

1 **Title:**

2 Delayed formation of neural representations of space in aged mice

3

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13 **Teaser:**

14 Representations of space in the aged mouse hippocampus are initially disrupted in novel
15 environments, but improve once environments become familiar.

16

17 **Abstract:**

18 Aging is associated with cognitive deficits, with spatial memory being very susceptible to

19 decline. The hippocampal dentate gyrus (DG) is important for processing spatial information in

20 the brain and is particularly vulnerable to aging, yet its sparse activity has led to difficulties in

21 assessing changes in this area. Using *in vivo* two-photon calcium imaging, we compared DG

22 neuronal activity and representations of space in young and aged mice walking on an unfamiliar

23 treadmill. We found that calcium activity was significantly higher and less tuned to location in

24 aged mice, resulting in decreased spatial information encoded in the DG. However, with

25 repeated exposure to the same treadmill, both spatial tuning and information levels in aged mice

26 became similar to young mice, while activity remained elevated. Our results show that spatial

27 representations of novel environments are impaired in the aged hippocampus and gradually
28 improve with increased familiarity. Moreover, while the aged DG is hyperexcitable, this does not
29 disrupt neural representations of familiar environments.

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31

32 **Main Text:**

33

34 **Introduction**

35 As the world's population ages, it becomes more important to understand the cognitive effects of
36 aging and to develop therapeutic strategies for the aging brain. While healthy aging has milder
37 cognitive effects than neurodegenerative states, several aspects of cognitive function exhibit
38 decline (1). Memory deteriorates with age, with spatial memory being particularly susceptible to
39 deficits in older humans and animals (2–4). Older adults show orientation deficits (5, 6), although
40 they tend to perform better in familiar locations or tasks than in novel ones (7–9). This aging-
41 related decay is thought to be caused by vulnerabilities in the hippocampal circuits that mediate
42 spatial memory (10, 11). The dentate gyrus (DG) is one of the hippocampal subregions that are
43 most vulnerable to aging (12–14), and the inputs from the entorhinal cortex to DG are a main area
44 of dysfunction in both Alzheimer's Disease (AD) patients and AD mouse models (15). During
45 aging, there are reduced synaptic contacts from both the medial and lateral entorhinal cortex (16)
46 onto DG neurons, as well as reduced synaptic plasticity of these inputs (17). However, there is very
47 limited data about how DG activity changes with aging, primarily because neuronal activity in this
48 region tends to be too sparse for *in vivo* electrophysiological recordings (18).

49 Here we investigated whether aging is associated with impaired DG activity and spatial
50 representations, which could account for the spatial memory deficits seen in aged individuals.

51 Since the DG is upstream of other hippocampal areas, changes in this region could help elucidate

52 the causes of aging-related changes in neuronal activity (19–22) and neural representations of
53 space (19, 23, 24) found in some areas of the hippocampus but not in others. Using *in vivo* two-
54 photon microscopy we imaged calcium activity in a large population of DG neurons of young and
55 aged mice as the animals walked head-fixed on a treadmill to which they had never been exposed.
56 We found that aged mice have increased DG single-cell calcium activity and disrupted neural
57 representations of space upon their first exposure to the treadmill setting. However, further
58 imaging sessions on subsequent days showed that spatial representations become similar to
59 those of young mice as the animals familiarize themselves with the treadmill, whereas single-cell
60 activity remains elevated. Our results highlight the importance of novelty and familiarity to spatial
61 encoding in aged animals.

62 **Results**

63 To record neuronal activity in DG excitatory neurons, we injected young (3-4 month old) and aged
64 (21-26 month old) C57Bl6 mice with an AAV encoding the calcium indicator jRGECO1a (25) under
65 the CaMKII promoter (Fig. 1A). We then implanted the mice with a hippocampal imaging window
66 above the DG (26, 27) to enable *in vivo* two-photon calcium imaging in this region (Fig. 1A,B).
67 After waiting 3-4 weeks to allow for viral expression and recovery from surgery, we imaged mice
68 on four consecutive days. During each imaging session the mice ran head-fixed along a treadmill
69 containing tactile cues with four distinct segments (Fig. 1A,C) that was manually moved (Fig. S1).
70 We recorded 10 minute videos of calcium activity and identified the regions corresponding to
71 active cells using Suite2p software (Fig. 1D). Previous studies found that hippocampal activity in
72 area CA3 becomes elevated during aging (19, 28). We determined single-cell calcium activity by
73 measuring the area under the curve of transients and normalizing to the distance run by each
74 mouse (29) (Fig. 1E-G). We found that the aged group had higher activity levels than the young
75 group (Fig. 1E, $p=0.00463$, nested bootstrap), which aligns with previous reports of hyperactivity.
76 We also estimated the fraction of active neurons during each imaging session and found no

77 changes between groups (Fig. S2). We next investigated whether there were differences between
78 the spatial representations of young and aged DG neurons. Both young and aged DG neurons
79 have a variety of spatial responses, including place cells that only respond to a specific location
80 and neurons whose activity shows low spatial preference (Fig. 2A,B). We determined the spatial

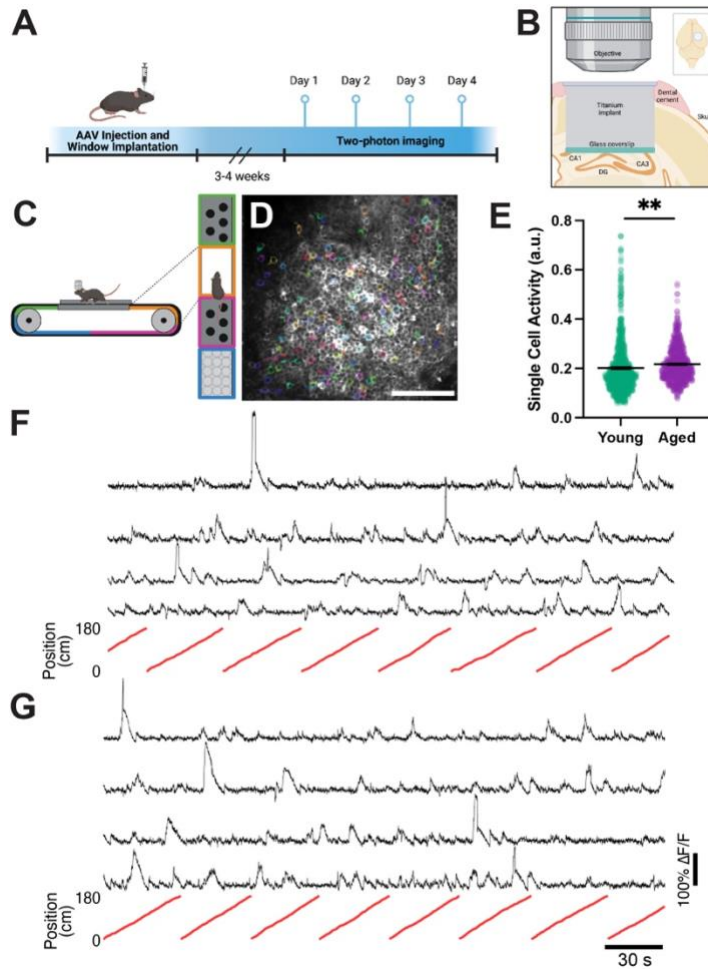


Figure 1. DG is hyperactive in aged animals. A) Experimental timeline, including AAV injection, window implantation, and two-photon imaging. B) Diagram of chronic window implant over the right hemisphere of the DG. C) Diagram of imaging setup, with side view (left) and top view (right) of mouse head-fixed to treadmill with multiple tactile zones. D) Example field of view with regions of interest of active cells shaded in color. Scale bar = 100 μ m. E) Mean single cell calcium activity. F) Example calcium traces (black) and corresponding treadmill positions (red) from young mice. G) Example calcium traces (black) and corresponding treadmill positions (red) from aged mice. a.u.= arbitrary units. Young N=8 mice, n=910 cells; Aged N=8 mice, n=699 cells. ** $p < 0.01$

81
82 selectivity of neuronal activity using a previously described spatial tuning index (see methods).
83 DG neurons in aged mice had significantly lower tuning indices than those in young mice (Fig.
84 2C, $p < 0.0001$, nested bootstrap). This difference indicates that the activity of DG neurons in aged
85 mice was not as place-specific as that of young DG neurons, suggesting an aging-related
86 degradation of the spatial code. We also computed single-cell Fisher Information (FI), as a
87 measure of how much spatial information was encoded by individual DG neurons. By plotting
88 whole-recording calcium activity raster plots for neurons in the 10th percentile of FI we verified that

89 neurons with high spatial information behave like ‘place cells’ (30) in both young and aged
90 animals, as their activity is concentrated at a single location on the treadmill (Fig. 2D,E). This
91 place-specific response was present across laps as neurons in both cohorts of mice largely kept
92 the same place response in both even and odd laps. Aged mice encoded less spatial information
93 than young mice as denoted by a significant decrease in FI (Fig. 2F, $p=0.00005$, nested
94 bootstrap).

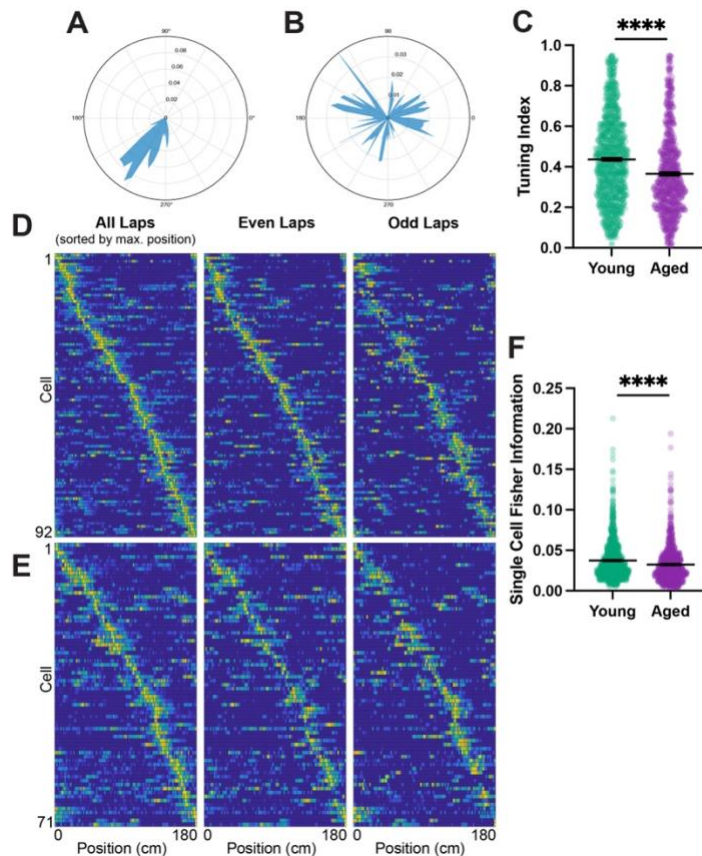


Figure 2. DG representations of space are impaired in aged mice. A) Tuning vector polar plots representing a highly spatially tuned cell and B) a cell with low spatial tuning. C) Spatial tuning index. D) Raster plots of tuning vectors of young mouse neurons in 10th percentile Fisher Information, sorted by the position of activity maximum for whole recording (All Laps). E) Same raster plots for aged mice. F) Single cell Fisher information. Statistics done with nested bootstrap analysis. Bars represent mean +/- SEM. Young N=8 mice, n=910 cells; Aged N=8 mice, n=699 cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. See also supplemental figures 1 and 2.

95

96 To confirm that the changes in spatial representations in aged mice corresponded to a change in
97 spatial memory, mice underwent behavioral testing using an object placement paradigm. This test
98 takes advantage of a mouse’s natural preference for novelty to assess how well animals can
99 remember the location of objects in space, a behavior that is thought to be hippocampus-
100 dependent (31). We allowed mice to explore an arena with visual cues and two novel and identical
101 objects during a training trial. We then displaced one object to another location in the arena and

102 again allowed the mice to explore during a test trial (Fig. S3A). Young mice had a preference for
103 the novel object that was significantly above chance ($p= 0.0017$, One sample Wilcoxon test)
104 whereas aged mice did not score above chance (Fig S3B-C, $p=0.6355$, One sample Wilcoxon
105 test). Additionally, significantly more aged mice than young mice failed the test (Fig. S3D,
106 $p=0.0393$, Chi squared test). This change in preference can also be seen in the exploration times
107 per objects, in which young mice spend more time engaged with the displaced object, while aged
108 mice spend about an even amount of time between objects (Fig. S3E, $p=0.0205$, Two-way
109 ANOVA with Sidak multiple comparisons test). These data confirm earlier findings of spatial
110 memory deficits in aged mice, which we now show is accompanied by impaired neural
111 representations of space in the DG.

112

113 Older individuals are better able to distinguish places in an environment that they are familiar with
114 than in a novel environment (7, 8), so we asked how the representations of space in the DG
115 changed across four consecutive days of imaging, as the treadmill belt became more familiar to
116 the mice. Single-cell calcium activity remained elevated in aged mice through all recording days,
117 even though this difference was not significant in days 2 and 3 (Fig. 3A, nested bootstrap with
118 Bonferroni correction). While in young mice there was a slight but significant decrease in activity
119 ($p=0.00005$) across days, in aged mice activity increased over time ($p<0.0001$) (Fig. 3B-C, nested
120 bootstrap). In contrast to activity, the differences in tuning index and FI were erased over the four
121 days of imaging, as young and aged groups converged. The spatial tuning index of young mice
122 remained approximately constant over the course of all imaging sessions ($p=0.19772$), whereas
123 the tuning of aged mice underwent an increase so that the differences between both groups were
124 no longer significant in days 3 and 4 ($p=0.00005$). (Fig. 3D-F, nested bootstrap, Bonferroni
125 corrected in 3D). FI rose in both young and aged groups over the four imaging days, but the
126 increase was more pronounced in aged animals so that the differences between young and aged

127 animals were no longer significant in days 3 and 4 (Fig. 3G-I, nested bootstrap, Bonferroni
 128 corrected in 3G). These data suggest that representations of space in aged animals can be
 129 rescued by repeated exposure to the same environment.

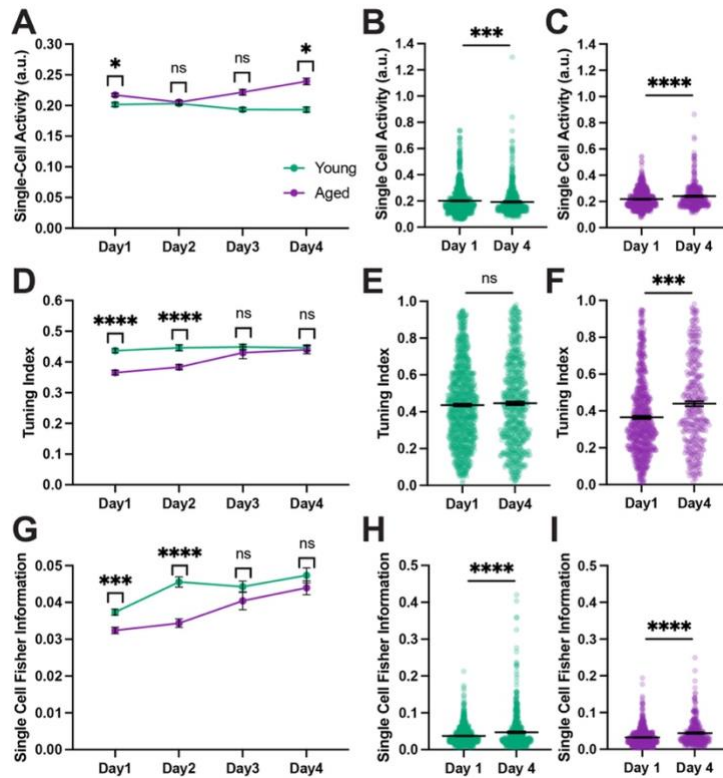


Figure 3. Deficits in aged spatial representations are rescued with increased familiarity. A) Mean single cell calcium activity across days. B) Single cell activity in young mice on day 1 versus day 4. C) Single cell activity in aged mice on day 1 versus day 4. D) Mean tuning index across days. E) Tuning index in young mice on day 1 versus day 4. F) Tuning index in aged mice on day 1 versus day 4. G) Mean single cell Fisher Information across days. H) Fisher Information in young mice on day 1 versus day 4. I) Fisher Information in aged mice on day 1 versus day 4. Statistics done with a nested bootstrap analysis. Bars represent mean +/- SEM. a.u.= arbitrary units. Young: day 1 N=8 , n=910; day 2 N=7, n=694; day 3 N=7, n=623; day 4 N=7, n=610; Aged: day 1 N=8, n=699; day 2 N=8, n=619; day 3 N=5, n=147; day 4 N=6, n=331. ns=not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

130
 131 Previous studies found the stability of neural representations of space was altered in aged animals
 132 (32, 33). We therefore tracked the activity of individual neurons across days to investigate the
 133 stability of the active cell ensemble (Fig. 4A-C). We first asked whether cells that were active in
 134 the initial imaging session were reactivated on subsequent days. Surprisingly, we found no
 135 difference between groups, suggesting that both DG neurons in young and aged mice have
 136 similar reactivation rates (Fig. 4D, mixed effects model with Sidak multiple comparisons test). We
 137 went on to compare the activity in the cells that were active both on day 1, when the treadmill was
 138 novel, and day 4, when it had become familiar. The single-cell activity of matched cells increased
 139 across days in the young mice ($p=0.00234$, nested bootstrap) but not in the old mice ($p=0.15981$,
 140 nested bootstrap) (Fig. 4E-F). This may reflect a ceiling for activity since the aged mice already

141 had higher activity on day 1. While the tuning index did not differ in either group across days (Fig.
142 4G-H, $p=0.07626$, $p=0.21341$), both groups saw higher spatial information, as FI was significantly
143 increased in reactivated cells on day 4 (Fig. 4I-J, $p=0.00015$, $p=0.00024$), which may simply
144 reflect the fact that FI increases sharply for the entire population across the four days. We then
145 asked whether the reactivated cells had a distinct profile compared to those neurons that were
146 not active across days. In day 1, cells that were to be reactivated did not differ from other cells in
147 single cell activity levels. Whether cells were reactivated or not, their activity levels were higher in
148 the aged mice on day 1, which recapitulates the pattern seen in the general population (Fig. 4K).
149 These effects were gone by day 4, again reflecting the trend in the general population (Fig. 4L).
150 In the aged group, cells that were reactivated showed higher tuning levels than other cells on day
151 1 (Fig. 4M), but this effect was also absent on day 4 (Fig. 4N). Reactivated cells had significantly
152 higher FI than other neurons in both the young and aged groups on both day 1 and day 4 (Fig.
153 4O-P). This suggests that higher spatial information is a predictor of whether neurons will be
154 reactivated over several days. Overall, we did not see evidence any changes in the stability of
155 coding ensembles in aged animals, when compared with the young cohort. However, since
156 reactivated neurons initially have higher tuning and spatial information than non-reactivated cells
157 in aged animals, and increase their FI over time, it's possible that they contribute to the
158 improvement of spatial representations in aged mice improve over the four days of imaging.

159 In summary, we have found that, in aged animals, DG neurons are hyperexcitable and impaired
160 in their encoding of spatial features in novel environments. Our results highlight how the defects
161 in spatial representations in the DG are specific to the first introduction to a novel context and can
162 be rescued by increasing familiarity with a given environment.

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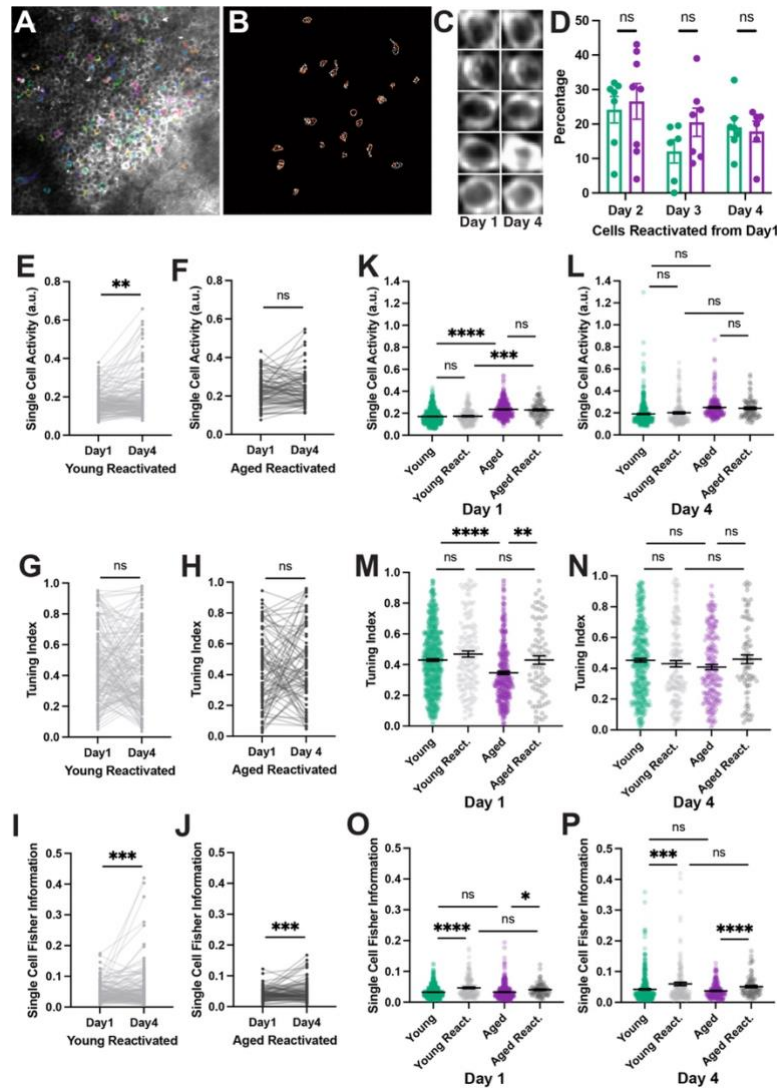


Figure 4. Reactivated cells do not have a distinct profile in aged animals. A) Example field of view used to match cells across days. Neurons that were active during recording session are shaded in color. B) Matched regions of interest from the field of view in panel A, red and white contours represent cells active on day 1 and 4. C) Examples of matched cells on day 1 and 4. D) Reactivation rate of neurons that were active on day 1. Percentages are based on imaging day versus day 1, regardless of whether the cell was active on other days. E-F) Matched single cell calcium activity in reactivated cells in young (E) and aged (F) mice. G-H) Matched tuning index in reactivated cells in young (G) and aged (H) mice. I-J) Matched single cell Fisher Information in reactivated cells in young (I) and aged (J) mice. K-L) Single cell activity of reactivated and non-reactivated cells on day 1 (K) and day 4 (L). M-N) Tuning of reactivated and non-reactivated cells on day 1 (M) and day 4 (N). O-P) Single cell Fisher Information of reactivated and non-reactivated cells on day 1 (O) and day 4 (P). Statistics done with nested bootstrap analysis. Bars represent mean \pm SEM. a.u.= arbitrary units. Young: N=6; day 1 n=617; day 1 react. n=140; day 4 n=396; day 4 react. n=140; Aged: N=5; day 1 n=375; day 1 react. n=80; day 4 n=196; day 4 react. n=80. ns=not significant, * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001.

164

165

166

167 **Discussion**

168 Aging is associated with cognitive decline in spatial memory in healthy aged humans and rodents
169 (2–4). In this study we asked whether aged mice have impaired representations of space in the
170 DG circuits that mediate spatial memory, which could account for the memory deficits seen in
171 aged individuals.

172 Several studies have found that neuronal activity is elevated in the hippocampus of aged animals
173 specifically in hippocampal area CA3 (19–22). Human studies using fMRI in older and younger
174 adults have generally confirmed the hyperexcitability findings in the DG-CA3 axis (5, 14), although
175 fMRI does not have the resolution to distinguish between areas DG and CA3. This aging-related
176 hyperexcitability phenotype is thought to contribute to the memory deficits seen in aged
177 individuals (34, 35). In order to understand the causes of the aging-related hyperexcitability it
178 would be important to record neuronal activity from the DG, as this region provides the main inputs
179 to CA3.

180 We used *in vivo* two-photon calcium imaging to record activity from young and aged DG neurons.
181 This allowed us to image a large population of cells and therefore have a better chance capturing
182 active cells in a sparsely active population. Additionally, using an imaging-based approach
183 allowed us to study the same group of cells across time. We have found that single-cell calcium
184 activity is indeed increased in aged DG neurons, when compared to young controls (Fig. 1E).

185 We also found that during the initial exposure to the treadmill, there is a significant reduction in
186 spatial information and tuning in aged DG neurons, as compared to their young counterparts (Fig.
187 2B-C). By using untrained mice for our experiments we were able to capture the very first neuronal
188 representations of a novel environment. We found that during the course of four consecutive
189 imaging days, spatial information and tuning in the aged mice converged with those of young
190 mice, which leads us to conclude that aged mice require additional exposure to a novel

191 environment in order to form adequate neural representations of space. It can therefore be said
192 that the spatial encoding defects seen in aged mice are specific to novel environments. This is in
193 agreement with previous reports that found that spatial representations in CA1 were disrupted
194 when novel environmental cues were introduced during a navigation task (33, 36). Interestingly,
195 both young and aged groups saw an increase in spatial information through the four days of
196 imaging, whereas spatial tuning increased in aged mice but was not significantly different in young
197 mice. We did not find a convergence in single-cell calcium activity levels between aged and young
198 groups, meaning that the hyperexcitability is no impediment to aged mice eventually forming
199 accurate spatial representations. There are several interesting parallels between our findings and
200 human data showing that aged individuals are better able to navigate familiar environments than
201 novel ones (8) leading them to avoid taking novel routes in favor of navigating familiar routes.

202

203 To verify that aged mice have impairments in spatial memory, we used an object placement test
204 that is not physically tasking and does not involve an aversive component. Our behavior data
205 confirmed that aged mice are more likely to fail at an object placement task than their younger
206 counterparts. This goes along with studies analyzing similar spatial memory tasks that have found
207 deficits in aged animals (37, 38). Our results also highlight the well-described heterogeneity
208 present during normal aging, as about half of aged mice were still able to pass the test (39, 40).

209 In order to better compare our data to previous studies of DG activity using IEG labelling, we
210 determined the percentage of active cells in our imaging fields but found no difference in the
211 number of active cells between young and aged animals. This may be due to differences in the
212 activation of ensembles based on the experimental designs. However, there are also some
213 caveats to our experimental data that must be kept in mind: we calculate the total number of cells
214 from mean projections of whole calcium imaging movies, which can introduce several biases, for
215 example active cells will tend to be brighter and therefore more visible. We found a correlation

216 between the percentage of active cells and the total number of cells in the imaging field (Fig.
217 S2B), as fields of view with fewer detected cells had much higher variance of the fraction of active
218 cells. Fields with more total cells generally had more cells that were dimmer and less active but
219 were still counted in the total. In contrast, in fields with few cells due to areas of lower viral
220 expression or obscured by blood vessels, there are likely more cells present than can be
221 quantified in the mean projection image. These differences in imaging fields across animals
222 makes it difficult to calculate exact percentages, limiting the usefulness of this approach.

223

224 Previous studies have found that increased hippocampal excitability contributes to aging-related
225 cognitive impairments. Our experimental design did not allow us to determine whether
226 hyperexcitability contributes to the deficits in spatial information seen in the novel environment
227 during the first day of imaging, but our results suggest that instead dysfunction in the plasticity
228 mechanisms that underlie spatial selectivity may be to blame. Spatial selectivity can develop very
229 fast in CA1 hippocampal neurons through a mechanism termed behavioral timescale synaptic
230 plasticity (41) that is mostly active in novel contexts, leading to the development and stabilization
231 of spatial tuning within the first few minutes of exposure to a new environment (42). While less is
232 known about the dynamics of spatial selectivity in the DG, spatially tuned cells also seem to
233 emerge and stabilize within the first few minutes of exposure to a novel environment, with further
234 refinement as the animals are re-exposed to the same environment over the following days (43).
235 This is in line with our finding that spatial information increases over several days both in young
236 and aged animals. Previous literature has shown that aging is associated with plasticity deficits in
237 the hippocampus (17, 44, 45), namely in long term potentiation and depression. Given that these
238 plasticity mechanisms are likely most active as mice map out new environments (42) we speculate
239 that this may explain the protracted development of spatial selectivity that we see in aged mice.
240 Expression of some IEGs, such as *Arc* and *Egr1*, which are thought to be regulators of neuronal

241 plasticity, is reduced in the DG of aged animals. Deficits in the expression of these genes following
242 neuronal activity could be at the origin of plasticity defects in aged animals. The increased
243 excitability of aged DG neurons could therefore also be seen as a compensation mechanism for
244 both the reduced synaptic input from the entorhinal cortex, and the reduced expression of IEGs.
245 Another factor potentially contributing to the delayed formation of spatial representations in the
246 aged DG is a reduction of adult neurogenesis. Adult-born neurons are known to enhance DG
247 neuronal plasticity (46, 47) but their numbers fall to almost zero in aged animals (48).

248 Aging-associated memory deficits have also been associated with changes in the stability of
249 spatial representations as some previous studies have found that the aged hippocampal spatial
250 code undergoes larger changes over different sessions than in young mice (32), whereas others
251 have found that spatial representations are more rigid (24, 33). We did not see changes in the
252 reactivation of cells from one session to another between groups (Fig. 4D). This is in contrast to
253 data showing that more distinct dentate populations express the IEG *Arc* when aged mice are re-
254 exposed to the same environment (49), which could be due to age-related changes in DNA
255 methylation rather than changes in neuronal excitability (50). In order to further investigate the
256 properties of cells in the aged DG over time, we matched cells that were active in our imaging
257 fields across days. We did not find major differences in the reactivated cells in any measures
258 between young and aged groups, suggesting that ensembles of neurons follow the same basic
259 mechanisms in the aged DG. When comparing reactivated neurons to the remainder of the
260 population, we found that reactivated cells had significantly higher spatial information (Fig. 4 O,P),
261 indicating that this may be a marker of whether a neuron will contribute to spatial representations
262 over time.

263 To summarize, our data shows that new DG spatial representations are initially impaired and take
264 longer to form in aged mice. These data expand our knowledge of the network activity and spatial
265 representations in the aged hippocampus and suggest that aging-associated hippocampal

266 hyperactivity is not an impediment to the formation of rich spatial representations. The protracted
267 refinement of spatial representations suggests that the plasticity mechanisms responsible for the
268 development of spatial selectivity are impaired in aged animals and are likely to be a relevant
269 therapeutic target for ameliorating the memory deficits associated with aging.

270

271

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282 **Author contributions:**

283 Conceptualization: KDM, JTG; Methodology: KDM, MAF, JTJ, JTG; Investigation: KDM, SSM,
284 JTG; Visualization: KDM, JTG; Supervision: JTG; Writing—original draft: KDM, JTG; Writing—
285 review & editing: KDM, JTG

286 **Competing interests:**

287 The authors have no competing interests.

288

289 **Data availability:**

290 The analysis code used in this study is openly available at
291 <https://github.com/GoncalvesLab/McDermott-et-al-aging>. All imaging data, consisting of calcium
292 traces for every active cell and the position of the mouse on the treadmill, will be made openly
293 available on a repository site before final publication. Due to their large size, raw calcium imaging
294 movies will be made available upon request.

295

296 **Methods**

297 **Animals** – Aged mice were 21-26 months old C57Bl6J females originally from Jackson Labs and
298 kindly gifted by Dr. Carolyn Pytte of Queens College. Young mice were 3-4 months old C57Bl6J
299 females purchased from Jackson Labs. All mice were housed in standard conditions with a 14/10
300 hour light/dark cycle. Mice were provided food and water ad libitum. All procedures were done
301 during the light part of the cycle and in accordance with the Einstein Institutional Animal Care and
302 Use Committee (Protocol #00001197).

303 **Viral injections** – Mice were anesthetized (induction: 5%, maintenance 0.5% isoflurane in O₂
304 vol/vol). Following anesthetization, mice were attached to a stereotactic apparatus and the right
305 hemisphere of the dentate gyrus (DG) was injected with 1µl of a DJ-serotype AAV vector
306 encoding the red-shifted calcium indicator jRGECO1a (25) under the CamKII promoter at 10¹²
307 GC/ml titer. Viral injections were done with a pulled glass pipette using a Nanoject III microinjector
308 (Drummond) at previously described coordinates (51).

309 **Window implantation** – Following viral injection, dexamethasone (1 mg/kg) was administered
310 via subcutaneous injection to minimize brain swelling. Optibond (Kerr Dental) adhesive was
311 applied to the skull and cured to help secure the implant once attached. A 3mm diameter

312 craniotomy was made over the right dorsal DG and the overlaying tissue was removed by
313 aspiration down to the hippocampal fissure where a custom-built cylindrical titanium implant with
314 a glass coverslip on the bottom was inserted over the dorsal surface of the DG (27). The implant
315 and a titanium bar for head-fixation were attached to the skull with dental cement. All mice were
316 given carprofen (5 mg/kg, subcutaneous) as a post-surgery analgesic.

317 **Two-photon Imaging** – *In vivo* calcium imaging movies were acquired with a custom two-photon
318 laser scanning microscope (based on Thorlabs Bergamo) using a femtosecond-pulsed laser
319 (Coherent Fidelity 2, 1070 nm) and a 16x water immersion objective (0.8 NA, Nikon). Imaging
320 sessions were started 3-4 weeks after surgery to allow for recovery and for optimal viral
321 expression. During imaging sessions, mice were head-fixed to the microscope with a titanium
322 headbar and the microscope stage was adjusted so that the hippocampal window was
323 perpendicular to the axis of the objective for optimal imaging conditions. Mice were awake and
324 walking on a manually rotated treadmill belt throughout imaging. The treadmill contained four
325 zones with different textures as previously described (52). Ten-minute videos were acquired at
326 15.253 fps with a 343.6 x 343.6 μm field of view. Treadmill data was acquired using National
327 Instruments analog-to-digital converter and synchronized with the imaging data using Thorsync
328 software. To track the same field of view, the initial location was noted on the first imaging day
329 using a coordinate system and by taking an image of the field. During following sessions, the
330 coordinates and initial field image were used to match as close as possible to the same field.

331 **Data Analysis**– The open-source Suite2p software (version 10.0) was used to register and
332 motion correct videos, for cell detection and spike deconvolution (53–55). Regions of interest
333 corresponding to active neurons were identified by Suite2p and further manually curated in order
334 to identify all active cells in the field of view. The ROIMatchPub (56) was used to identify and
335 match cells in the same field of view across imaging sessions. Matched ROIs were manually
336 confirmed after automated detection.

337 Single-cell calcium activity was determined as previously described (29). Briefly, $\Delta F/F$ data was
338 filtered with a zero phase shift, third order Butterworth lowpass filter. This filtered $\Delta F/F$ was
339 thresholded to 2 standard deviations(σ) above a rolling-mean baseline. Single cell activity was
340 determined as the cumulative sum of the thresholded trace for each cell. This was then normalized
341 to the total distance traveled by each mouse.

342 Tuning indices were using deconvolved calcium traces, which were thresholded to 2σ above the
343 baseline. Any point not significantly above that noise threshold was set to zero. Videos were cut
344 to only include periods of movement $>1s$ in duration. The treadmill band was segmented into 100
345 position bins, and transients were mapped to these bins according to the location of the mice on
346 the treadmill in order to generate a tuning vector for each cell (26).The mean of the thresholded
347 activity at each location was calculated for every neuron and normalized to the time the mouse
348 spent at that position. The tuning index was defined as the modulus of this normalized tuning
349 vector.

350 Single-cell Fisher Information (FI) was calculated as previously described (29, 57). A bias-
351 corrected signal to noise ratio was computed using the unfiltered $\Delta F/F$ fluorescence data for each
352 individual cell, where the signal is the square of the difference of the mean activity at two locations
353 on the treadmill, and the noise is the average variance of the activity at each location. Position
354 data was segmented into 20 bins.

355 **Behavior** – Object Placement –A square-shaped arena 42x42x30 (LxWxH) was set up with visual
356 cues along the walls and with two novel and identical objects on the floor. Mice were individually
357 placed in the arena for a 4 minute training trial. Following a 45 minute retention period, one object
358 was displaced to another location in the arena and the mice were placed again for a 4 minute test
359 trial. Exploration of the objects was quantified by an experimenter and confirmed with computer
360 tracking.

361 **Statistics** – Nested bootstrap analysis – When pooling cell data from different mice into a single
362 experimental group, significance testing was done using a multi-level bootstrapped approach as
363 previously described (29). To assess whether differences between two experimental groups are
364 significant, a null surrogate distribution was constructed for each mouse in each group by
365 resampling with substitution from the pool of all imaged cells. The difference between the null
366 distributions generated for each group was calculated and the previous steps repeated to
367 generate 100,000 bootstrap estimates of the difference between two groups' null distributions.
368 The empirically observed value of the difference between conditions was then compared to the
369 null distribution values with a statistical significance level (α) set at 0.05. If the empirical group
370 differences fall outside of the 95th percentile of the 100,000 bootstrap estimates of the difference
371 between the null distributions, then it is considered to be a statistically significant difference. The
372 p-value is the proportion of bootstrap different estimates that are larger than the empirical
373 difference between groups (or smaller, if the difference is negative). Whenever comparing more
374 than two groups, Bonferroni's correction for multiple comparisons was applied.

375 - Mixed-effects models with Geisser-Greenhouse correction for matched values were used to
376 compare mean values by mouse across imaging days. Sidak's multiple comparisons test was
377 used to compare groups on each imaging day.

378 - Nonparametric one sample Wilcoxon tests were used to determine if group medians were
379 significantly above chance levels in behavior experiments.

380 - A two-sided Chi squared test with 95% confidence interval was used to compare pass/fail rates
381 in behavior experiments.

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535 **Figure legends:**

536 **Figure 1. DG is hyperactive in aged animals.** A) Experimental timeline, including AAV injection,
537 window implantation, and two-photon imaging. B) Diagram of chronic window implant over the
538 right hemisphere of the DG. C) Diagram of imaging setup, with side view (left) and top view (right)
539 of mouse head-fixed to treadmill with multiple tactile zones. D) Example field of view with regions
540 of interest of active cells shaded in color. Scale bar = 100 μm . E) Mean single cell calcium activity.
541 F) Example calcium traces (black) and corresponding treadmill positions (red) from young mice.
542 G) Example calcium traces (black) and corresponding treadmill positions (red) from aged mice.
543 a.u.= arbitrary units. Young N=8 mice, n=910 cells; Aged N=8 mice, n=699 cells. **p<0.01

544 **Figure 2. DG representations of space are impaired in aged mice.** A) Tuning vector polar
545 plots representing a highly spatially tuned cell and B) a cell with low spatial tuning. C) Spatial
546 tuning index. D) Raster plots of tuning vectors of young mouse neurons in 10th percentile Fisher
547 Information, sorted by the position of activity maximum for whole recording (All Laps). Raster plots
548 of partial recording (Even Laps and Odd Laps) keep same sorting used for whole recording. E)
549 Same raster plots for aged mice. C) Single cell Fisher information. Statistics done with nested
550 bootstrap analysis. Bars represent mean +/- SEM. a.u.= arbitrary units. Young N=8 mice, n=910
551 cells; Aged N=8 mice, n=699 cells. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. See also
552 supplemental figures 1 and 2.

553 **Figure 3. Deficits in aged spatial representations are rescued with increased familiarity.** A)
554 Mean single cell calcium activity across days. B) Single cell activity in young mice on day 1 versus
555 day 4. C) Single cell activity in aged mice on day 1 versus day 4. D) Mean tuning index across
556 days. E) Tuning index in young mice on day 1 versus day 4. F) Tuning index in aged mice on day
557 1 versus day 4. G) Mean single cell Fisher Information across days. H) Fisher Information in young
558 mice on day 1 versus day 4. I) Fisher Information in aged mice on day 1 versus day 4. Statistics
559 done with a nested bootstrap analysis. Bars represent mean +/- SEM. a.u.= arbitrary units. Young:

560 day 1 N=8 , n=910; day 2 N=7, n=694; day 3 N=7, n=623; day 4 N=7, n=610; Aged: day 1 N=8,
561 n=699; day 2 N=8, n=619; day 3 N=5, n=147; day 4 N=6, n=331. ns=not significant, *p<0.05,
562 **p<0.01, ***p<0.001, ****p<0.0001.

563 **Figure 4. Reactivated cells do not have a distinct profile in aged animals.** A) Example field
564 of view used to match cells across days. Neurons that were active during recording session are
565 shaded in color. B) Matched regions of interest from the field of view in panel A, red and white
566 contours represent cells active on day 1 and 4. C) Examples of matched cells on day 1 and 4. D)
567 Reactivation rate of neurons that were active on day 1. Percentages are based on imaging day
568 versus day 1, regardless of whether the cell was active on other days. E-F) Matched single cell
569 calcium activity in reactivated cells in young (E) and aged (F) mice. G-H) Matched tuning index in
570 reactivated cells in young (G) and aged (H) mice. I-J) Matched single cell Fisher Information in
571 reactivated cells in young (I) and aged (J) mice. K-L) Single cell activity of reactivated and non-
572 reactivated cells on day 1 (K) and day 4 (L). M-N) Tuning of reactivated and non-reactivated cells
573 on day 1 (M) and day 4 (N). O-P) Single cell Fisher Information of reactivated and non-reactivated
574 cells on day 1 (O) and day 4 (P). Statistics done with nested bootstrap analysis. Bars represent
575 mean +/- SEM. a.u.= arbitrary units. Young: N=6; day 1 n=617; day 1 react. n=140; day 4 n=396;
576 day 4 react. n=140; Aged: N=5; day 1 n=375; day 1 react. n=80; day 4 n=196; day 4 react. n=80.
577 ns=not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

578

579 **Figure S1. Velocity and laps run during two photon imaging.** A) Average velocity of mice
580 walking along treadmill belt. B) Average number of laps run per imaging session. Statistics done
581 with mixed effects model. Each point represent an individual animal. Bars represent mean +/-
582 SEM. ns=not significant.

583 **Figure S2. No difference in active cell number between young and aged mice.** A) Percentage
584 of cells active out of the total cells in a field of view, by mouse. Statistics done with mixed effects
585 model. Bars represent mean +/- SEM. ns=not significant. B) Correlation of percentage active cells
586 versus the total number of cells pooled from all imaging days. Data are shown by mouse.

587 **Figure S3. Aged mice show a deficit in a spatial memory task.** A) Schematic of the object
588 placement behavioral paradigm. B) Novelty preference of mice measured by percentage of total
589 time spent exploring the novel object. C) Discrimination index calculated by dividing the difference
590 in exploration time between objects by the total exploration time $[(\text{novel}-\text{familiar})/(\text{novel}+\text{familiar})]$.
591 D) Pass/fail rate of task, where passing was defined as at least a 53% preference for novel object.
592 E) Exploration time dedicated to each object. Statistics in B and C were done with one sample
593 Wilcoxon tests, D with Chi squared test and E with mixed effects model. Bars represent mean +/-
594 SEM. Young N=13, Aged N=13. ns=not significant, * $p<0.05$, ** $p<0.01$.

595

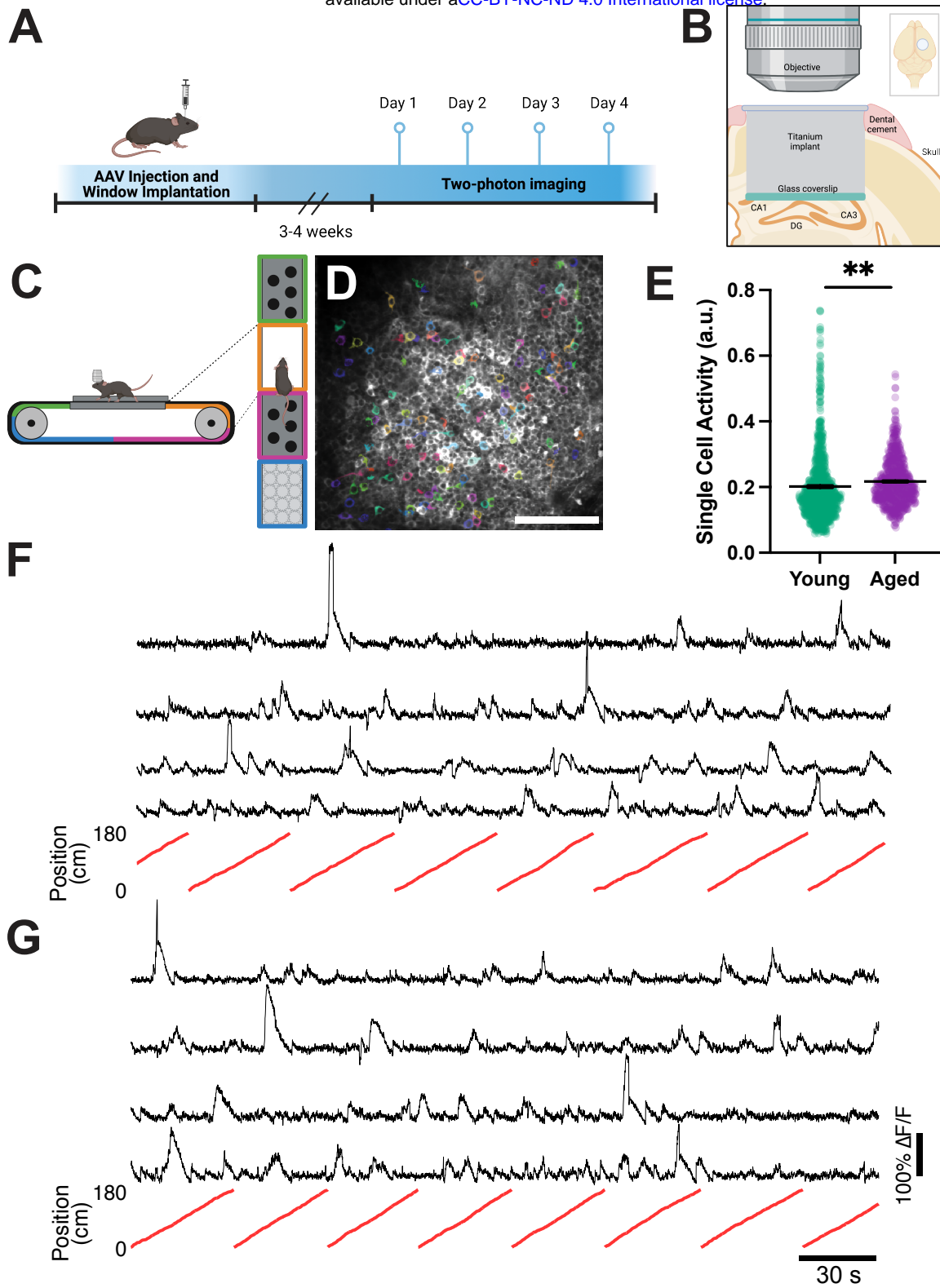


Figure 1

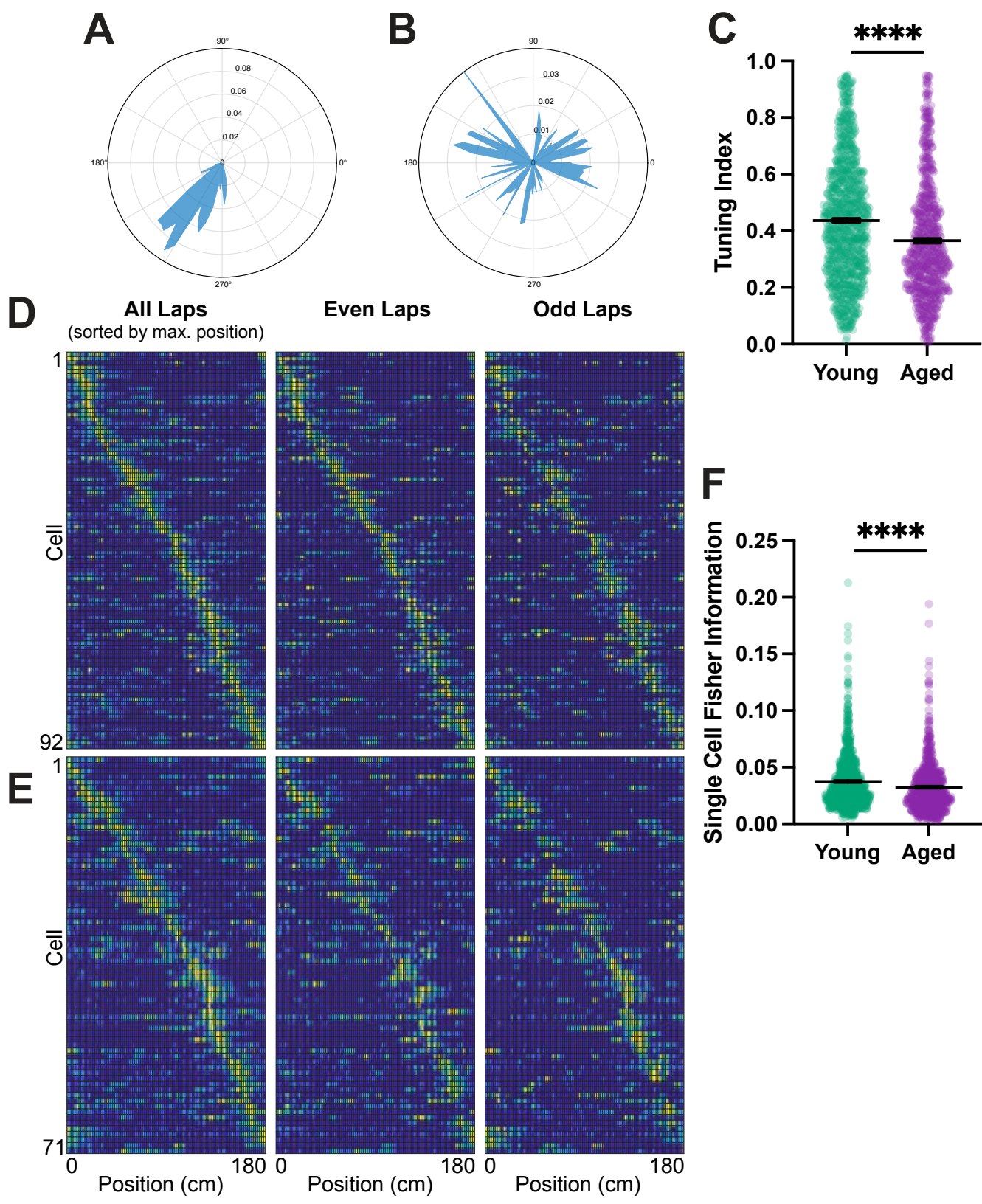


Figure 2

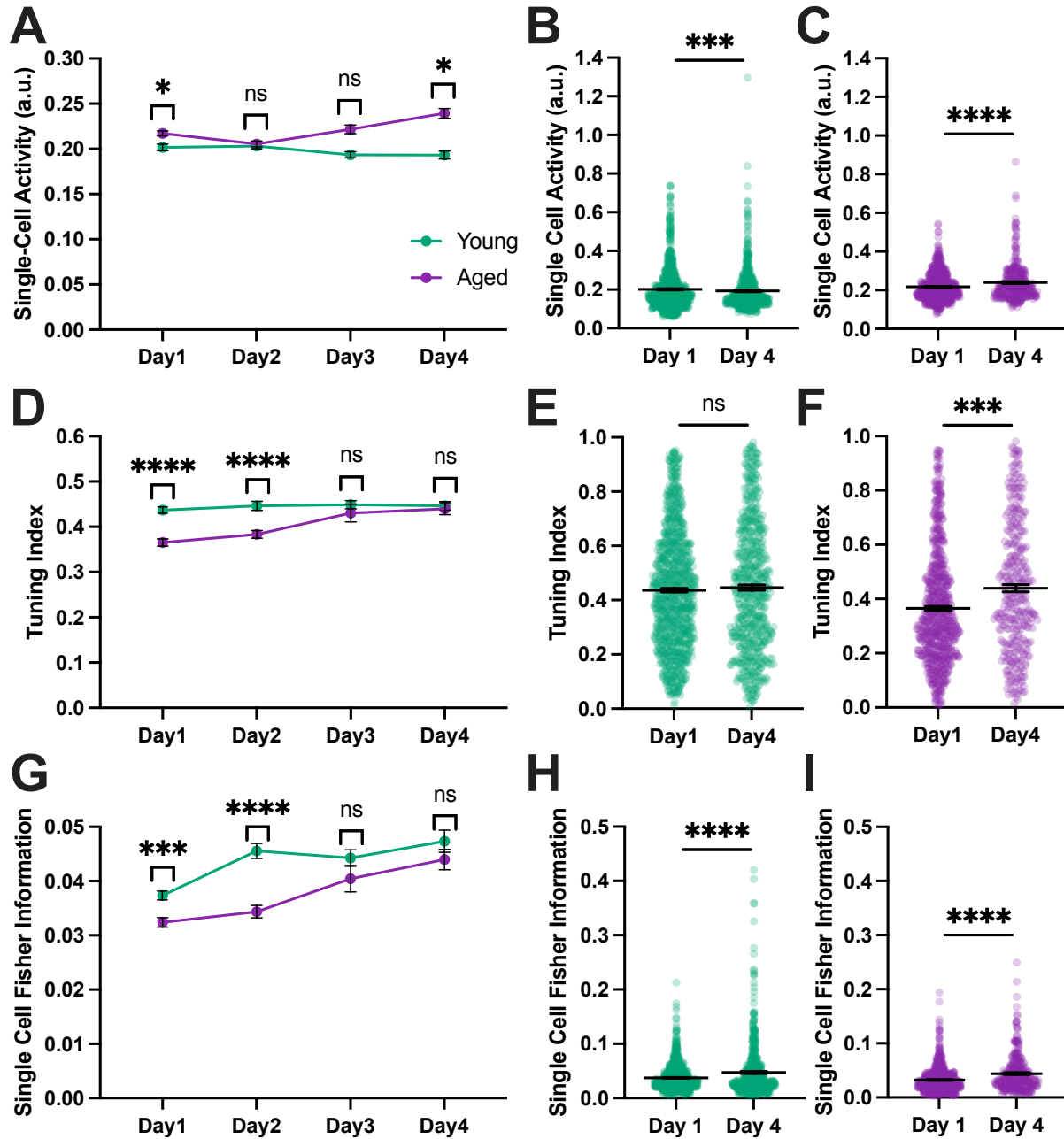


Figure 3

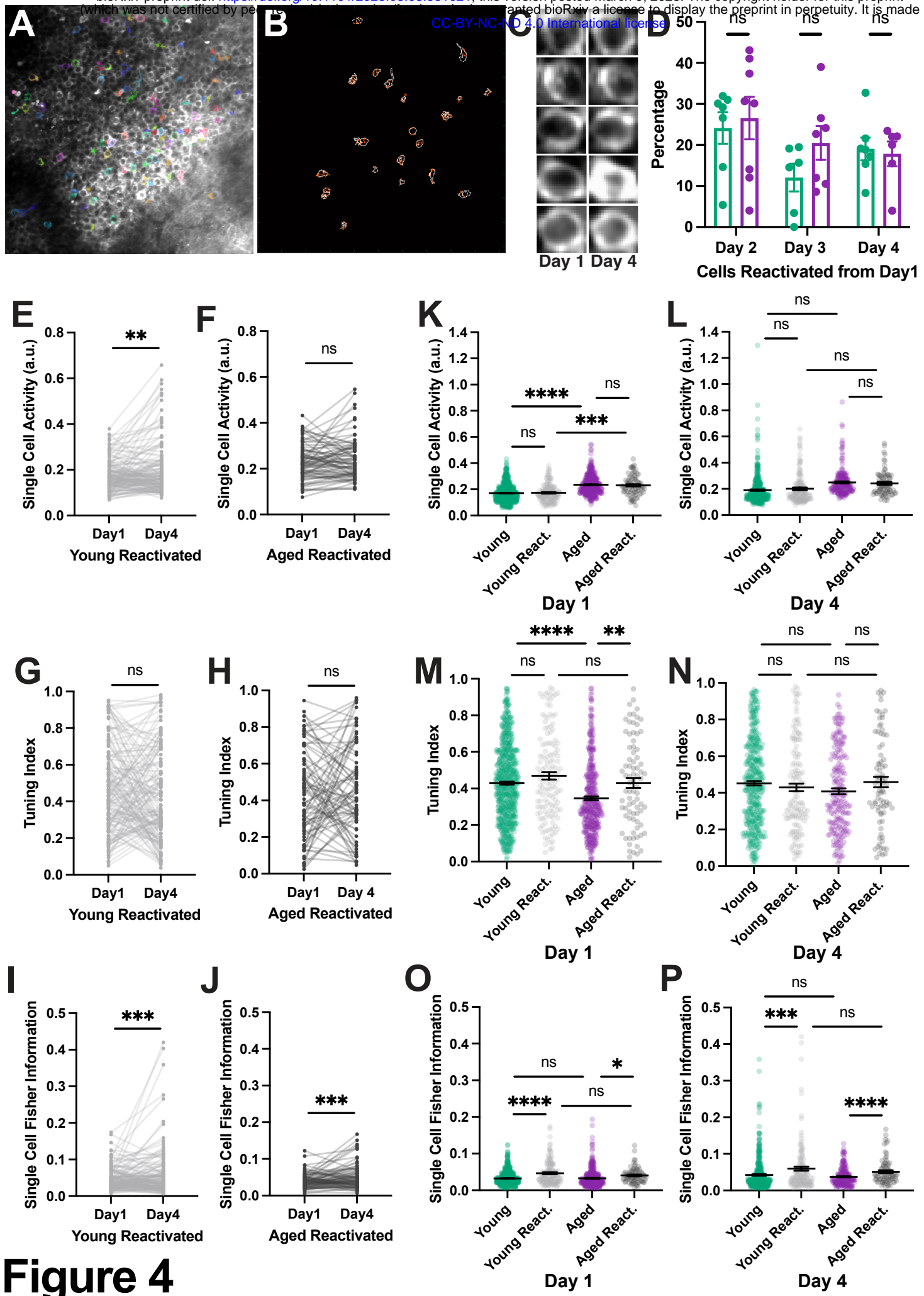


Figure 4