

Aged Rats Exhibit Altered Behavior-Induced Oscillatory Activity, Place Cell Firing Rates, and Spatial Information Content in the CA1 Region of the Hippocampus

 Lindsey M. Crown,^{1,2} Daniel T. Gray,^{1,3} Lesley A. Schimanski,¹  Carol A. Barnes,^{1,2,3} and  Stephen L. Cowen^{1,2}

¹Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, Arizona 85724, ²Department of Psychology, University of Arizona, Tucson, Arizona 85721, and ³Departments of Neurology and Neuroscience, University of Arizona, Tucson, Arizona 85724

Hippocampal gamma and theta oscillations are associated with mnemonic and navigational processes and adapt to changes in the behavioral state of an animal to optimize spatial information processing. It has been shown that locomotor activity modulates gamma and theta frequencies in rats, although how age alters this modulation has not been well studied. Here, we examine gamma and theta local-field potential and place cell activity in the hippocampus CA1 region of young and old male rats as they performed a spatial eye-blink conditioning task across 31 d. Although mean gamma frequency was similar in both groups, gamma frequency increased with running speed at a slower rate in old animals. By contrast, theta frequencies scaled with speed similarly in both groups but were lower across speeds in old animals. Although these frequencies scaled equally well with deceleration and speed, acceleration was less correlated with gamma frequency in both age groups. Additionally, spike phase-locking to gamma, but not theta, was greater in older animals. Finally, aged rats had reduced within-field firing rates but greater spatial information per spike within the field. These data support a strong relationship between locomotor behavior and local-field potential activity and suggest that age significantly affects this relationship. Furthermore, observed changes in CA1 place cell firing rates and information content lend support to the hypothesis that age may result in more general and context-invariant hippocampal representations over more detailed information. These results may explain the observation that older adults tend to recall the gist of an experience rather than the details.

Key words: cross-frequency coupling; gamma; hippocampus; oscillation; place cell; theta

Significance Statement

Hippocampal oscillations and place cell activity are sensitive to sensorimotor input generated from active locomotion, yet studies of aged hippocampal function often do not account for this. By considering locomotion and spatial location, we identify novel age-associated differences in the scaling of oscillatory activity with speed, spike-field coherence, spatial information content, and within-field firing rates of CA1 place cells. These results indicate that age has an impact on the relationship between locomotion and hippocampal oscillatory activity, perhaps indicative of alterations to afferent input. These data also support the hypothesis that aged hippocampal place cells, compared with young, may more often represent more general spatial information. If true, these results may help explain why older humans tend to recall less specific and more gist-like information.

Received Sep. 12, 2021; revised Mar. 18, 2022; accepted Apr. 14, 2022.

Author contributions: L.A.S. and C.A.B. designed research; L.A.S. performed research; L.M.C. and S.L.C. analyzed data; L.M.C., D.T.G., C.A.B., and S.L.C. wrote the paper.

This work was supported by the Evelyn F. McKnight Brain Research Foundation, National Institutes of Health Grant AG012609, Canadian Institutes of Health Research Grant SIB78537, and Alberta Heritage Foundation for Medical Research Grant 20060436.

The authors declare no competing financial interests.

L.M. Crown's present address: Keck School of Medicine, University of Southern California, Los Angeles, Los Angeles, California.

D.T. Gray's present address: Department of Physiology, University of California, Los Angeles, Los Angeles, California.

L.A. Schimanski's present address: Department of Psychology, Capilano University, Roberts Creek, British Columbia, Canada.

Correspondence should be addressed to Stephen L. Cowen at scowen@email.arizona.edu.

<https://doi.org/10.1523/JNEUROSCI.1855-21.2022>

Copyright © 2022 the authors

Introduction

Hippocampus-dependent functions such as spatial navigation and other forms of episodic memory necessitate the rapid integration of sensory cues with vestibular information. It is believed that this integration occurs in large part within the hippocampus and through its interactions with the surrounding cortex. Changes to hippocampus-dependent memory function are common features of normative brain aging (Glisky, 2007; Lester et al., 2017; Berron et al., 2018). For example, studies of episodic memory in aged individuals have found that older adults tend to recall more gist-like information at the expense of details (Koutstaal and Schacter, 1997; Paige et al., 2016; Wank et al., 2021). Importantly, aging in the absence of

neurodegenerative disease is not associated with hippocampal neuron loss (West et al., 1994; Rapp and Gallagher, 1996; Rasmussen et al., 1996; Keuter et al., 2003), suggesting that altered hippocampal function with age instead results from physiological changes that have an impact on network function.

Within the rodent hippocampus, both theta (5–10 Hz) and gamma (30–120 Hz) oscillations are known to organize the activity of place cells (O'Keefe and Recce, 1993; Bieri et al., 2014; Fernández-Ruiz et al., 2017), cells that fire with high spatial precision at particular locations within an environment (O'Keefe and Dostrovsky, 1971). Despite a wealth of data supporting the importance of theta and gamma oscillations to hippocampal physiological processes, few studies have investigated the degree to which these oscillations are altered with normal brain aging. Experiments examining theta in young and old animals have shown mixed results, with some reporting no differences between age groups (Abe and Toyosawa, 1999) and others reporting reduced theta frequency in old rats (Shen et al., 1997; Insel et al., 2012; Jacobson et al., 2013). Arguably, even less is known about age-related changes to hippocampal gamma oscillations. Although a previous study found no differences in the frequency of hippocampal gamma oscillations between young and old animals (Insel et al., 2012), it did not take into account the degree to which hippocampal (Maurer et al., 2005, 2012) and entorhinal dynamics (Kropff et al., 2015; Hinman et al., 2016) are modulated by running speed and acceleration (Kropff et al., 2021). Like theta, hippocampal gamma increases with running speed, suggesting that these oscillations are modulated by incoming sensory input (Ahmed and Mehta, 2012). Whether the frequency of gamma is modulated by running speed to the same degree in old and young rats remains an open question.

A number of studies have examined changes in behavior-induced CA1 single-unit activity in aged and young rats and have found no overall firing rate differences between age groups (Barnes and McNaughton, 1983; Oler and Markus, 2000; Wilson et al., 2005; Schimanski et al., 2013). One study that did identify age differences in CA1 firing rates examined firing rate with respect to velocity and found that rates increased with running speed in both young and aged rats, although the relative increase was significantly less in the old animals (Shen et al., 1997). The spatial information content of cell spiking can be calculated to quantify the extent to which the firing of a neuron corresponds to the spatial location of an animal (Skaggs et al., 1993). The few studies to examine spatial information in CA1 found no difference between middle-aged and old rats (Markus et al., 1994; Wilson et al., 2005; Robitsek et al., 2015), although these studies did not assess spatial information with respect to speed.

Here, we investigate the relationship between locomotor activity parameters, such as speed and acceleration, and oscillatory properties of gamma and theta while young and old rats performed a spatial eye-blink conditioning task across 31 d. In addition, we investigate the impact of age on single-unit firing rates, spatial information, and phase-locking to gamma and theta in these same animals. We hypothesize that in aged animals, CA1 gamma frequencies will be less tuned to locomotor activity, which may result in reduced place cell spatial information content.

Materials and Methods

Subjects and behavioral pretraining

Data were acquired from 12 male Fisher 344 rats ($n = 6$ young, 9–12 months; $n = 6$ old, 25–28 months). Animals were purchased from the National Institute on Aging colony at Charles River Laboratories and are the same as those used in Schimanski et al. (2013). Experiments were

performed as described in Schimanski et al. (2013) following the guidelines of the United States National Institutes of Health *Guide for the Care and Use of Laboratory Animals* using protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Rats were individually housed and kept on a 12/12 h reversed light cycle. Following acclimation, before behavioral training, animals were tested on the Morris water maze (Morris, 1984) across 4 consecutive days with six trials per day to assess motor ability, vision, and spatial learning of the animals (Schimanski et al., 2013). This procedure is described fully by Barnes et al. (1996). Before eye-blink conditioning, animals were pretrained on a linear track for an Ensure reward until reaching the criterion of 80 laps within 45 min. Training on the spatial eye-blink conditioning task (described below) and electrophysiological recordings began following recovery from electrode implantation and once tetrodes were lowered to CA1.

Behavioral procedures

Before spatial eye-blink conditioning each day, rats were brought to a dedicated room and the amount of electrical stimulation necessary to induce an eye-blink in each rat was calibrated (typical range, 0.1–0.6 mA). The eyelid stimulus was a 100 ms, 100 Hz train of bipolar square pulses 5 ms long, delivered through the wires implanted in the right eyelid and stimulated using a Master-8 from AMPI and stimulus isolator (model A365) from World Precision Instruments. Following calibration, rats were brought to a dimly lit room containing a circular track (85 cm diameter), a towel-lined clay flowerpot, and numerous visual cues (Schimanski et al., 2013). Rats were allowed to rest in the pot for ~30 min before and after both behavior epochs on each day across all 31 d of the task (Fig. 1A). For the task, rats ran from the barrier at one end of the track to the other end of the track (one lap). Rats completed 10 laps for food reward without eye shocks after which rats received an eye-blink-inducing electrical stimulation pseudo-randomly on 50% of laps at two locations, one in the clockwise and another in the counter-clockwise direction (~140 and 250° relative to the barrier; Fig. 1B). Rats ran a maximum of 74 laps or until they stopped. The number of laps was yoked by young/old pairs so that the pairs ran the same number of laps each day. The two within-day recording sessions were separated by an average of 157.5 ± 4.0 min. This protocol was repeated for 31 d.

The spatial eye-blink conditioning task was chosen in the original study by Schimanski et al. (2013) to determine the effects of age on hippocampal place cell remapping. The eye-blink behavior was used to measure the perception of the rat of its location in space. In the current study, only trials without eyelid stimulation were analyzed because of the presence of significant artifact from the stimulus. Running behavior was influenced by the conditioned stimulus (eye shock); some rats tended to slow down before the shock zone and reward site and accelerate after exiting the shock zone; however, the effect on running behavior was variable in both groups.

Animals ran along the circular track at a variety of speeds and acceleration/deceleration values. Figure 1C shows the distribution of speeds achieved by young and old rats as they moved across the track between reward zones. One old rat fell ill on day 15 and was removed from the study. Analysis of speed data from his 15 d of recording showed that he was not able to reliably reach speeds >30 cm/s and was therefore removed from further analysis. The probability distribution for speeds from 1 to 80 cm/s is shown for each rat in thin lines, whereas the thick line represents the mean. Young rats ran, on average, faster than the old rats ($t_{(8,6)} = 2.61$, $p = 0.029$, Cohen's $d = 1.58$). Figure 1D also shows the distribution of acceleration and deceleration values for young and old rats. Young rats accelerated faster, on average, than old rats ($t_{(8,6)} = 3.45$, $p = 0.008$, Cohen's $d = 2.05$) and decelerated faster ($t_{(8,4)} = -3.02$, $p = 0.016$, Cohen's $d = -1.79$). Because old rats were unable to reliably run at speeds >40 cm/s or accelerate/decelerate faster than 30 cm/s^2 or -30 cm/s^2 , analyses were limited to speeds from 1 to 40 cm/s and acceleration/deceleration values between -30 cm/s^2 and 30 cm/s^2 .

Surgical and electrophysiological recording procedures

Before experimentation, animals were implanted with eyelid stimulation electrodes and chronic electrode arrays (hyperdrives) holding 12

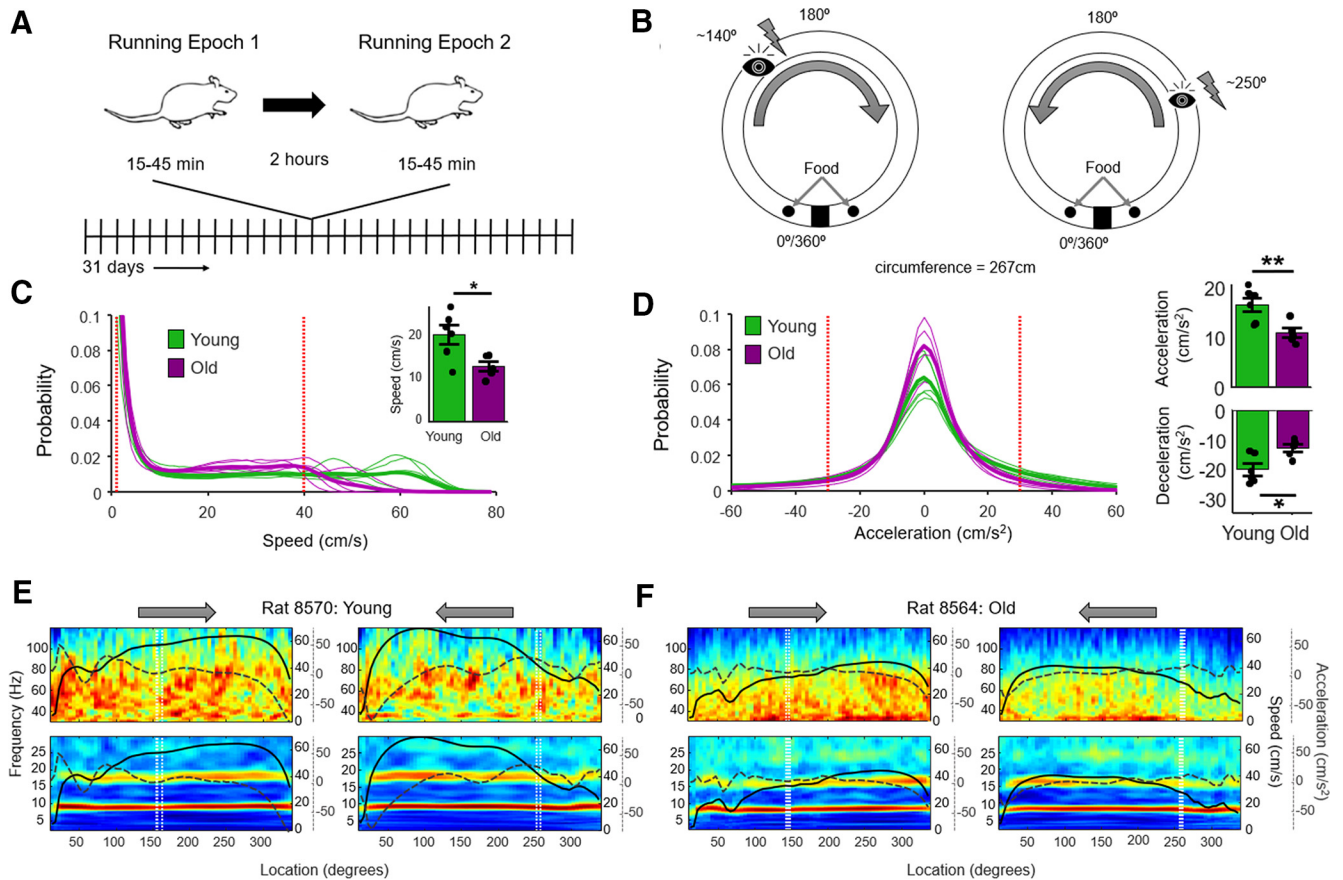


Figure 1. Task design. **A**, Animals ran twice daily on a circular track for 31 consecutive days. The two daily sessions were separated by 2 h of rest. Each running epoch lasted between 15 and 45 min. Rats ran until they reached 74 laps total or stopped running. The number of laps was yoked by young/old pairs so that the pairs ran the same number of laps each day. **B**, Animals ran back and forth for reward on a circular track. After 10 trials without eyelid stimulation, rats received a brief stimulation pseudo randomly on 50% of trials at the two locations indicated. **C**, Distribution of speeds achieved by young and old rats across 31 d of track running. Inset, The bar plot shows that young rats ran, on average, faster than old rats ($t_{(8,6)} = 2.61$, $p = 0.029$). Thin lines indicate values from individual rats, and the thick lines indicate the mean. Because old rats rarely reached speeds >40 cm/s, analyses were typically limited to speeds between 1 and 40 cm/s (delineated by red line in distribution plot). **D**, Average acceleration values achieved throughout track running across 31 experimental days. Thin lines indicate values from individual rats, and the thick lines indicate the mean. Acceleration values >30 cm/s² and lower than -30 cm/s² were rarely achieved. Thus, analyses were restricted to this range (dashed red lines). Bar plots show that the magnitudes of acceleration ($t_{(8,6)} = 3.45$, $p = 0.008$) and deceleration ($t_{(8,4)} = -3.02$, $p = 0.016$) were greater in young rats. Error bars represent mean $\pm$ SEM. **E**, Example time-frequency plots for average gamma range (30–120 Hz, top) and subgamma range (2–29 Hz, bottom) activity for a young animal during an example recording session across the linearized track. Average speed is indicated by the black line (right axis), and average acceleration is indicated by the gray dashed line (second right axis). Warmer colors indicate higher power. **F**, The same average time-frequency plots for gamma and subgamma activity as in **E**, but for an old rat.

independently adjustable tetrodes (McNaughton et al., 1983b; Wilson and McNaughton, 1993). Tetrodes consisted of four twisted polyimide-coated nichrome wires (13 μ m diameter). Before surgery, rats received antibiotic treatment (either a 10 d ampicillin cycle or a 5 d sulfamethoxazole and trimethoprim oral suspension cycle). On the day of surgery, rats were anesthetized using 1.0–2.0% isoflurane in oxygen (flow rate 1.5 L/min) and placed into a stereotaxic apparatus. Craniotomies were centered over the right hippocampus (2.0 mm lateral and 3.8 mm posterior to bregma). Tetrodes were lowered to an initial depth of 1 mm at surgery and lowered over the next 14 d to optimally record extracellular spikes from CA1 pyramidal cells. Two additional tetrodes in which the four wires were shorted together served as references and were placed in or near the corpus callosum and hippocampal fissure. Dental acrylic was used to secure all wires and a ground screw to the skull.

Neural signals were amplified through a unity-gain headstage and programmable amplifiers (Neuralynx). Electrophysiological signals were recorded using the Cheetah Data Acquisition System (Neuralynx). A 1 ms window was recorded surrounding each candidate action potential. Single-unit activity was digitized at 32 kHz, amplified 500–5000 times, and bandpass filtered between 600 and 6000 Hz. Local field potential (LFP) activity was recorded from a subset of tetrodes, bandpass filtered from 0.5 to 600 Hz, and sampled at 1893 Hz. LFP channels from each rat on each session were chosen by visual inspection for optimization of the

following metrics: 1) polarity of sharp wave deflection was positive and low (to keep anatomic depth of recording as constant), 2) $<20\%$ of the recording was above artifact threshold (800 μ V), and 3) visual inspection of power spectral densities (PSDs) of LFP activity during the task showed a normal 1/f distribution. LFP channels were chosen blinded to rat age.

Video tracking data were obtained with an overhead CCD camera at 32 frames per second. Speed was calculated by finding the first derivative of the tracking data acquired from the overhead camera, squaring and smoothing the result. Acceleration and deceleration values were calculated by then taking the first derivative of speed data. Thirty-one recording sessions were acquired from 11 rats (six young and five old).

Analysis of local field potentials

Measures of power across specific frequency ranges (theta, 5–10 Hz; broadband gamma, 30–120 Hz; low gamma, 30–55 Hz; high gamma, 65–120 Hz) were obtained by bandpass filtering signals (Butterworth, 12th order) and taking the absolute value of the Hilbert transformed data. Mean power was determined after restricting analysis to trial running on non-eye-blink-stimulated trials and periods when the rat was running between 1 and 40 cm/s. Peak frequency was determined using the MATLAB `instfreq()` function on signals also restricted to these behavioral epochs.

Measures of power across varying speeds during nonstimulated trials were obtained using a bump wavelet (`cwt()` function in the MATLAB Signal Processing Toolbox). Figure 1, *E* and *F*, shows the average gamma (30–120 Hz) and subgamma (2–30 Hz) activity across the maze for a single example session (day 12, first run) from one young (Fig. 1*E*) and one old (Fig. 1*F*) animal. The average speed of the animal across the maze is overlaid in black, and average acceleration/deceleration is shown by the dark gray dashed line.

To quantify the relationship between speed and peak oscillatory frequency, normalized wavelet power spectra as rats moved between speeds of 1 and 40 cm/s were obtained for each rat on each day. Power values were normalized by taking the *z*-score of the power for each frequency (180 frequencies between 30 and 120 Hz for gamma and 20 frequencies between 5 and 9 Hz for theta). These spectra were then binned by running speed from 1 to 40 cm/s in bins of 1 cm/s for a total of 39 bins. An average power spectral density by running speed plot was thus obtained for each rat on each day. The frequency of maximum *z*-scored power was identified for each running speed, and then a robust regression (`robustfit()` in MATLAB) was performed across these values to obtain beta values for slope and intercept for each rat. This same procedure was used to quantify the relationship between theta/gamma power, acceleration (>0 cm/s² and <30 cm/s², 29 bins) and deceleration (<0 cm/s² and more than -30 cm/s², 29 bins).

Quantification of single-unit activity

Selection of neurons for analysis

Analysis was restricted to putative principal neurons as determined by analysis of waveform shape, the autocorrelogram, and mean firing rate (Csicsvari et al., 1999; Barthó et al., 2004; Cowen and McNaughton, 2007). Neurons with a short-latency peak in the autocorrelogram (~ 10 ms) and wide waveforms were classified as principal cells. Neurons with either narrow waveforms or a late peak in the autocorrelogram (~ 100 ms) were classified as interneurons. Putative interneurons were not included in the analysis because of the relatively low abundance of interneurons recorded. Analysis of place-specific activity was further restricted to neurons with spatial information per spike values of >0.4 bits. To reduce the contribution of double counting the same neuron across days, analyses described below were performed on datasets acquired every other day (starting on day 2 of recording). Tetrodes were repositioned on a nearly daily basis, so it is likely that double counting was virtually eliminated.

Data collected during periods of eyelid stimulation were eliminated from the analysis to avoid contamination from electrical artifact. Analysis of neural activity was restricted to times in which rats were moving ≥ 4 cm/s to reduce the potential contribution of activity related to sharp-wave ripples or activity during rest or grooming. To facilitate comparisons between old and young animals, place fields were excluded from analysis if the mean running speed within the field boundaries was >40 cm/s (Fig. 1*C*). Individual neurons often express unique fields in different running directions (McNaughton et al., 1983a). To constrain analyses so that only one field was analyzed per neuron, all calculations of firing properties and spatial information were restricted to trials/running directions associated with the field with the greatest spatial information content.

Firing rate measures

Two measures of firing rate were analyzed. The mean firing rate was measured as the total spikes observed divided by the total time spent while actively moving on the track. The peak firing rate of a given place cell was determined as the maximum firing rate when the rat was within the boundaries of the place field (see below).

Place field measurement

Place fields were computed by first binning the linearized position of the track into 3 cm bins (4° of circumference) and then determining the vector of the time spent in each bin (occupancy, *Occ*) and the vector of the number of action potentials per bin (*V_{spk}*). The place field was calculated as *V_{spk}*/*Occ*. The boundaries of a place field were determined by first smoothing the occupancy normalized linearized place field (third-degree

polynomial filter, window = 30° on track; `sgolayfilt()` function in MATLAB). Starting from the location of peak activity of the place field, the two locations at 20% of the peak were identified (preceding and following the peak). From these two points, the locations of the field boundaries were extended to the locations where the firing rates stopped decreasing (e.g., reached zero or started increasing). These points marked the start and end of the field.

Spatial information

Spatial information content by time (bits per second) was calculated as follows:

$$I_t = \sum P_i \left(\frac{f_{r_i}}{\bar{f}_r} \right) \log_2 \left(\frac{f_{r_i}}{\bar{f}_r} \right),$$

and spatial information by spike was calculated as follows:

$$I_{sp} = \sum P_i \left(\frac{f_{r_i}}{\bar{f}_r} \right) \log_2 \left(\frac{f_{r_i}}{\bar{f}_r} \right)$$

(Skaggs et al., 1993). In these formulas, *i* is the place field bin, and *P_i* is the probability of occupancy in that bin, *f_r* indicates mean firing rate of the neuron, and *f_{r_i}* indicates the firing rate within a spatial bin. These two measures were used as they convey different computational interpretations. Specifically, *I_t* measures the overall spatial content of the place field or how much information per second a cell is capable of transmitting to downstream neurons. In contrast, *I_{sp}* measures the information content per action potential, which controls for differences in firing rate and indicates increased specificity and compactness of the place field (Skaggs et al., 1993).

Analysis of spike-field coherence

Spike-field coherence (SFC) measures the degree to which spikes phase lock to specific frequencies of the local field potential (Bragin et al., 1995; Womelsdorf et al., 2006; Jutras et al., 2009; Benchenane et al., 2010). SFC was computed by first measuring the phase of the oscillation at the time of each action potential. The Rayleigh *z* measure of phase locking (Fisher, 1993) was computed for the resulting distribution of phases, and this value was then compared with a computed null distribution. This null distribution was generated by recalculating the Rayleigh *z* value 400 times after randomly sampling phases with replacement within a ± 0.5 s window surrounding each action potential. This local-window approach, as opposed to randomly selecting phases across the entire recording, was done to reduce possible contamination from slow nonstationary changes in the distribution of phases that can bias measures of phase locking. The *p* value of phase locking for each neuron was determined using a kernel smoothing density estimate (`ksdensity()` in MATLAB) of the null distribution.

Modifications to the analysis of spike-field coherence to increase the precision of phase-locking measures. Although the general procedure described above for analyzing spike-field coherence was followed, modifications were made to address the complication raised by the wide-band response of gamma observed in the data (30–120 Hz; see Fig. 3*A*). Traditional measures typically use a single bandpass filter to identify a single measure of phase across a range of frequencies. This is not ideal for the wide-band response observed here as gamma frequencies varied as a function of running speed (see Fig. 3*A*), and phase can vary considerably within the wide gamma band (see Fig. 6*C*). To address this, we augmented the procedure described above. First, the Morelet wavelet spectrogram was determined for the ± 0.5 s window surrounding each action potential (30–120 Hz; see Fig. 6*A*). This spectrogram was normalized by subtracting the mean wavelet power spectral density (computed over the entire track-traversal period) to reduce the 1/*f* component present in neural spectra. The frequency at which power was maximal at the time of the action potential was then identified (see Fig. 6*A*, white X). Third, the phase at this frequency (see Fig. 6*B,C*, magenta and yellow Xs) was identified and used to compute the distribution of phases needed for the Rayleigh *z* measure of phase locking.

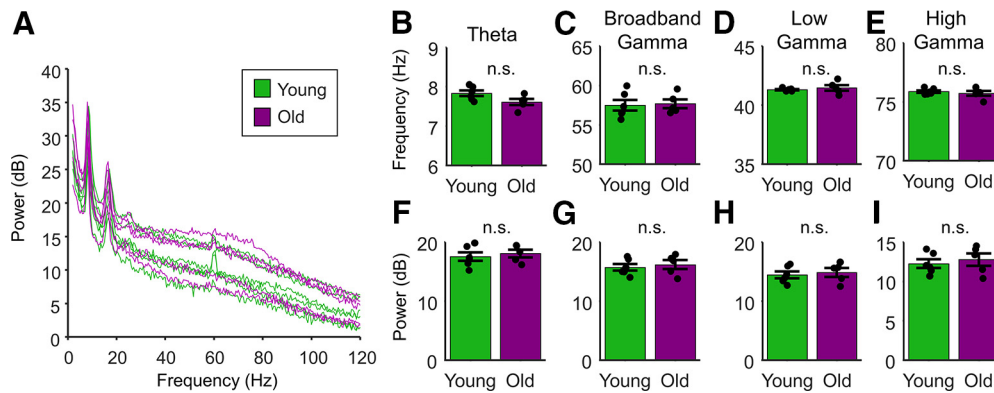


Figure 2. No differences in oscillatory frequency and power are seen when data are collapsed across running speeds. **A**, Average PSDs for CA1 local-field activity during the eye-blink conditioning task at speeds >1 cm/s and <40 cm/s. Individual lines represent averages across all 31 sessions for each animal. **B**, Theta (5–10 Hz) frequencies were not significantly (n.s.) different between young and old animals ($t_{(8.5)} = 2.10$, $p = 0.067$). **C**, Broadband gamma frequencies (30–120 Hz) were not statistically different between age groups ($t_{(9.0)} = -0.22$, $p = 0.829$). **D**, Low gamma frequencies (30–55 Hz) were not statistically different between age groups ($t_{(4.4)} = -0.65$, $p = 0.546$). **E**, High gamma (65–120 Hz) frequencies were not significantly different between age groups ($t_{(6.0)} = 0.67$, $p = 0.526$). **F**, Theta power was not significantly different between young and old animals ($t_{(9.0)} = -0.54$, $p = 0.602$). **G**, Broadband gamma power was not significantly different between age groups ($t_{(7.6)} = -0.48$, $p = 0.642$). **H**, There was no significant difference in low gamma power between age groups ($t_{(8.0)} = -0.42$, $p = 0.685$). **I**, There was no significant difference in high gamma power between age groups ($t_{(7.3)} = -0.60$, $p = 0.570$). All error bars represent mean $\pm$ SEM.

To better ensure that phase locking was measured during periods where oscillatory activity was present, spikes were eliminated from analysis if maximum oscillatory power at the time of the spike fell below the mean power measured during the track-running period. In addition, neurons with <30 spikes during behavior were eliminated from analysis. Although this approach was developed to address the wide-band gamma response, it was also used to measure phase locking to theta (see Fig. 6F) to facilitate comparison between these different rhythms.

Statistical analyses

Data are presented as mean $\pm$ SE. Standard parametric tests such as Student's t tests, regression, and ANOVA and nonparametric tests such as Wilcoxon rank sum tests were used. Tukey–Kramer (ANOVA) and Bonferroni–Holm corrections were performed to adjust for familywise error. Alpha was set at 0.05 (two tailed). Statistical analyses were performed using MATLAB (MathWorks) and R software (version 4.0.4).

Results

Old rats exhibited spatial memory impairments on the Morris water maze but no impairments in spatial eye-blink conditioning

As reported in Schimanski et al. (2013), aged animals had a significantly longer mean corrected integrated path length (Gallagher et al., 1993) for the last day of water maze testing (average of six trials) than did young animals ($p = 0.017$, Wilcoxon rank sum). Conversely, as reported in Schimanski et al. (2013), aged animals did not exhibit deficits in their ability to blink near the stimulation zone ($t_{(7.2)} = -1.49$, $p = 0.179$).

No differences in theta and gamma frequency or power between age groups were detected when locomotor activity was not taken into account

Average power spectral densities during nonstimulated trials for speeds between 1 and 40 cm/s for each rat are shown in Figure 2A. When analyzed this way, theta frequency was not significantly different between young and old animals ($t_{(8.5)} = 2.10$, $p = 0.067$, Cohen's $d = 1.27$; Fig. 2B). As previously reported by Insel et al. (2012), there were also no significant age-associated differences in average broadband gamma frequency ($t_{(9.0)} = -0.22$, $p = 0.829$; Fig. 2C). Different functional roles have been proposed for low (~ 30 –55 Hz) and high (~ 65 –120 Hz) gamma (Colgin et al., 2009; Bieri et al., 2014; Dvorak et al., 2018). Consequently, we

investigated the possibility that low and high gamma are differentially modulated by age. We saw no differences in peak low gamma frequency between age groups when all speeds were collapsed between 1 and 40 cm/s ($t_{(4.4)} = -0.65$, $p = 0.546$; Fig. 2D) or peak high gamma frequency between age groups ($t_{(6.0)} = 0.67$, $p = 0.526$; Fig. 2E). Theta power did not differ between age groups ($t_{(9.0)} = -0.54$, $p = 0.602$; Fig. 2F), nor did broadband gamma power ($t_{(7.6)} = -0.48$, $p = 0.642$; Fig. 2G), power for low gamma ($t_{(8.0)} = -0.42$, $p = 0.685$; Fig. 2H), or high gamma ($t_{(7.3)} = -0.60$, $p = 0.570$; Fig. 2I).

Gamma frequency increased with running speed at a slower rate in old animals

To determine whether age affects the relationship between gamma frequency and speed, we created a normalized power spectrogram at each running speed for each recording session. This was accomplished by subtracting the mean spectral power across frequencies and dividing by the standard deviation to obtain z-scored power at a given speed. Frequencies were binned in 0.5 Hz steps between 30 and 120 Hz. These normalized spectrograms were computed at each speed (1–40 cm/s) to create an average speed by relative gamma power matrix for each day. Matrices were averaged across all 31 d for each animal. The frequency of maximum gamma power was found for each speed, and a robust regression analysis (robustfit() in MATLAB) was performed across the maxima to compute the slope and intercept for each animal (Fig. 3A).

Figure 3B shows the regression lines for each rat overlaid on the average normalized speed by frequency power plots for young and old rats. Old animals had a significantly higher value for the y-axis intercept than young animals, indicating that peak gamma frequency was higher for lower running speeds in older animals ($t_{(8.9)} = -3.35$, $p = 0.009$, Cohen's $d = 2.00$; Fig. 3C). Additionally, slopes were significantly lower for older animals, indicating that the rate of increase in gamma frequency by speed is reduced in aged animals ($t_{(8.3)} = 2.8$, $p = 0.022$, Cohen's $d = 1.71$; Fig. 3D). To determine whether the relationship between running speed and gamma frequency was weaker in older animals, we compared R^2 values (goodness of fit) of the regression lines between young and old animals, but we saw no significant differences ($t_{(9.0)} = 1.33$, $p = 0.217$). Together, these results

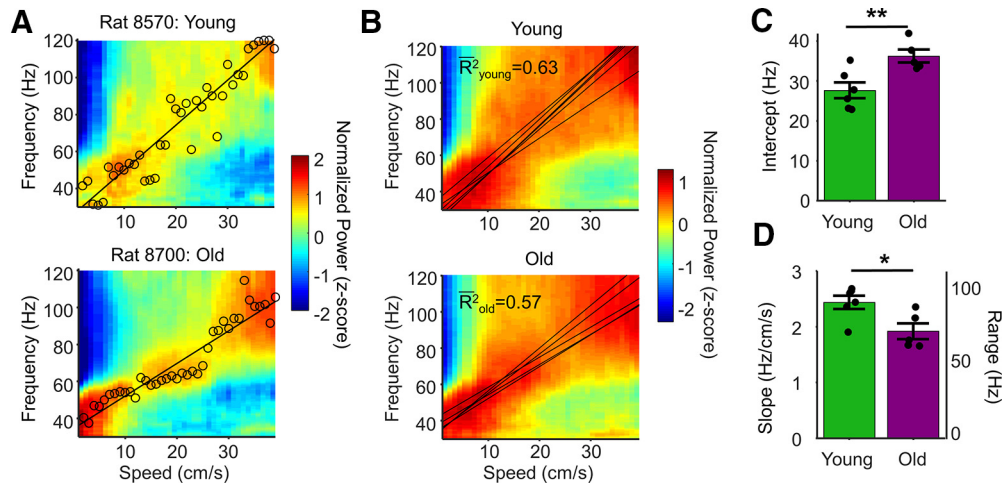


Figure 3. Gamma frequency increases with running speed in both young and old rats but at a slower rate and over a reduced range in old rats. **A**, To quantify the relative change in peak gamma power across running speeds (1–40 cm/s), PSDs were z-scored and binned according to speed. They were then averaged for each rat. The frequency for maximum gamma power was identified for each speed bin (black circle). A robust regression (black line) was then performed to obtain a slope and intercept value for each animal. Color plots show data from one young (top) and one old (bottom) rat. Warmer colors indicate higher power. **B**, The average normalized power spectra for speeds from 1 to 40 cm/s is shown for all animals grouped by age. The overlaid black lines show regression lines for individual animals. Inset, Average R^2 values did not differ between age groups. **C**, The mean intercept of the regression lines shown in **B** was significantly higher for older animals than for younger animals ($t_{(8,9)} = -3.35$, $**p = 0.009$). **D**, The slope (Hz/cm/s) and range of frequencies spanned (Hz) by these regression lines were significantly reduced for older animals compared with younger animals ($t_{(8,3)} = 2.8$, $*p = 0.028$). Error bars represent mean $\pm$ SEM.

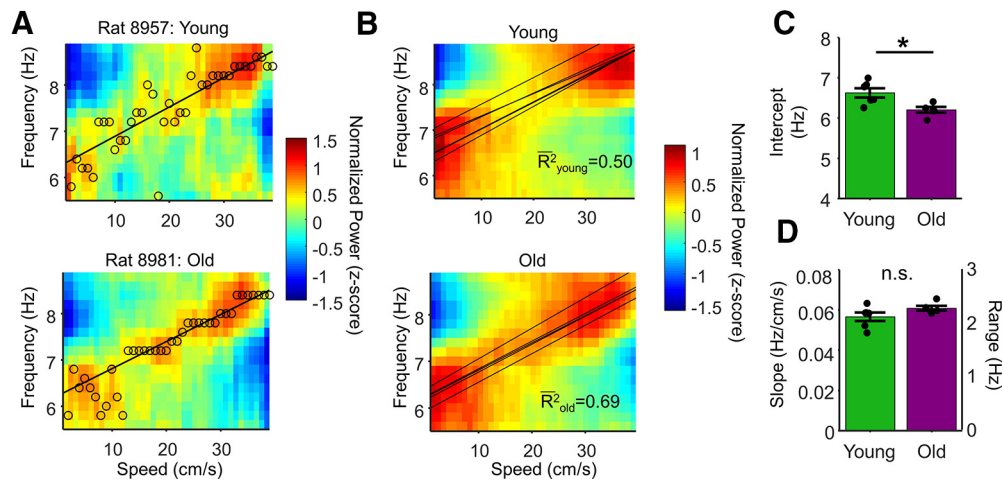


Figure 4. Theta frequency is higher across all speeds in young animals but increases at the same rate across running speeds in aged and young rats. **A**, The same analysis that was conducted for gamma frequencies was conducted for theta frequencies. PSDs are binned across running speeds from 1 to 40 cm/s. Like gamma, theta frequency increases with running speed. Black circles show the frequency of maximum power, and the black line shows the regression line for an example young (top) and old (bottom) animal. Warmer colors indicate higher power. **B**, Average running speed by theta frequency plots for all young and old animals. Regression lines from individual animals in each age group are overlaid. Warmer colors indicate higher power. **C**, The mean intercept of the regression line for young animals was significantly higher than that for the old animals ($t_{(8,2)} = 3.10$, $*p = 0.014$). **D**, There were no significant (n.s.) age differences between the slope (Hz/cm/s) and frequency range (Hz; $t_{(7,8)} = -1.70$, $p = 0.129$). Error bars represent mean $\pm$ SEM.

indicate that hippocampal CA1 network activity in aged rats reliably scales with running speed but over a smaller range of frequencies compared with younger rats (Fig. 3D, right axis).

Theta frequency is higher in young than in old animals across running speeds, but in both age groups frequency scales with speed at the same rate

To assess whether theta is differentially modulated by speed in young and old rats, the same speed by frequency analysis was performed for theta frequencies (5–9 Hz) in steps of 0.2 Hz. Figure 4A shows an example of this analysis for a young and an old rat. Figure 4B shows the average speed by theta frequency plots for young and old animals with regression lines of individual animals overlaid in black. The mean intercept of the regression line was higher for younger animals ($t_{(8,2)} = 3.10$, $p = 0.014$, Cohen's $d =$

1.89; Fig. 4C); however, there was no difference in the slope and range of these regression lines between young and old animals ($t_{(7,8)} = -1.70$, $p = 0.129$; Fig. 4D). The R^2 measure trended toward being greater for old animals, but this was not statistically significant ($t_{(7,9)} = -2.27$, $p = 0.054$). These observations indicate that theta frequency in old animals scales with running speed at the same rate as it does for young animals but that, on average, young animals have a higher theta frequency given the same running speed as old animals.

Theta is modulated by speed, deceleration, and acceleration, whereas gamma is principally modulated by speed and deceleration in both aged and young rats

Recent reports have indicated that acceleration is the best predictor of theta frequency (Kropff et al., 2021). Thus, we investigated acceleration and deceleration as potential predictors of both theta

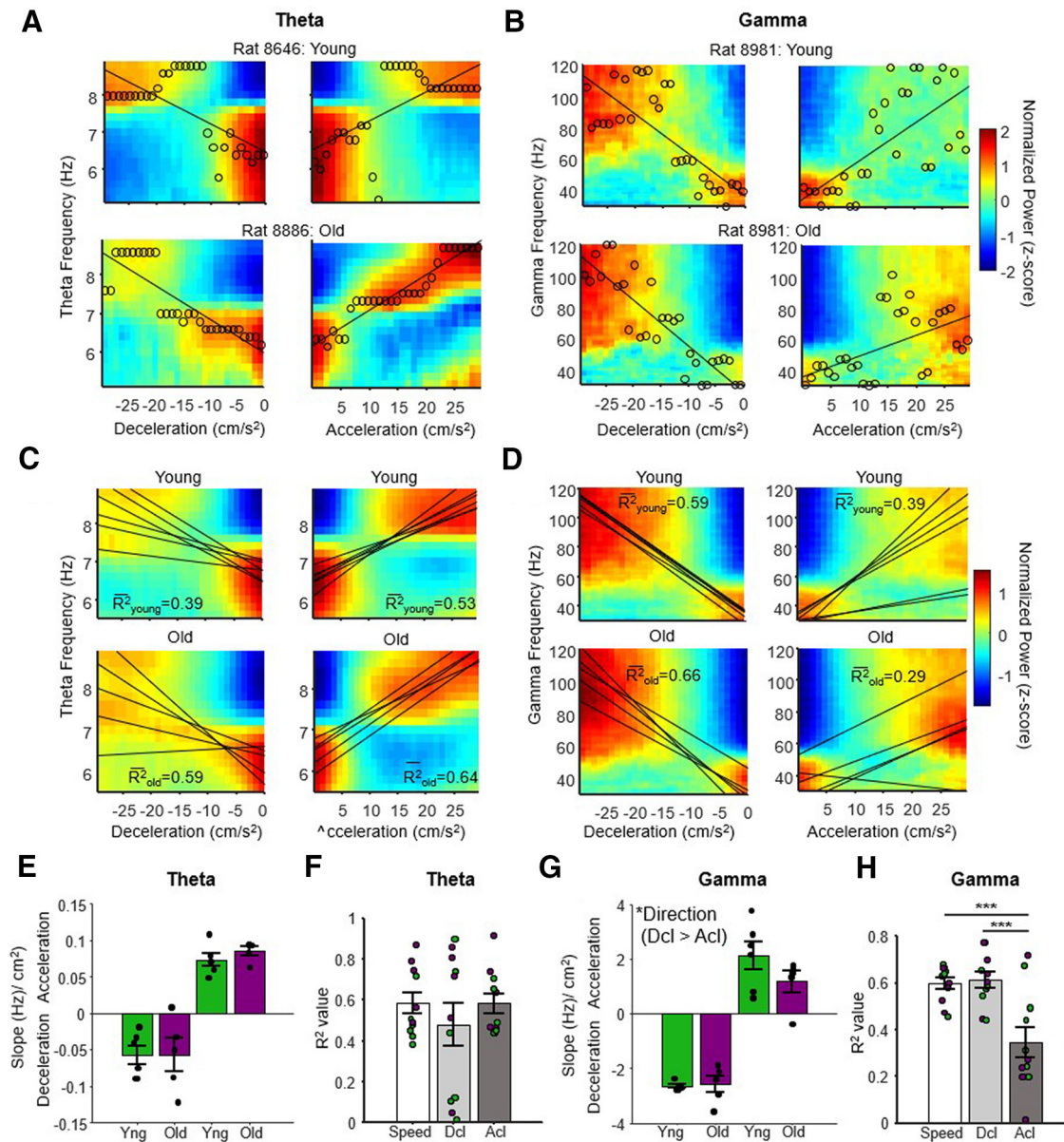


Figure 5. Acceleration is associated with increasing theta frequency, whereas deceleration is associated with decreasing gamma frequency. **A**, Examples of the relationship between deceleration, acceleration, and theta frequency across all sessions for a young (top) and an old (bottom) rat. Black circles indicate peak theta frequency at each acceleration/deceleration bin. Robust regression lines are overlaid (black lines). Warmer colors indicate higher power (**A–E**). **B**, Examples of the relationship across all sessions between deceleration, acceleration, and gamma frequency for a young (top) and an old (bottom) rat. Black circles indicate peak gamma frequency at each acceleration/deceleration bin. Robust regression lines are overlaid (black lines). **C**, Average relationship between deceleration, acceleration, and theta frequency for all young and old animals. Regression lines for individual animals are overlaid in black. **D**, Average relationship between deceleration, acceleration, and gamma frequency for all young and old animals. Regression lines for individual animals are overlaid in black. **E**, Two-way repeated-measures ANOVA (deceleration/acceleration by age) of the slope of the relationship between deceleration, acceleration, and theta frequency was not different between young and old animals ($F_{(1,9)} = 0.17$, $p = 0.690$), nor was there a difference in the deceleration rate and acceleration rate ($F_{(1,9)} = 3.04$, $p = 0.115$) or any age by direction (acceleration/deceleration) interaction ($F_{(1,9)} = 0.27$, $p = 0.618$). Slopes for deceleration were multiplied by -1 to allow comparison with acceleration values. **F**, Comparison of R^2 values for the relationships among theta frequency, speed, acceleration, and deceleration. Because there was no effect of age, values from all animals were combined for R^2 comparisons; dot color represents age group (young, green; purple, old). No difference between R^2 values was observed among theta frequency and speed, deceleration, and acceleration ($F_{(2,20)} = 0.71$, $p = 0.506$). **G**, Two-way repeated-measures ANOVA (deceleration/acceleration by age) of the slopes of the relationships among deceleration, acceleration, and gamma frequency revealed a main effect of direction on the magnitude of the slope such that the slope was significantly steeper for deceleration than acceleration ($F_{(1,9)} = 8.37$, $*p = 0.018$). However, there was no effect of age ($F_{(1,9)} = 0.22$, $p = 0.218$), nor was there an interaction ($F_{(1,9)} = 1.69$, $p = 0.226$). Slopes for deceleration were multiplied by -1 to allow comparison with acceleration values. **H**, Comparison of R^2 values among gamma frequency, speed, acceleration, and deceleration. Because there was no effect of age, values from all animals were combined; dot color represents age group (young, green; purple, old). A significant difference in R^2 values was observed among measures of speed, acceleration, and deceleration ($F_{(2,20)} = 14.99$, $***p = 0.0001$). *Post hoc* comparisons with Bonferroni–Holm corrections for multiple comparisons found that R^2_{speed} was significantly greater than $R^2_{\text{acceleration}}$ ($***p = 0.0004$), and $R^2_{\text{deceleration}}$ was significantly greater than $R^2_{\text{acceleration}}$ ($***p = 0.0005$), but no significant differences were present between speed and deceleration R^2 values ($p = 0.823$). Error bars represent mean $\pm$ SEM.

and gamma frequency in young and old rats. Because rats rarely achieved acceleration and deceleration values >30 cm/s² or less than -30 cm/s², we restricted our analyses to this range. Figure 5A shows example plots of deceleration and acceleration by theta frequency

across all session from an example young and old animal. Figure 5B shows example plots for gamma range activity. Figure 5C and D, show average deceleration and acceleration plots for animals grouped by age for theta and gamma range activity, respectively.

To determine whether the relationship between acceleration/deceleration and theta frequency was different with age or acceleration/deceleration, we performed a mixed 2×2 ANOVA. We found no main effects of age ($F_{(1,9)} = 0.17$, $p = 0.690$) or acceleration/deceleration on slope values ($F_{(1,9)} = 3.04$, $p = 0.115$) or any interaction ($F_{(1,9)} = 0.27$, $p = 0.618$; Fig. 5E). Additionally, the R^2 values for young and old animals showed no significant differences for deceleration ($t_{(8,9)} = -0.96$, $p = 0.363$) or acceleration ($t_{(6,37)} = -1.11$, $p = 0.307$) across theta frequencies.

We were also interested in whether our data replicate recent observations that acceleration is a better determinant of theta frequency than speed (Kropff et al., 2021). Because we had observed no age effects, we compared R^2 values for speed, deceleration, and acceleration across all animals. We found no significant difference in R^2 measures for these three different movement parameters ($F_{(2,20)} = 0.71$, $p = 0.506$; Fig. 5F).

To our knowledge, no one has yet reported how gamma frequency scales with acceleration. Comparing slopes for deceleration and acceleration, we found no significant main effect of age ($F_{(1,9)} = 0.22$, $p = 0.218$); however, the frequency decreased more per unit of deceleration (Hz/cm/s²) than it increased per unit of acceleration ($F_{(1,9)} = 8.37$, $p = 0.018$, partial $\eta^2 = 0.27$; Fig. 5G). There was no age by acceleration/deceleration interaction ($F_{(1,9)} = 1.69$, $p = 0.226$).

The R^2 value of the relationships between gamma frequency and both deceleration and speed was greater than the R^2 value of the relationship between gamma frequency and acceleration ($F_{(2,20)} = 14.99$, $p = 0.0001$, $\eta^2 = 0.48$; *post hoc* speed versus acceleration, $p = 0.0004$; Bonferroni–Holm corrected; *post hoc* speed versus deceleration, $p = 0.0005$; Bonferroni–Holm corrected; Fig. 5H).

Aged CA1 neurons show increased phase locking to gamma

Our analysis thus far has emphasized the degree to which gamma frequency changes with locomotor behavior. Traditional measures of phase locking estimate phase using a single bandpass filter (Bragin et al., 1995; Jutras et al., 2009). Assessing phase locking across the wide range of gamma frequencies observed here (30–120 Hz; Fig. 3A,B) poses problems for this approach as phase could vary as a function of frequency within the band. To address this, the approach illustrated in Figure 6 was adopted. Briefly, at the time of each action potential, we identified the frequency within the 30–120 Hz band where power was maximal (Fig. 6A) and used this frequency to determine the phase assigned to that action potential (Fig. 6A,B; see above, Materials and Methods).

Gamma oscillations are thought to group cell assemblies (Lisman and Jensen, 2013). We hypothesized that a reduced responsiveness of gamma frequency to running speed in older animals (Fig. 3) would be accompanied by a reduced capacity of

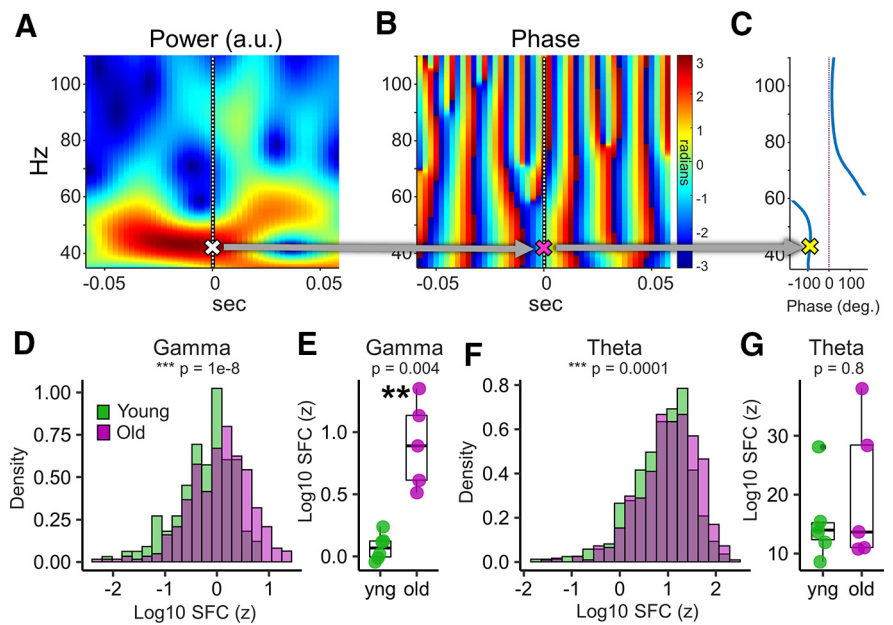


Figure 6. Aged rat CA1 neurons show increased phase locking to gamma. **A**, Illustration of the method for assigning the phase from a wide-band gamma signal (30–120 Hz) to the time of a single action potential. The dashed line indicates the time of a single action potential (time = 0). The frequency with the greatest power at $t = 0$ is determined (white X in the plot) and used to estimate phase (gray arrow). **B**, The frequency identified in **A** (indicated as the white X) is used to identify the phase in **B** (magenta X) when the action potential occurred (colors indicate phase in radians). **C**, The yellow X and gray arrows indicate the phase at $t = 0$ of the same action potential from subplots **A** and **B**. This plot illustrates that phase can vary considerably as a function of frequency, emphasizing the need to select phase based on the frequency of peak power to increase the precision of phase-locking measures. **D**, When all cells are considered (neurons, $n = 617$ old, $n = 437$ young, $n = 1054$ total), phase locking of spikes to gamma was increased in aged rats relative to that of young rats ($p = 1e-8$, Wilcoxon rank sum test, Cohen's $d = -0.40$). **E**, When data are considered by animal, the significant effect remained ($p = 0.004$, Cohen's $d = -2.8$). **F**, **G**, When all cells are considered, phase locking of spikes to theta was larger in neurons from aged relative to young rats ($p = 0.0001$, Wilcoxon rank sum test, Cohen's $d = -0.26$); however, at the animal level (**G**), there was no significant effect of age on theta-band spike-field coupling ($p = 0.80$, Wilcoxon rank sum test).

these oscillations to entrain neurons. Intriguingly, we found that neurons from older animals were more phase locked to gamma relative to those from young animals ($p = 0.0007$, Wilcoxon rank sum test, Cohen's $d = 0.46$; Fig. 6D). This effect was also significant at the animal-level of analysis ($p = 0.004$, Cohen's $d = -2.8$; Fig. 6E). The same approach was used to assess phase locking to theta. Although phase locking to theta was greater in neurons from aged relative to young rats ($p = 0.0034$, Wilcoxon rank sum test, Cohen's $d = -0.2$, $n = 585$ old, $n = 335$ young neurons; Fig. 6F), this effect was not significant at the animal level ($p = 0.80$, Wilcoxon rank sum test; Fig. 6G).

Within-field firing rates are reduced in aged rats, but spatial information per action potential is increased

One hypothesis put forward by Ahmed and Mehta (2012) is that increased gamma frequency at higher running speeds allows for a quicker transition between cell assemblies so that spatial information is not lost. Because we observed a reduced scaling of gamma frequency with running speed in old animals, we hypothesized that place cells in older animals carry less spatial information. To test this hypothesis, we examined the information content of place cells ($n = 754$ old, $n = 466$ young, $n = 1220$ total fields) from young and old animals. Two measures of spatial information content were considered (Skaggs et al., 1993; see above, Materials and Methods). Information-per-time (I_t) measures how much information per second a cell is capable of transmitting to downstream neurons. In contrast, information-

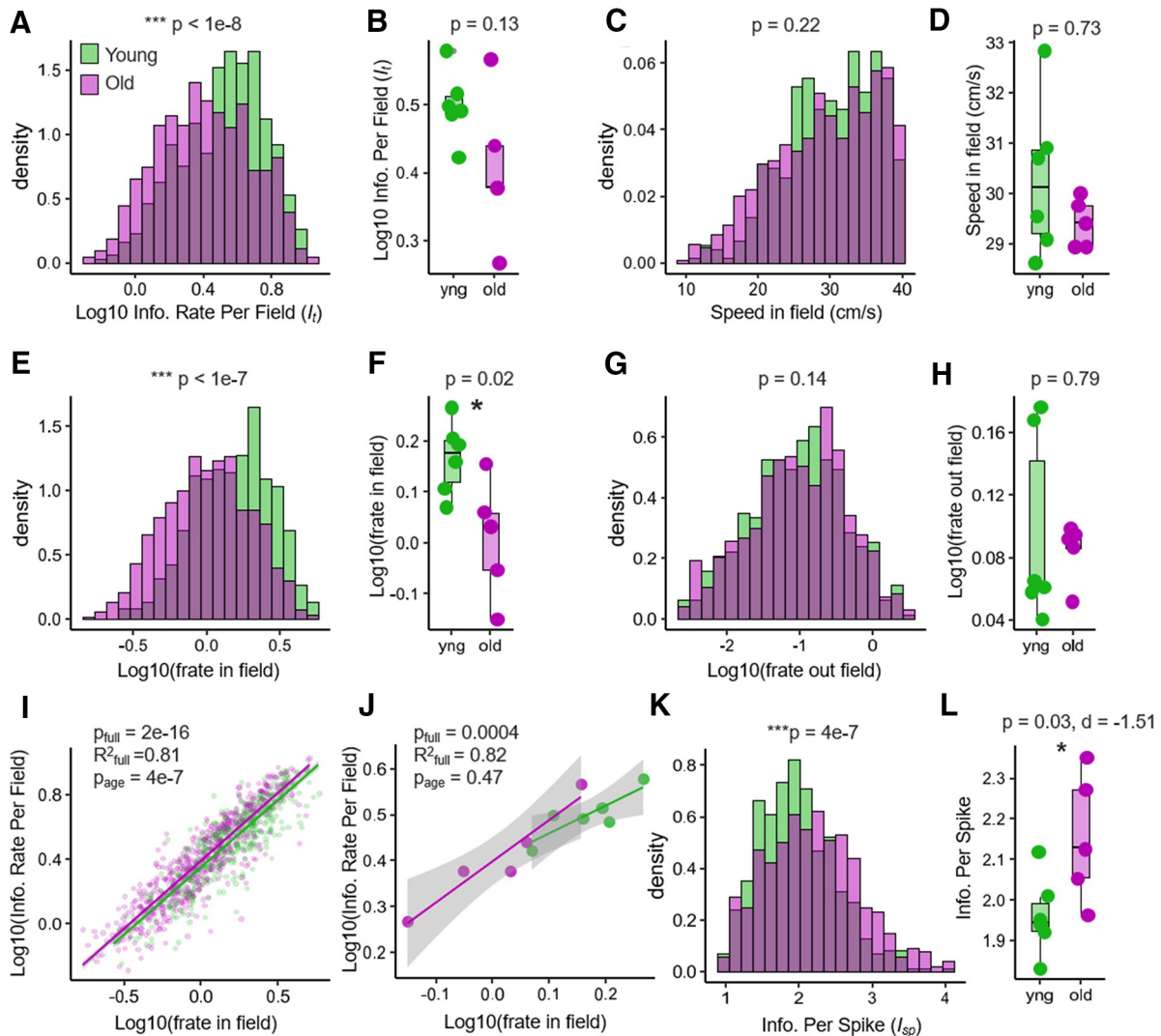


Figure 7. Within-field firing rates are reduced in aged rats, but spatial information per action potential is increased in aged rats. **A**, When all cells are considered, there is decreased spatial information content per place field (I_t) in aged when compared with young rats (*** $p < 1e-8$, Wilcoxon rank sum test, Cohen's $d = 0.40$). Histograms show the distributions of spatial information values for place fields (neurons/fields, $n = 617$ old, $n = 437$ young, $n = 1054$ total) in aged and young rats. Dark purple indicates overlap between old and young distributions. **B**, When averages of information content per field were calculated per animal ($n = 6$ young, $n = 5$ old rats), there was no significant difference between age groups ($p = 0.13$, Wilcoxon rank sum test). **C**, When all cells are considered, the mean within-field running speeds per place field were not different between old and young rats ($p = 0.22$, Wilcoxon rank sum test). Data are restricted to within-field running speeds between 4 and 40 cm/s. **D**, No difference between groups was also observed when averages were calculated per animal ($p = 0.73$). **E**, When all cells are considered, within-field firing rates were higher in young rats compared with old ($p = 1e-7$, Wilcoxon rank sum test, Cohen's $d = 0.61$). **F**, When data are considered by animal, the significant effect remained (* $p = 0.02$, Cohen's $d = 1.68$). **G**, When all cells are considered, no difference in the out-of-field firing rates was observed between aged and young rats ($p = 0.14$, Wilcoxon rank sum test). **H**, When data are considered by animal, no significant age effect was observed ($p = 0.79$). **I**, When all cells are considered, a significant relationship was observed between within-field firing rate and spatial information per field (I_t), along with a significant effect of age ($R^2_{full} = 0.81$, $p_{full} = 2e-16$, *** $p_{age} = 4e-7$, $p_{age:frate} = 0.56$). **J**, When data are considered by animal, a significant relationship was observed between the mean within-field firing rate and spatial information, but there was no effect of age ($p_{full} = 0.0004$, $R^2_{full} = 0.82$, $p_{age} = 0.47$). **K**, When all cells are considered, spatial information per spike (I_{sp}) was increased in aged rats when compared with young (*** $p = 4e-7$, Cohen's $d = -0.33$). **L**, When data are considered by animal, aged animals had significantly more information per spike (* $p = 0.03$, Cohen's $d = -1.51$).

per-spike (I_{sp}) indicates the information content per action potential, thus controlling for differences in firing rate. Furthermore, higher values of I_{sp} suggest increased specificity of the neural response to spatial location and the presence of compact place field (Skaggs et al., 1993).

Using the I_t measure of information per second, we found that place cells recorded from old rats had significantly lower spatial information rates than those recorded from young animals ($p < 1e-8$; Wilcoxon rank sum test; Fig. 7A); however, this effect was not significant at the animal level of analysis ($p = 0.13$, Wilcoxon rank sum test; Fig. 7B). I_t can be affected by running speed and firing rate. Consequently, we investigated whether the within-field running speed (Fig. 7C,D) and

within-field firing rates (Fig. 7E,F) by age group. No group-level difference in within-field running speed was observed at the neuron ($p = 0.22$, Wilcoxon rank sum test; Fig. 7C) nor the animal level of analysis ($p = 0.73$, Wilcoxon rank sum test; Fig. 7D). Contrastingly, within-field firing rates were reduced when measured both at the neuron ($p = 1e-7$, Wilcoxon rank sum test; Fig. 7E) and animal levels of analysis ($p = 0.02$, Wilcoxon rank sum test; Fig. 7F) with old animals displaying reduced within-field firing rates of hippocampal place cells. This age-associated reduction in firing rates was not observed for out-of-field activity at the individual unit ($p = 0.14$; Fig. 7G) nor at the animal level of analysis ($p = 0.79$, Wilcoxon rank sum test; Fig. 7H).

Within-field firing rates correlated strongly with I_t at the neuron ($R^2_{\text{full}} = 0.81$, $p_{\text{frate}} = 2 \times 10^{-16}$, $p_{\text{age}} = 4 \times 10^{-7}$, $p_{\text{age:frate}} = 0.56$, Fig. 7I) and the animal levels of analysis ($R^2_{\text{full}} = 0.82$, $p_{\text{full}} = 0.0004$, $p_{\text{age}} = 0.47$; Fig. 7J). Although there was a significant effect of age at the neuron level, the overlapping and parallel regression lines, the lack of a significant interaction, and the absence of an effect at the animal level indicated that the effect of age on I_t was small at best. The strong overall relationship between I_t and firing rate, however, indicated that measures of information per spike (I_{sp}), could provide additional information on the spatial processing capacity of CA1 neurons in aged rats as this measure adjusts for differences in firing rate. Using this metric, we found, intriguingly, that cells from older animals had significantly more spatial information per spike ($p = 4 \times 10^{-7}$, Cohen's $d = -0.33$; Fig. 7K). This effect remained significant at the animal level of analysis ($p = 0.03$, Cohen's $d = -1.51$; Fig. 7L). Together, these results imply that the age-associated reductions in information rate are the consequence of reduced within-field firing in older animals. Yet, despite this reduction in within-field firing rate, information per spike is significantly greater in older animals, indicating that these cells have the capacity of transmitting at least as much information as neurons in young rats.

Discussion

Several novel findings emerged from this study of the hippocampus CA1 region of young and aged rats. First, gamma frequency increased with higher running speeds in both age groups but did so over a reduced range of frequencies in the older animals. Second, overall theta frequency was lower in aged rats but similarly modulated by running speed. Third, although gamma and theta scaled equally well with deceleration and speed, acceleration was significantly less correlated with gamma frequency in both age groups. Fourth, single-unit phase locking to gamma, but not theta, was significantly increased in aged rats. Fifth, spatial information per spike was greater in older animals. Finally, within-field but not out-of-field firing rates were reduced in older animals. Previous studies of the impact of age on oscillatory dynamics and place cell activity have averaged across speed, frequencies, and maze locations. By taking these factors into account, we were able to identify specific age-associated changes that may have otherwise been obscured.

Gamma and theta modulation by running speed

Without regard to the speed of the animal, no age-related differences were observed in gamma or theta frequency or power (Fig. 2), consistent with previous reports that did not account for running speed (Abe and Toyosawa, 1999; Insel et al., 2012). When speed was taken into account, gamma frequency increased with running speed in both young and old animals equally reliably, replicating the findings first reported by Ahmed and Mehta (2012). The slope of this relationship, however, was reduced in older animals (Fig. 3). The scaling of gamma frequencies by running speed is thought to facilitate the transition between elements of a spatial sequence to keep up with changes in sensory flow Ahmed and Mehta (2012). Reductions of the frequency range over which gamma scales with running speed in old rats may limit the ability of hippocampal networks to flexibly adjust to changes in sensory input, potentially degrading sequence information.

One potential reason for the observed reductions of gamma scaling in old rats is a sparser set of inputs from afferent regions that convey sensory information to CA1 pyramidal cells. An

example of this is a reduced EPSP response in CA1 elicited by stimulation of CA3 inputs (Landfield et al., 1986; Barnes et al., 1992; Deupree et al., 1993). Because there is no age difference in the depolarization of CA1 pyramidal cells elicited by stimulation of a single CA3 axon (unitary EPSP), it was hypothesized that a subset of synapses might be silent (Barnes et al., 1992). Subsequent anatomic studies found a reduction in postsynaptic density sizes in some CA1 synapses of old rats, which may provide an explanation for the observed weakening of transmission in this structure (Nicholson et al., 2004). It remains to be determined whether the direct input from layer 3 entorhinal cortex into CA1 is also altered with age.

Theta frequency is reported to increase with running speed (McFarland et al., 1975), and we confirmed that this occurred in both young and old animals in the present study. Consistent with previous studies (Shen et al., 1997), theta frequency was significantly lower across all running speeds in older animals. One possible explanation for reduced theta frequency in older rats is a change in cholinergic and GABAergic modulation of theta from medial septal inputs to CA1 (Sun et al., 2014). This possibility has yet to be examined but is supported by work demonstrating that modulation of cholinergic and GABAergic tone similarly alters the relationship between running speed and theta frequency (Burgess, 2008; Wells et al., 2013; Monaghan et al., 2017). In fact, there is a 50–60% reduction in cholinergic transmission observed across hippocampal subfields in aged rats (Shen and Barnes, 1996). Future experiments should explore whether modulating cholinergic activity in aged animals would increase theta frequencies and improve memory.

Gamma and theta frequency modulation by speed, acceleration, and deceleration

A recent report by Kropff et al. (2021) suggests that theta frequency is primarily modulated by acceleration. In the present study, theta frequency was equally related to speed, deceleration, and acceleration in both age groups. The task implemented by Kropff et al. (2021) used a bottomless car that allowed independent control of speed and acceleration and resulted in the finding that theta was only related to acceleration but not speed or deceleration. The apparent discrepancy with the present study might be explained by the fact that these variables could not be separated in our paradigm. Although not examined by Kropff et al. (2021), we observed a decoupling of the impact of speed and deceleration on gamma frequency from that of acceleration. Higher acceleration values tended to occur with the initiation of the trial before animals reached the shock zone, whereas greater speeds and deceleration co-occurred as the animal neared the reward zone. Viewed in this way, perhaps these different task components (e.g., anticipating reward vs anticipating shock) modulate gamma in distinct ways.

Gamma and theta spike-field coherence

We observed that CA1 place cells from aged animals were more phase locked to gamma than those from young animals (Fig. 6E). This was very robust at the animal level, with every old animal showing greater gamma phase locking than every young animal. This increased phase locking happened in conjunction with a decrease in the range of frequencies over which gamma was modulated by speed (Fig. 3). The decreased range of gamma frequencies across speeds suggests that the same gamma frequency will be induced by a greater number of speeds in the old animals. Conversely, at the animal level, young and aged rats showed a similar pattern of pyramidal cell phase locking to the theta

rhythm (Fig. 6G), and the range of theta frequencies over which theta was modulated by speed was also not different. Together, these observations indicate that when the range of frequencies is reduced, as for gamma in older rats, neurons maintain a stronger phase relationship with the oscillation, which may facilitate network function.

Place cell spatial information content

Because gamma modulation by running speed was reduced in older animals (Fig. 3), we predicted that this would compromise the spatial information carried by place cells. In accord with this prediction, we found that the overall spatial information rate (I_s ; Skaggs et al., 1993) was reduced in place cells from aged rats. Because this measure is heavily influenced by firing rate (Fig. 7I) and because aged CA1 neurons were observed to have reduced within-field firing (Fig. 7E), we also measured spatial information content per spike (I_{sp}). This measure takes into account firing rate (Skaggs et al., 1993) and revealed that I_{sp} was greater in aged animals within a given place field. This suggests that age does not degrade the capacity of place cells to represent spatial information.

Within-field and out-of-field firing rates of CA1 place cells

Although previous studies have suggested no age-associated differences in the overall behavior-induced firing rate of CA1 place cells (Barnes et al., 1983; Markus et al., 1994; Wilson et al., 2005; Robitsek et al., 2015), when within- and out-of-field firing rates are assessed separately, a selective decrease in within-field firing rates was observed in old animals (Fig. 7F). Because firing rate follows a log-normal distribution (Mizuseki and Buzsáki, 2013), it is proposed (Buzsáki and Mizuseki, 2014) that a minority of well-connected, fast-firing neurons carry the greatest amount of spatial information, form the backbone of spatial representations, and have more consistent place field activity (Wilson and McNaughton, 1994; Ziv et al., 2013). In contrast, the majority of place cells carry less information, fire less, participate in fewer cell assemblies, but are more plastic. Consistent with the hypothesis that higher firing cells carry more information, we observed a strong correlation between firing rate and spatial information rate (content per field; Fig. 7I). One possible consequence of the observed reduced within-field firing rate (Fig. 7E) is that a subset of the low-firing majority no longer meaningfully contributes to a spatial representation. If this hypothesis is correct, then a greater relative proportion of the neurons participating in a spatial representation would come from the higher firing rate cells that carry more information. If this is the case, then this may explain our observation that place cells in aged animals had greater spatial information per spike, despite reduced firing rates.

It has been hypothesized that the ability of animals to distinguish between different contexts relies largely on the contribution of the more plastic slow-firing majority as their firing patterns differ to a greater extent between contexts (Buzsáki and Mizuseki, 2014). Observations of inappropriate remapping (Barnes et al., 1997) and reduced goal-finding accuracy (Rosenzweig et al., 2003) in aged rats support the hypothesis that their spatial memory impairments may result from a weaker contribution of the more plastic majority of cells. A greater relative contribution of cells representing general context as opposed to specific details may also help explain studies of episodic memory in aging humans, which find older individuals often recall the gist of a memory at the expense of

details (Koutstaal and Schacter, 1997; Paige et al., 2016; Wank et al., 2021).

References

- Abe Y, Toyosawa K (1999) Age-related changes in rat hippocampal theta rhythms: a difference between type 1 and type 2 theta. *J Vet Med Sci* 61:543–548.
- Ahmed OJ, Mehta MR (2012) Running speed alters the frequency of hippocampal gamma oscillations. *J Neurosci* 32:7373–7383.
- Barnes CA, McNaughton BL (1983) Excitability changes in hippocampal granule cells of senescent rat. *Progress in Brain Res* 58:445–451.
- Barnes CA, McNaughton BL, O'Keefe J (1983) Loss of place specificity in hippocampal complex spike cells of senescent rat. *Neurobiol Aging* 4:113–119.
- Barnes CA, Rao G, Foster TC, McNaughton BL (1992) Region-specific age effects on AMPA sensitivity: electrophysiological evidence for loss of synaptic contacts in hippocampal field CA1. *Hippocampus* 2:457–468.
- Barnes CA, Rao G, McNaughton BL (1996) Functional integrity of NMDA-dependent LTP induction mechanisms across the lifespan of F-344 rats. *Learn Mem* 3:124–137.
- Barnes CA, Suster MS, Shen J, McNaughton BL (1997) Multistability of cognitive maps in the hippocampus of old rats. *Nature* 388:272–275.
- Barthó P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsáki G (2004) Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. *J Neurophysiol* 92:600–608.
- Benchenane K, Peyrache A, Khamassi M, Tierney PL, Gioanni Y, Battaglia FP, Wiener SI (2010) Coherent theta oscillations and reorganization of spike timing in the hippocampal-prefrontal network upon learning. *Neuron* 66:921–936.
- Berron D, Neumann K, Maass A, Schütze H, Fließbach K, Kiven V, Jessen F, Sauvage M, Kumaran D, Düzel E (2018) Age-related functional changes in domain-specific medial temporal lobe pathways. *Neurobiol Aging* 65:86–97.
- Bieri KW, Bobbitt KN, Colgin LL (2014) Slow and fast γ rhythms coordinate different spatial coding modes in hippocampal place cells. *Neuron* 82:670–681.
- Bragin A, Jando G, Nádasdy Z, Hetke J, Wise K, Buzsáki G (1995) Gamma (40–100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci* 15:47–60.
- Burgess N (2008) Grid cells and theta as oscillatory interference: theory and predictions. *Hippocampus* 18:1157–1174.
- Buzsáki G, Mizuseki K (2014) The log-dynamic brain: how skewed distributions affect network operations. *Nat Rev Neurosci* 15:264–278.
- Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser M-B, Moser EI (2009) Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature* 462:353–357.
- Cowen SL, McNaughton BL (2007) Selective delay activity in the medial prefrontal cortex of the rat: contribution of sensorimotor information and contingency. *J Neurophysiol* 98:303–316.
- Csicsvari J, Hirase H, Czúrkó A, Mamiya A, Buzsáki G (1999) Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J Neurosci* 19:274–287.
- Deupree DL, Bradley J, Turner DA (1993) Age-related alterations in potentiation in the CA1 region in F344 rats. *Neurobiol Aging* 14:249–258.
- Dvorak D, Radwan B, Sparks FT, Talbot ZN, Fenton AA (2018) Control of recollection by slow gamma dominating mid-frequency gamma in hippocampus CA1. *PLoS Biol* 16:e2003354.
- Fernández-Ruiz A, Oliva A, Nagy GA, Maurer AP, Berényi A, Buzsáki G (2017) Entorhinal-CA3 dual-input control of spike timing in the hippocampus by theta-gamma coupling. *Neuron* 93:1213–1226.e5.
- Fisher NI (1993) Statistical analysis of circular data. Cambridge: Cambridge University Press.
- Gallagher M, Burwell R, Burchinal M (1993) Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* 107:618–626.
- Glisky EL (2007) Changes in cognitive function in human aging. In *Brain aging: Models, methods, and mechanisms* (Riddle DR ed), pp 3–20. Boca Raton (FL): CRC Press/Taylor & Francis.
- Hinman JR, Brandon MP, Climer JR, Chapman GW, Hasselmo ME (2016) Multiple running speed signals in medial entorhinal cortex. *Neuron* 91:666–679.

- Insel N, Patron LA, Hoang LT, Nematollahi S, Schimanski LA, Lipa P, Barnes CA (2012) Reduced gamma frequency in the medial frontal cortex of aged rats during behavior and rest: implications for age-related behavioral slowing. *J Neurosci* 32:16331–16344.
- Jacobson TK, Howe MD, Schmidt B, Hinman JR, Escabi MA, Markus EJ (2013) Hippocampal theta, gamma, and theta-gamma coupling: effects of aging, environmental change, and cholinergic activation. *J Neurophysiol* 109:1852–1865.
- Jutras MJ, Fries P, Buffalo EA (2009) Gamma-band synchronization in the macaque hippocampus and memory formation. *J Neurosci* 29:12521–12531.
- Keuler JIH, Luiten PGM, Fuchs E (2003) Preservation of hippocampal neuron numbers in aged rhesus monkeys. *Neurobiol Aging* 24:157–165.
- Koutstaal W, Schacter DL (1997) Gist-based false recognition of pictures in older and younger adults. *J Mem Lang* 37:555–583.
- Kropff E, Carmichael JE, Moser M-B, Moser EI (2015) Speed cells in the medial entorhinal cortex. *Nature* 523:419–424.
- Kropff E, Carmichael JE, Moser EI, Moser M-B (2021) Frequency of theta rhythm is controlled by acceleration, but not speed, in running rats. *Neuron* 109:1029–1039.e8.
- Landfield PW, Pitler TA, Applegate MD (1986) The effects of high Mg^{2+} -to- Ca^{2+} ratios on frequency potentiation in hippocampal slices of young and aged rats. *J Neurophysiol* 56:797–811.
- Lester AW, Moffat SD, Wiener JM, Barnes CA, Wolbers T (2017) The aging navigational system. *Neuron* 95:1019–1035.
- Lisman JE, Jensen O (2013) The θ - γ neural code. *Neuron* 77:1002–1016.
- Markus EJ, Barnes CA, McNaughton BL, Gladden VL, Skaggs WE (1994) Spatial information content and reliability of hippocampal CA1 neurons: effects of visual input. *Hippocampus* 4:410–421.
- Maurer AP, VanRhoads SR, Sutherland GR, Lipa P, McNaughton BL (2005) Self-motion and the origin of differential spatial scaling along the septo-temporal axis of the hippocampus. *Hippocampus* 15:841–852.
- Maurer A, Burke S, Lipa P, Skaggs W, Barnes C (2012) Greater running speeds result in altered hippocampal phase sequence dynamics. *Hippocampus* 22:737–747.
- McFarland WL, Teitelbaum H, Hedges EK (1975) Relationship between hippocampal theta activity and running speed in the rat. *J Comp Physiol Psychol* 88:324–328.
- McNaughton BL, O'Keefe J, Barnes CA (1983a) The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. *J Neurosci Methods* 8:391–397.
- McNaughton BL, Barnes CA, O'Keefe J (1983b) The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. *Exp Brain Res* 52:41–49.
- Mizuseki K, Buzsáki G (2013) Preconfigured, skewed distribution of firing rates in the hippocampus and entorhinal cortex. *Cell Rep* 4:1010–1021.
- Monaghan CK, Chapman GW, Hasselmo ME (2017) Systemic administration of two different anxiolytic drugs decreases local field potential theta frequency in the medial entorhinal cortex without affecting grid cell firing fields. *Neuroscience* 364:60–70.
- Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11:47–60.
- Nicholson DA, Yoshida R, Berry RW, Gallagher M, Geinisman Y (2004) Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments. *J Neurosci* 24:7648–7653.
- O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res* 34:171–175.
- O'Keefe J, Recce ML (1993) Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* 3:317–330.
- Oler JA, Markus EJ (2000) Age-related deficits in the ability to encode contextual change: A place cell analysis. *Hippocampus* 10:338–350.
- Paige LE, Cassidy BS, Schacter DL, Gutchess AH (2016) Age differences in hippocampal activation during gist-based false recognition. *Neurobiol Aging* 46:76–83.
- Rapp PR, Gallagher M (1996) Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc Natl Acad Sci U S A* 93:9926–9930.
- Rasmussen T, Schliemann T, Sørensen JC, Zimmer J, West MJ (1996) Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. *Neurobiol Aging* 17:143–147.
- Robitsek J, Ratner MH, Stewart T, Eichenbaum H, Farb DH (2015) Combined administration of levetiracetam and valproic acid attenuates age-related hyperactivity of CA3 place cells, reduces place field area, and increases spatial information content in aged rat hippocampus. *Hippocampus* 25:1541–1555.
- Rosenzweig ES, Redish AD, McNaughton BL, Barnes CA (2003) Hippocampal map realignment and spatial learning. *Nat Neurosci* 6:609–615.
- Schimanski LA, Lipa P, Barnes CA (2013) Tracking the course of hippocampal representations during learning: when is the map required? *J Neurosci* 33:3094–3106.
- Shen J, Barnes CA (1996) Age-related decrease in cholinergic synaptic transmission in three hippocampal subfields. *Neurobiol Aging* 17:439–451.
- Shen J, Barnes CA, McNaughton BL, Skaggs WE, Weaver KL (1997) The effect of aging on experience-dependent plasticity of hippocampal place cells. *J Neurosci* 17:6769–6782.
- Skaggs WE, McNaughton BL, Gothard KM, Markus EJ (1993) An information-theoretic approach to deciphering the hippocampal code. In: *Advances in neural information processing systems 5* (Hanson SJ, Cowan JD, Giles CL, eds), pp 1030–1037. Burlington: Morgan-Kaufmann.
- Sun Y, Nguyen AQ, Nguyen JP, Le L, Saur D, Choi J, Callaway EM, Xu X (2014) Cell-type-specific circuit connectivity of hippocampal CA1 revealed through Cre-dependent rabies tracing. *Cell Rep* 7:269–280.
- Wank AA, Andrews-Hanna JR, Grilli MD (2021) Searching for the past: exploring the dynamics of direct and generative autobiographical memory reconstruction among young and cognitively normal older adults. *Mem Cognit* 49:422–437.
- Wells CE, Amos DP, Jeewajee A, Douchamps V, Rodgers J, O'Keefe J, Burgess N, Lever C (2013) Novelty and anxiolytic drugs dissociate two components of hippocampal theta in behaving rats. *J Neurosci* 33:8650–8667.
- West MJ, Coleman PD, Flood DG, Troncoso JC (1994) Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *The Lancet* 344:769–772.
- Wilson IA, Ikonen S, Gallagher M, Eichenbaum H, Tanila H (2005) Age-associated alterations of hippocampal place cells are subregion specific. *J Neurosci* 25:6877–6886.
- Wilson MA, McNaughton BL (1993) Dynamics of the hippocampal ensemble code for space. *Science* 261:1055–1058.
- Wilson MA, McNaughton BL (1994) Reactivation of hippocampal ensemble memories during sleep. *Science* 265:676–679.
- Womelsdorf T, Fries P, Mitra PP, Desimone R (2006) Gamma-band synchronization in visual cortex predicts speed of change detection. *Nature* 439:733–736.
- Ziv Y, Burns LD, Cocker ED, Hamel EO, Ghosh KK, Kitch LJ, Gamal AE, Schnitzer MJ (2013) Long-term dynamics of CA1 hippocampal place codes. *Nat Neurosci* 16:264–266.