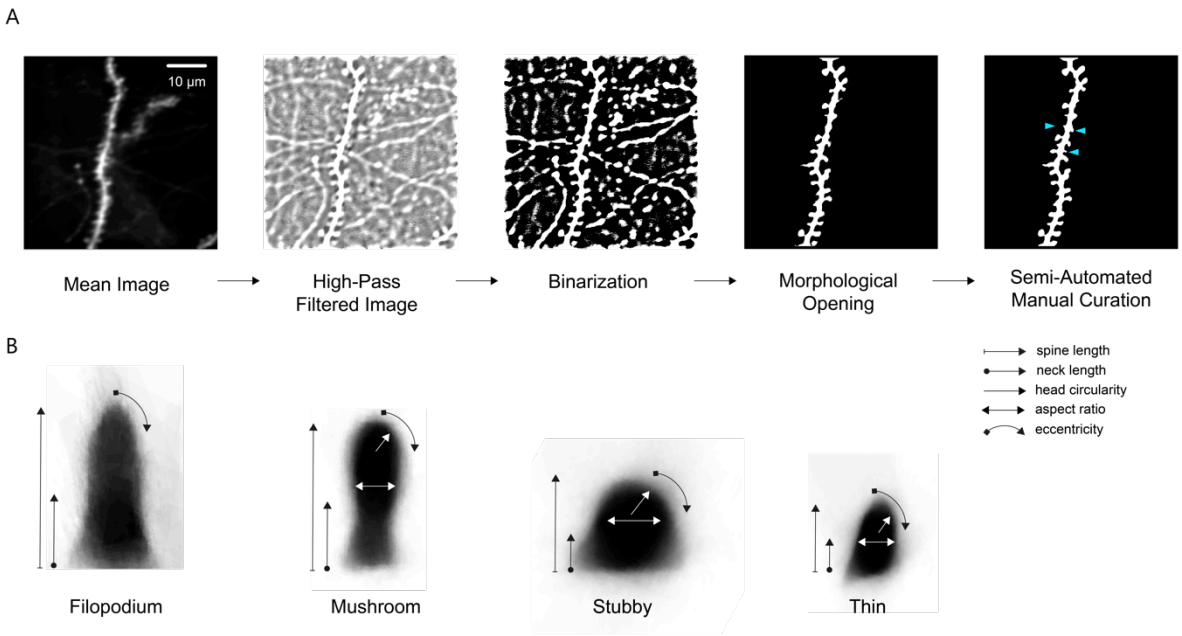

Figure S2: Dendritic morphology image processing pipeline and spine classification.

(A) Steps in dendritic morphology image processing pipeline (see Methods). Weighted mean image is obtained by acquiring average projections from 4 planes spaced by 3 µm and weighting the images around the brightest part of the dendrite. Next, the mean image is high-pass filtered to reveal fine structures. The image is then binarized using a global threshold, to filter out less prominent dendrites. Morphological opening is applied to remove any elements of the high-pass filtered image that survive the binarization process that are too small to be the principal dendrite. A semi-automated manual curation process is then used to add back any individual spines that were lost during binarization and remove any unwanted dendritic segments. Cyan arrowheads indicate manually added spines. To isolate the spines, the image is skeletonized and structures that protruded laterally from the dendritic shaft and had a total area of >1 μm^2 were identified as spines. Scale bar = 10 μm.

(B) Mean profile of all filopodium, mushroom, stubby, and thin spines (*n* = 47, 431, 695, and 495, respectively), computed by averaging across the registered spines of each morphological classification. Spines were classified according to spine length, neck length, neck width, head circularity, and eccentricity (see Methods).

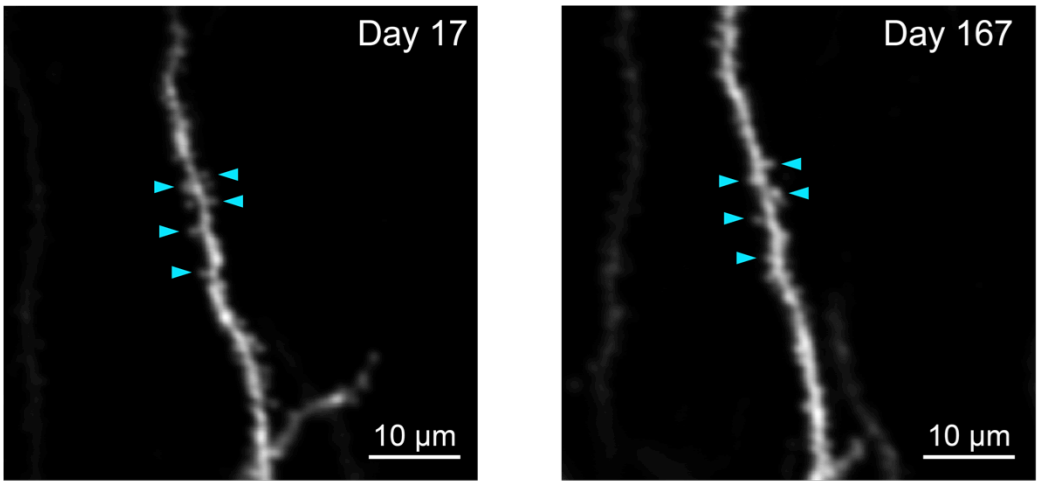

Figure S3: Long-term imaging of the same dendrite.

Weighted average projection of the same dendritic process 150 days apart. Spines present at both time points indicated by cyan arrowheads. Scale bar = 10 μm.

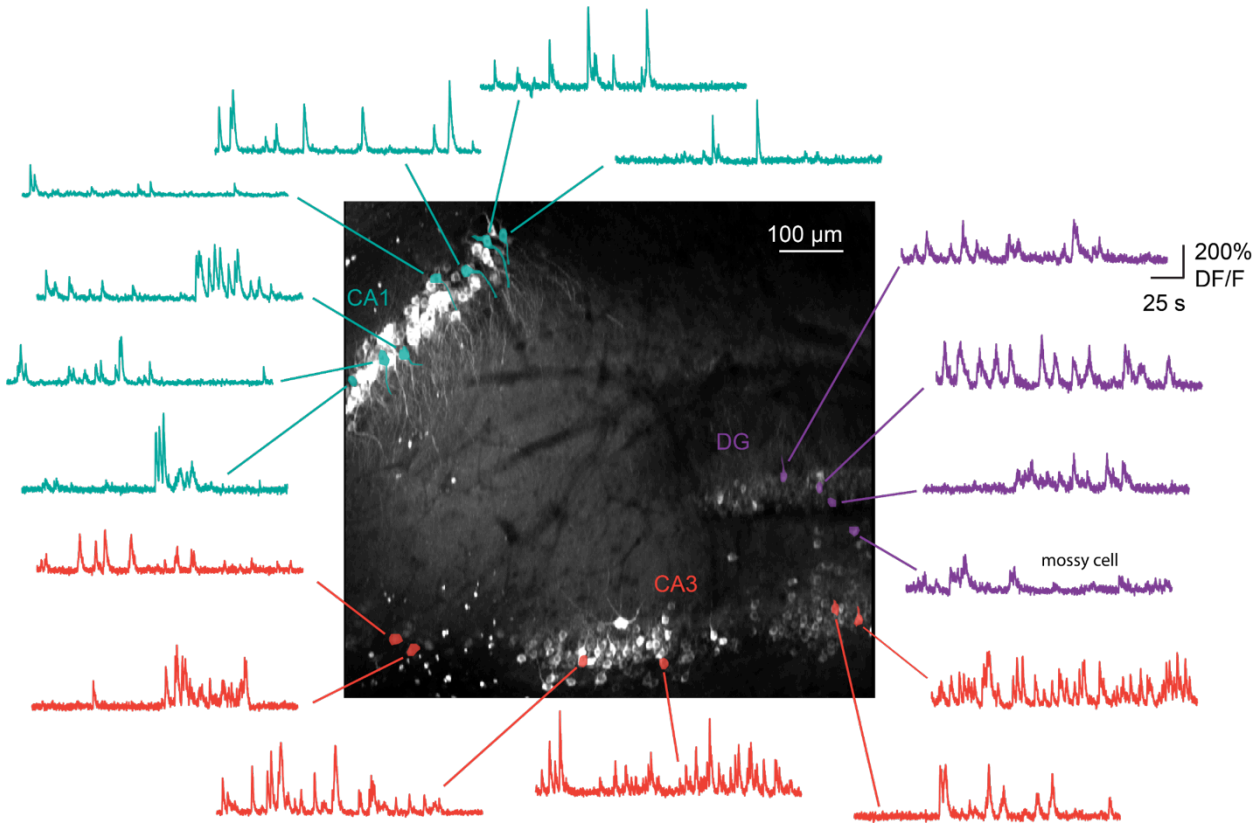

Figure S4. Simultaneous imaging of all three hippocampal subfields.

Maximum projection, and example GCaMP6s fluorescence time courses for identified cells, from a recording in which CA1 (n = 55 cells), CA3 (n = 158), and DG (n = 28) were simultaneously recorded from the same image plane. Scale bar = 100 μm.

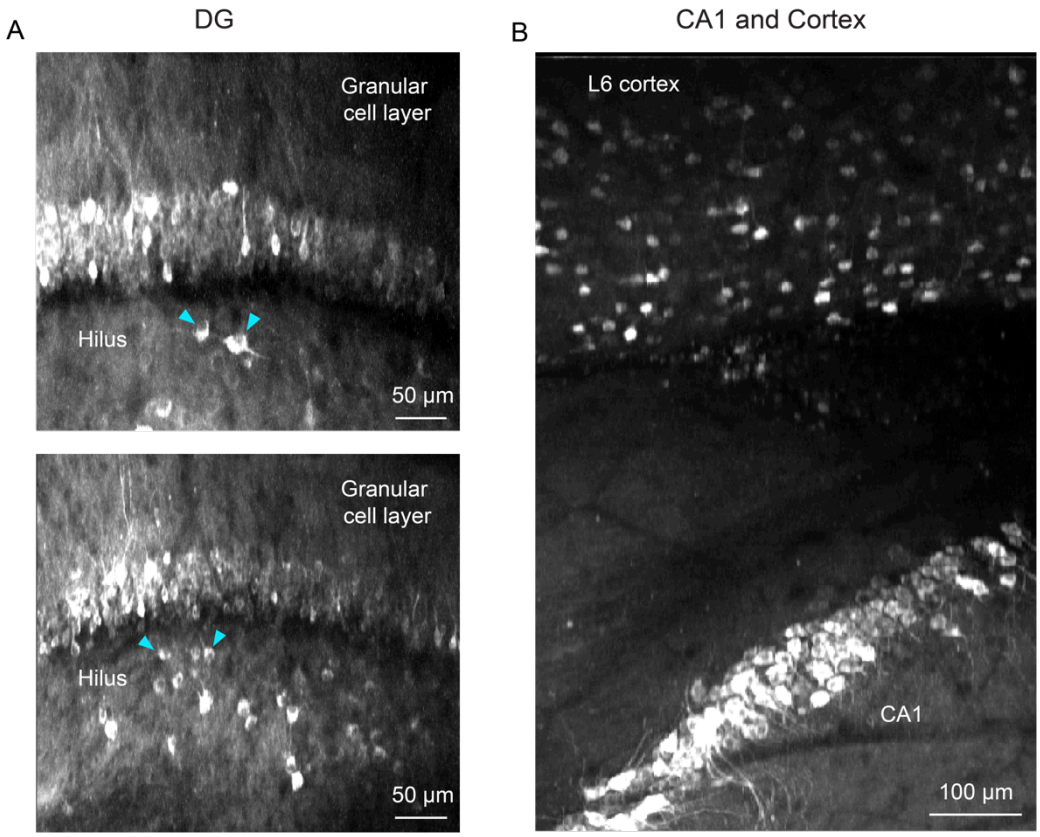

Figure S5. Additional applications for microperiscope imaging.

(A) Two maximum projections of imaging planes in which both the granular cell layer and the hilus in the DG could be imaged simultaneously. Example putative mossy cells indicated with cyan arrowheads. Scale bar = 50 μm.

(B) Maximum projection from an imaging session in which both CA1 and L6 of the neocortex are visible, allowing simultaneous imaging of neurons in CA1 and deep layers of the parietal cortex. Scale bar = 100 μm.

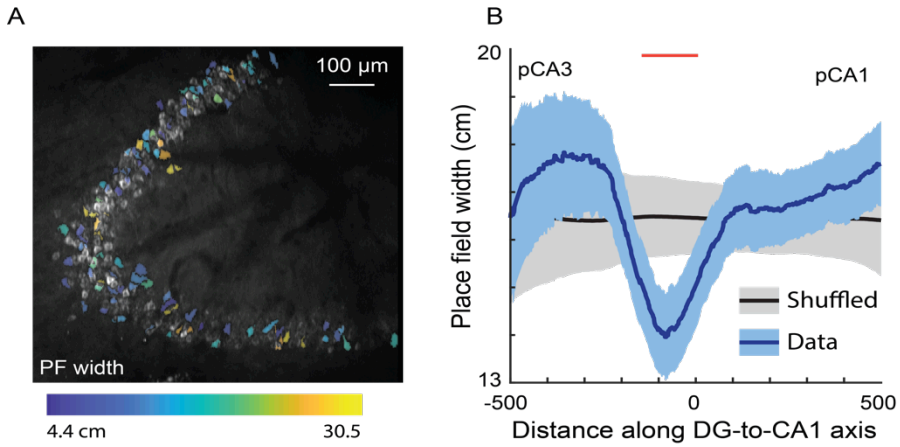

Figure S6. Place field width along the DG-to-CA1 axis.

(A) Place field width pseudo-colored for each place cell overlaid on the maximum projection from Fig. 5A. Any cell that was not a place cell is not pseudo-colored. Scale bar = 100 μm.

(B) Place field width, as a function of distance along the DG-to-CA1 axis (pCA3 to pCA1), of real data (blue) vs. shuffled control (black). Shaded area is s.e.m. Red lines indicate individual positions with place field values that were outside of the shuffled distribution ($p < 0.05$). However, a General Linear F-test revealed no significant non-uniformity: $F(4, 221) = 1.61$, $p = 0.17$.

SUPPLEMENTAL VIDEOS

Video S1. Demonstration of two-photon imaging of a Slc17a7-GCaMP6s mouse through the microperiscope. Recording starts in the superficial cortex in front of microperiscope, then moves through the microperiscope to the hippocampus, zooming in on subfields CA1, CA3, and DG.

Video S2. Calcium activity in subfield CA1 in a Slc17a7-GCaMP6s mouse imaged through the microperiscope.

Video S3. Calcium activity in subfield DG in a Slc17a7-GCaMP6s mouse imaged through the microperiscope.

Video S4. Mouse running in the floating chamber circular track. Ambient light is higher than usual levels for improved video quality.

327 ## METHODS

328 **Animals**

329 For dendritic morphology experiments, Thy1-GFP-M (Jax Stock #007788) transgenic mice (*n* =
330 7) were used for sparse expression of GFP throughout the forebrain. For forebrain-wide calcium
331 indicator expression, Emx1-IRES-Cre (Jax Stock #005628) x ROSA-LNL-tTA (Jax Stock
332 #011008) x TITL-GCaMP6s (Jax Stock #024104) triple transgenic mice (*n* = 2) or Slc17a7-
333 IRES2-Cre (Jax Stock #023527) x TITL2-GC6s-ICL-TTA2 (Jax Stock #031562) double
334 transgenic mice (*n* = 6) were bred to express GCaMP6s in excitatory neurons. For imaging
335 experiments, 8-51 week old (median 17 weeks) mice of both sexes (6 males and 9 females)
336 were implanted with a head plate and cranial window and imaged starting 2 weeks after
337 recovery from surgical procedures and up to 10 months after microperiscope implantation. The
338 animals were housed on a 12 hr light/dark cycle in cages of up to 5 animals before the implants,
339 and individually after the implants. All animal procedures were approved by the Institutional
340 Animal Care and Use Committee at University of California, Santa Barbara.

341

342 **Surgical Procedures**

343 All surgeries were conducted under isoflurane anesthesia (3.5% induction, 1.5 - 2.5%
344 maintenance). Prior to incision, the scalp was infiltrated with lidocaine (5 mg kg-1,
345 subcutaneous) for analgesia and meloxicam (2 mg kg-1, subcutaneous) was administered
346 preoperatively to reduce inflammation. Once anesthetized, the scalp overlying the dorsal skull
347 was sanitized and removed. The periosteum was removed with a scalpel and the skull was
348 abraded with a drill burr to improve adhesion of dental acrylic.

349 For hippocampal imaging, we used two types of custom-designed glass microperiscope
350 (Tower Optical). The first ($v1_{CA1}$), for imaging the upper part of the hippocampus (CA1/CA2)
351 consisted of a 1 x 1 x 1 mm square base and a 1 mm right angle prism, for a total length of 2
352 mm on the longest side (Fig. 1B, left). The second ($v2_{HPC}$), for imaging the entire transverse
353 circuit (CA1-3, DG) had a 1.5 x 1.5 x 1.0 mm (L x W x H) square base and a 1.5 mm right angle
354 prism, for a total length of 2.5 mm on the longest side (Fig. 1B, right). The hypotenuse of the
355 right angle prisms were coated with enhanced aluminum for internal reflectance. The
356 microprism was attached to a 5 mm diameter coverglass (Warner Instruments) with a UV-cured
357 optical adhesive (Norland, NOA61). Prior to implantation, the skull was soaked in sterile saline
358 and the cortical vasculature was inspected to ensure that no major blood vessels crossed the
359 incision site. If the cortical vasculature was suitable, a 3-4 mm craniotomy was made over the
360 implantation site (centered at 2.2 mm posterior, 1.2-1.7 mm lateral to Bregma). For the smaller

361 microperiscope ($v1_{CA1}$), a 1 mm length anterior-to-posterior incision centered at -2.1 mm
362 posterior, 1.2 mm lateral to Bregma was then made through the dura, cortex, and mediodorsal
363 tip of the hippocampus to a depth of 2.2 mm from the pial surface with a sterilized diamond
364 micro knife (Fine Science Tools, #10100-30) mounted on a manipulator. For the larger
365 microperiscope ($v2_{HPC}$), two overlapping 1.0 mm length anterior-to-posterior incisions were
366 made centered at -1.8 mm posterior / 1.7 mm lateral and -2.4 mm posterior / 1.7 mm lateral to
367 Bregma to a depth of 2.7 mm, with a total anterior-to-posterior incision length of 1.6 mm. Note
368 that placements in the regions shown in Fig. 1B required incision coordinates slightly posterior
369 to those indicated on the atlas. Care was taken not to sever any major cortical blood vessels.
370 Gelfoam (VWR) soaked in sterile saline was used to remove any blood from the incision site.
371 Once the incision site had no bleeding, the craniotomy was submerged in cold sterile saline,
372 and the microprism was lowered into the cortex using a manipulator, with the imaging face of
373 the prism facing lateral. Once the microprism assembly was completely lowered through the
374 incision until the coverglass was flush with the skull, the edges of the window were sealed with
375 silicon elastomer (Kwik-Sil, World Precision Instruments), then with dental acrylic (C&B-
376 Metabond, Parkell) mixed with black ink. Care was taken that the dental cement did not protrude
377 over the window, as it could potentially scratch the objective lens surface. Given the working
378 distance of the objective used in this study (3 mm), the smaller microperiscope ($v1_{CA1}$) implant
379 enabled imaging from 2250 - 2600 μm below the coverglass surface, corresponding to
380 approximately 150 - 500 μm into the lateral hippocampus (the 150 μm of tissue nearest to the
381 prism face was not used for imaging). The larger microperiscope ($v2_{HPC}$) implant enabled
382 imaging from 2650 - 2850 μm below the coverglass surface, corresponding to approximately
383 150 - 350 μm into the lateral hippocampus (the 150 μm of tissue nearest to the prism face was
384 not used for imaging). The microprism implantations were stable for up to ten months following
385 the surgery.
386 After microperiscope implantation, a custom-designed stainless steel head plate
387 (eMachineShop.com) was affixed using dental acrylic (C&B-Metabond, Parkell) mixed with
388 black ink. After surgery, mice were administered carprofen (5 - 10 mg kg-1, oral) every 24 hr for
389 3 days to reduce inflammation. Microperiscope designs and head fixation hardware are
390 available on our institutional lab web site (https://goard.mcdb.ucsb.edu/resources).

391 **Point Spread Function Measurements**

392 To measure empirical point spread functions, fluorescent microspheres (0.2 μm yellow-green
393 fluorescent microspheres; ThermoFisher F8811) were embedded 1:2000 in 0.5% agar and
394 placed under the cranial window or on the face of the microperiscope. Image stacks were taken

395 through the microspheres (0.06 μm per pixel in XY; 0.5 μm per plane in Z), and candidate
396 microspheres were isolated using a watershed algorithm (Matlab image processing toolbox).
397 Only microspheres that were >20 pixels (1.2 μm) away from nearest neighbor microspheres and
398 completely contained within the Z-stack were used for further analysis. We registered isolated
399 microspheres at their centroids and measured the full width at half maximum (FWHM) of the
400 average XY and XZ profiles to determine the lateral and axial resolution, respectively.
401 Since the geometry of the microperiscope limits the angle of the focusing light cone
402 through the microperiscope, it predominately determines the functional numerical aperture at
403 the imaging plane. Based on the microperiscope geometry, we calculated the effective NA of
404 the $v1_{CA1}$ microperiscope and $v2_{HPC}$, and used it to calculate the theoretical lateral and axial
405 resolution according to the following formulae[89]

$$\omega_{XY} = \begin{cases} \dfrac{0.320\,\lambda}{\sqrt{2}\,NA} & NA < 0.7 \\ \dfrac{0.325\,\lambda}{\sqrt{2}\,NA^{0.91}} & NA > 0.7 \end{cases}$$

$$\omega_Z = \frac{0.532\,\lambda}{\sqrt{2}}\left[\frac{1}{n - \sqrt{n^2 - NA^2}}\right].$$

406 To perform aberration correction with adaptive optics, a deformable mirror (Multi-3.5,
407 Boston Micromachines Corporation) was set at a plane conjugate to the raster scanning mirrors
408 and the back aperture of the objective lens in the two-photon imaging system. Fluorescent
409 microspheres (0.2 μm) were imaged, and the standard deviation of the image brightness was
410 maximized under different configurations of the deformable mirror. Twelve selected Zernike
411 modes are applied and modulated sequentially over a total of three rounds. The 12 zernike
412 modes are: 1) oblique astigmatism, 2) vertical astigmatism, 3) vertical trefoil, 4) vertical coma, 5)
413 horizontal coma, 6) oblique trefoil, 7) oblique quadrafoil, 8) oblique secondary astigmatism, 9)
414 primary spherical, 10) vertical secondary astigmatism, 11) vertical quadrafoil, 12) secondary
415 spherical. For each Zernike mode, 21 steps of amplitudes were scanned through, and images
416 were acquired for each step. The amplitude that resulted in the largest standard deviation was
417 saved and set as the starting point of the DM configuration for the scanning of the next Zernike
418 mode. The brightness, the lateral resolution, and the axial resolution are compared with and
419 without the application of the deformable mirror correction.

420 **Air-floated Chamber**

421 For measurement of spatial responses, mice were head-fixed in a floating carbon fiber

422 chamber[34] (Mobile Homecage, NeuroTar, Ltd). The chamber base was embedded with magnets
423 to allow continual tracking of the position and angular displacement of the chamber. Behavioral
424 data was collected via the Mobile HomeCage motion tracking software (NeuroTar, versions
425 2.2.0.9, 2.2.014, and 2.2.1.0 beta 1). During imaging experiments, image acquisition was
426 triggered using a TTL pulse from the behavioral software to synchronize the timestamps from
427 the 2P imaging and chamber tracking.

428 A custom carbon fiber arena (250 mm diameter) was lined with four distinct visual
429 patterns (5.7 cm tall, 18.1 cm wide) printed on 7 mil waterproof paper (TerraSlate) with black
430 rectangles (5.7 cm tall and 1.5 cm wide) placed in between the four patterns. A circular track
431 (Fig. 4A; Video S4) was made by adding a removable inner circle (14 cm in diameter and 4.2
432 cm tall) with visual cues that were matched to the outer wall printed on 7 mil waterproof paper.
433 Transparent tactile stickers (Dragon Grips) were placed on the arena floors to give differential
434 tactile stimuli along the track. In between each recording and or behavioral session, the arena
435 walls and floors were thoroughly cleaned.

436 Mice were acclimated to the arena by the following steps: 1) On the first day the mice
437 were placed into the chamber and allowed to freely explore without head fixation for 15 - 20
438 minutes. A piece of plexiglass with holes drilled through was placed on top of the arena to keep
439 the mice from climbing out. 2) On the second day, the mice were head-fixed to a crossbar
440 extending over the floating chamber (Fig. 4A) and allowed to freely explore the floating chamber
441 freely for 15 minutes. Air flow (3 - 6.5 psi) was adjusted to maximize steady walking/running. On
442 subsequent days, the head fixation time was increased by increments of 5 minutes, as long as
443 the mice showed increased distance walked and percent time moving. This was continued until
444 the mice would explore for 30 - 40 minutes and run for greater than 15% of the time. 3) Mice
445 were head-fixed in the floated chamber for 20 minutes with a custom light blocker attached to
446 their headplate. 4) Mice were head-fixed and placed on the 2P microscope to allow habituation
447 to the microscope noise. 5) After mice were fully habituated, 20 - 40 minute duration recording
448 sessions on the 2P microscope were performed.

449 If at any point during the above acclimation protocol the mouse significantly decreased
450 distance traveled or percentage of time moving, then the mouse was moved back to the
451 previous step.

452 Custom software was written to process the behavioral data output by the Mobile
453 HomeCage motion tracking software. Because the Mobile HomeCage motion tracking software
454 sampling rate was faster than the frame rate of our 2P imaging, all behavioral variables (speed,

455 location, polar coordinates, and heading) that were captured within the acquisition of a single 2P
456 frame were grouped together and their median value was used in future analysis. For the polar
457 angle (which we used as the location of the mouse in 1D track), the median was computed
458 using an open source circular statistics toolbox (CircStat 2012a) written for Matlab[90]. We
459 removed any time points when the mouse was not moving, as is standard for measurement of
460 place fields[25]. This helps separate processes that are related to navigation from those that are
461 related to resting state. To do this, we smoothed the measured instantaneous speed and kept
462 time periods > 1 s that had speeds greater than 20 mm/s (adding an additional 0.5 s buffer on
463 either side of each time period).

464 **Two-photon Imaging**

465 After recovery from surgery and behavioral acclimation, GFP or GCaMP6s fluorescence was
466 imaged using a Prairie Investigator 2P microscopy system with a resonant galvo scanning
467 module (Bruker). For fluorescence excitation, we used a Ti:Sapphire laser (Mai-Tai eHP,
468 Newport) with dispersion compensation (Deep See, Newport) tuned to λ = 920 nm. Laser power
469 ranged from 40-80 mW at the sample depending on GCaMP6s expression levels.
470 Photobleaching was minimal (<1% min^{-1}) for all laser powers used. For collection, we used
471 GaAsP photomultiplier tubes (H10770PA-40, Hamamatsu). A custom stainless-steel light
472 blocker (eMachineShop.com) was mounted to the head plate and interlocked with a tube around
473 the objective to prevent light from the environment from reaching the photomultiplier tubes. For
474 imaging, we used a 16x/0.8 NA microscope objective (Nikon) to collect 760 x 760 pixel frames
475 with field sizes of 829 x 829 μm or 415 x 415 μm. Images were collected at 20 Hz and stored at
476 10 Hz, averaging two scans for each image to reduce shot noise.

477 For imaging spines across days, imaging fields on a given recording session were
478 aligned based on the average projection from a reference session, guided by stable structural
479 landmarks such as specific neurons and dendrites. Physical controls were used to ensure
480 precise placement of the head plate, and data acquisition settings were kept consistent across
481 sessions. Images were collected once every day for 5 - 10 days.

482 **Two-photon Post-processing**

483 Images were acquired using PrairieView acquisition software (Bruker) and converted into multi-
484 page TIF files.

485 For spine imaging, registration and averaging was performed for each z-plane spanning
486 the axial width of the dendrite to ensure all spines were captured across z-planes. The resulting

projections were weighted according to a Gaussian distribution across planes. Non-rigid registration was used to align dendritic segments across consecutive recording sessions. The registered images underwent high-pass filtering to extract low amplitude spine features using code adapted from Suite2P's enhanced mean image function[91] (Fig. S2A). The resulting ROIs were binarized using Otsu's global threshold method for spine classification (Fig. S2A). In most cases, the global threshold successfully isolated the single most prominent dendrite. In fields with higher background dendrites that were not desired, these extraneous dendrites were manually excluded. To identify spines that fall below the global threshold, the user manually specifies incrementally lower thresholds from which to select spines that were excluded in the initial binarization. (Fig. S2A). Spines above the global threshold with an area of >1 μm^2 were included in our analysis. To classify each spine as one of the four major morphological classes, we performed the following steps. First, we found the base of the spine by identifying the region closest to the dendritic shaft. Second, we calculated the length of the spine by taking the Euclidean distance between the midpoint of the spine base and the most distant pixel. Third, this vector was divided evenly into three segments to find the spine head, neck, and base areas respectively. Finally, spines were classified in the four categories, considering the following threshold parameters (Fig. S2B): stubby (neck length < 0.2 μm and aspect ratio < 1.3), thin (neck length > 0.2 μm, spine length < 0.7 μm, head circularity < 0.8 μm), mushroom (neck length > 0.2 μm, head circularity > 0.8 μm), and filopodium (neck length > 0.2 μm, spine length < 0.8 μm, aspect ratio >1.3).

For calcium imaging sessions, the TIF files were processed using the Python implementation of Suite2P[91]. We briefly summarize their pipeline here. First, TIFs in the image stack undergo rigid registration using regularized phase correlations. This involves spatial whitening and then cross-correlating frames. Next, regions of interest (ROIs) are extracted by clustering correlated pixels, where a low-dimensional decomposition is used to reduce the size of the data. The number of ROIs is set automatically from a threshold set on the pixel correlations. We manually checked assigned ROIs based on location, morphology and DF/F traces.

Since the hippocampal pyramidal cells are densely packed and the prism reduces the axial resolution, we perform local neuropil subtraction using custom code (https://github.com/ucsb-goard-lab/two-photon-calcium-post-processing) to avoid neuropil contamination. The corrected fluorescence was estimated according to

$$F_{corrected}(n) = F_{soma}(n) - \alpha\,(F_{neuropil}(n) - \bar{F}_{neuropil}),$$

where $F_{neuropil}$ was defined as the fluorescence in the region <30 μm from the ROI border (excluding other ROIs) for frame n. $\bar{F}_{neuropil}$ is $F_{neuropil}$ averaged over all frames. α was chosen from [0, 1] to minimize the Pearson's correlation coefficient between $F_{corrected}$ and $F_{neuropil}$. The ΔF/F for each neuron was then calculated as

$$\frac{\Delta F}{F} = \frac{F_n - F_0}{F_0},$$

where F_n is the corrected fluorescence ($F_{corrected}$) for frame n and F_0 is defined as the first mode of the corrected fluorescence density distribution across the entire time series.

We deconvolved this neuropil subtracted ΔF/F to obtain an estimate for the instantaneous spike rate, which we used (only) for the computation of neurons' spatial information (see below). This inferred spike rate was obtained via a MATLAB implementation of a sparse, nonnegative deconvolution algorithm (OASIS) used for Ca^{2+} recordings[92]. We used an auto-regressive model of order 1 for the convolution kernel.

Spine Imaging Data Analysis

After nonrigid registration, high pass filtering, and binarization of the dendritic segment, individual spines were extracted based on standard morphological criteria[93]. Spines projecting laterally from the dendritic segment were extracted and analyzed as individual objects, as described previously (Fig. S2). The sum of the members of each spine class, as well as the total number of all spines, was recorded for each session. Spine totals (S_{total}) were then broken down into 10 μm sections of the dendritic segment ($S_{section}$) using the following calculation:

$$S_{section}(n) = \frac{S_{total}(n)}{\left(D_{length} * \frac{F_{\mu m}}{F_{pixels}}\right)} * 10,$$

where length of the dendritic segment, D_{length}, was determined by skeletonizing the dendritic shaft to 1 pixel in diameter, then taking the area of the pixels. F_{pixels} is the FOV in pixels, which here was 760 x 760 at 16x magnification, and $F_{\mu m}$ is the FOV in microns, which was 52 x 52 μm.

Turnover was estimated at 24 h increments; turnover here is defined as the net change in spines per day for each morphological class (Fig. 2F). To determine which specific spines were involved in turnover across days, segments recorded 24 h apart were aligned and overlaid

using a custom MATLAB interface, which allowed the user to manually select new or removed spines. Percent addition/subtraction $S_{a/s}$ was calculated as:

$$S_{a/s} = \frac{N_{a/s}(t)}{N(t)} * 100,$$

where $N_{a/s}(t)$ is spines that have been added or subtracted and $N(t)$ is the total average number of spines. To account for variance in spine classification across days, turnover of specific classes of spines was normalized to total cumulative turnover per day.

To calculate the survival fraction curve $S(t)$, we determined which spines were present at time t_n that were not present at time t_0[43,44]. The dendritic segment from t_0 was transparently overlaid with segments from t_n, and replacement spines that were present in t_0 but not t_n were manually identified. Survival fraction was quantified as

$$S(t) = \frac{N_r(t_n)}{N(t_0)} * 100,$$

where $N_r(t_n)$ are the total spines at t_n that were also found in t_0, and $N(t_0)$ are the total number of spines that were present in t_0. Survival fraction, as well as % addition and subtraction, was calculated in 10 μm sections to control for segment length.

Calcium Imaging Data Analysis

For calcium imaging experiments during exploration of the air-floated chamber, processed and synchronized behavioral data and 2P imaging data were used to identify place and speed cells as follows.

First, the 1D track was divided into 72 equal bins (each ~0.85 cm in length). Activity as a function of position (we refer to these as spatial tuning curves) was computed for each lap, with activity divided by occupancy of each binned location. To avoid misattribution of slow calcium signals to spatial bins, any lap where the average instantaneous speed was greater than 180 mm/s, or where the total length of the lap took less than 1 second, were removed and not considered for further analysis. To assess the consistency of spatial coding of each cell, we randomly split the laps into two groups and computed the correlation coefficient between the averaged spatial tuning curves. We then did the same for shuffled data in which each lap's spatial tuning curve was circularly permuted by a random number of bins. This was performed 500 times, and the distribution of actual correlation coefficient values was compared to the

distribution of circularly shuffled values using a two-sample Kolmogorov-Smirnov test (α = 0.01). A cell that passed this test was considered a "consistent" cell.

To identify a neuron as a place cell, the neuron had to pass the consistency test and also be well fit by a Gaussian function, $R_{DF/F} = A_0 + A\, e^{\left(\frac{(X-B)}{C}\right)^2}$, with FWHM = $2C\sqrt{\log 2}$. Specifically, we required that: 1) the adjusted $R^2 > 0.375$; 2) 2.5cm < FWHM < 30.6 cm (50% of track length); 3) A > 0; 4) $A/A_0 > 0.50$. Cells that met these conditions were characterized as place cells; with place fields at the location of maximal activity and width defined as the FWHM.

Speed cells were identified using a standard process developed for identification of speed cells in medial enthorinal cortex and hippocampus[62–64]. We computed the Pearson correlation of each cells' DF/F trace with the mouse's speed across the experiment. This value is considered as a "speed score". We then circularly shuffled the DF/F 100 times (making sure that the amount shuffled was greater than 10 frames to ensure that the shuffled distribution did not have artificially high correlations). Cells whose speed score was greater than 99%, or less than 1%, of the shuffled distribution were considered speed cells.

To compute the spatial information[94] of cell j (SI_j), we used the following formula

$$SI_j = \frac{1}{\bar{a}_j} \sum_{k=1}^{72} p(k) a_j(k) \log_2 \left[\frac{a_j(k)}{\bar{a}_j} \right]$$

where $\bar{a}_j$ is the mean inferred spike rate of cell j, $a_j(k)$ is the mean inferred spike rate of cell j at position bin k, and $p(k)$ is the probability of being at position bin k. We divide by $\bar{a}_j$ to have SI in units of bits/inferred spike.

To align recordings where we recorded along the CA1-DG axis, we found the inflection point of the axis and then computed the distance of each cell to that point. To do this, we performed the following steps. 1) We extracted the position of each identified cell using Suite2p's centroid output. 2) We then fit a function of the form $a(x-b)^2 + c$ to the cell positions by rotating the field-of-view from 0 to 180 degrees and finding the rotation that maximized the R^2 value of the fit. 3) We determined the inflection point as the peak of the curve and de-rotated the fit to determine the inflection point and curve in the original coordinates. The distance of each cell to the inflection point was found by finding the point along the fit curve that had the minimal distance to the cells centroid.

600 **Immunohistochemistry**

601 Samples were perfusion fixed using 4% paraformaldehyde in 0.1M sodium cacodylate buffer
602 (pH = 7.4) for 10 mins, and then immersion fixed overnight at 4ºC. Next, sections were rinsed in
603 cold PBS 5 x 5 mins and 1 x 1hr. Whole brains were then embedded in 10% low-melting
604 agarose. Subsequently, 100 μm coronal sections were cut using a vibratome (Leica, Lumberton,
605 NJ). Sections were then blocked overnight in normal donkey serum (Jackson ImmunoResearch;
606 West Grove, PA) diluted 1:20 in PBS containing 0.5% bovine serum albumin, 0.1% Triton-X
607 100, and 0.1% sodium azide, hereafter, PBTA at 4ºC. Next, primary antibodies anti-GFAP
608 (1:500; abcam; ab53554), anti-S100 (1:1000; DAKO; Z0311) were diluted in PBTA and
609 incubated overnight at 4ºC. Then, sections were rinsed 5 x 5 mins and 1 x 1hr before
610 corresponding secondary antibodies along with the nuclear stain Hoechst33342 (1:5000;
611 Molecular Probes; H-3570) were incubated overnight at 4ºC. Lastly, secondary antibodies were
612 rinsed and sections mounted using Vectashield (Vector laboratories Inc; H-1200) and sealed
613 under #0 coverslips.

614 High resolution wide-field mosaics of brain samples were then imaged with a 20x oil
615 immersion lens and an Olympus Fluoview 1000 laser scanning confocal microscope (Center
616 Valley, PA) at a pixel array of 800 x 800 and then registered using the bio-image software
617 Imago (Santa Barbara CA).

618 We then calculated glial cell density as a function of distance from the prism face. First,
619 each mosaic was rotated so that the medial-lateral axis of the brain sample was aligned to be
620 parallel with the horizontal axis of the image. Then each mosaic was cropped to remove
621 extraneous pixels outside of the imaged brain slice. Next, a line denoting the face of the prism
622 was manually drawn parallel to the dorsal-ventral axis aligned with the location of the prism
623 face. We then used a custom cell-counting algorithm that identified potential regions of interest
624 (ROIs). We limited the ROIs to be within the hippocampal formation in the brain slices. The
625 euclidean distance between the closest point on the defined prism face and each ROI's centroid
626 was calculated. Afterward, a similar procedure was performed on the contralateral side of the
627 brain slice, with a mock "prism face" defined at symmetric coordinates to the true prism face, to
628 serve as a control. These steps were repeated for each channel of the mosaic.

629 After extracting each ROIs distance from the prism face, we counted the number of cells
630 in each 50 µm distance bin. To account for basal glial cell density, we calculated the percent
631 change of glial cell density on the prism side with respect to the control side. This procedure

632 was repeated 1000 times using randomly sampled distances, with replacement, to bootstrap the
633 sample variance.

634 **Statistical Information**

635 Violin plots were made using an open source Matlab package[95]. Statistical tests for spine
636 morphological types were calculated using a one-way ANOVA. Reliability across laps was
637 tested with a two-sample Kolmogorov-Smirnov test. Comparisons between model fits for spatial
638 distribution of spatial information and place field width used a General Linear F-test.

DATA AVAILABILITY

Microperiscope designs can be found on our institutional lab website (https://goard.mcdb.ucsb.edu/resources). Spine imaging data from Fig. 2 and neuronal response data from Figs. 4-5 are available on Dryad (DOI: TBA, final version will be uploaded upon manuscript acceptance). Code for spine analysis and place/speed cell identification is available on Github (DOI: TBA, final version will be uploaded upon manuscript acceptance).

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AUTHOR CONTRIBUTIONS

W.T.R. and M.J.G. designed the experiments; M.J.G. performed the surgical implants; W.T.R., N.S.W., L.M., and M.J.G. conducted the imaging experiments and analyzed the data; G.L. and T.D.M. performed immunohistochemistry and K.K.S. analyzed the resulting images; C.H.Y. and S.L.C. performed optical modeling; W.T.R., N.S.W., and M.J.G. wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing financial interests.

MATERIALS AND CORRESPONDENCE

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