Hippocampal "Time Cells": Time versus Path Integration

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Supplemental Information

Movie S1: Firing activity of three simultaneously recorded "time cells" during treadmill running.

Movie S1 shows two laps of spatial alternation on a figure-eight maze, including two runs on the treadmill embedded in the stem of the maze (Figure 1). One red (left) and one green (right) LED are mounted on the microdrive and used to track the rat's position. The firing activity of three different neurons is indicated both by a blinking dot that follows the location of the rat, as well as an audible clicking noise. The dot blinks three different colors (magenta, green, and blue) and the audible click has three different frequencies. The first neuron (indicated by the color magenta and the lowest frequency clicks) fires early during both runs on the treadmill and has a place field located just to the left of the choice point on the maze. The second neuron (indicated by the color green and the middle frequency clicks) fires in the middle of each run on the treadmill and has a place field located just to the right of the choice point on the maze. The third neuron (indicated by the color blue and the highest frequency clicks) fires at the end of each run on the treadmill and has a place field at the start of the center stem. A red triangle appears in the lower left corner when the treadmill is active.

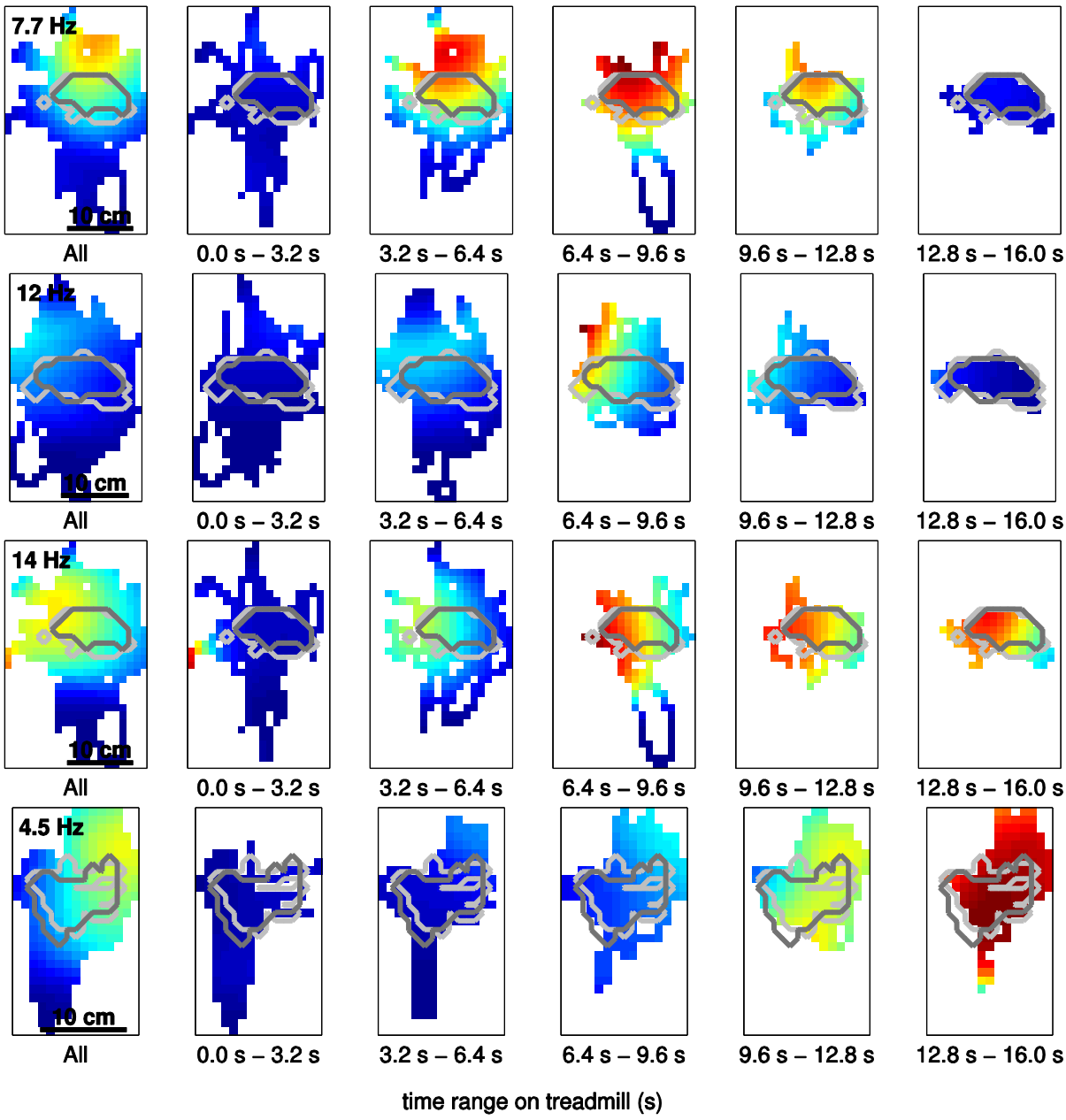

Figure S1: Spatial firing patterns on the treadmill depend on time (additional examples).

As in Figure 5, each row represents the spatial distribution of firing rates for a single neuron during treadmill running. The left-most panel on each row shows the overall firing rate map (spatial tuning curve) for the entire time on the treadmill. The remaining 5 panels show the firing rate map for each of 5 evenly divided bins of time spent on the treadmill. The light gray and dark gray outlines in each panel indicate the extent of A_{AT} and A_{75} (respectively) for that

session. Both outlines are duplicated across panels to aid comparison. In each rate map: white represents areas that were not visited by the rat during that period of time; blue represents no firing (zero spikes per second) in a visited location; red represents peak firing for that particular neuron. The number in the upper-left corner of the first panel indicates peak firing rate in spikes per second (Hz). The color scale is consistent across panels within a row to allow for comparison across panels.

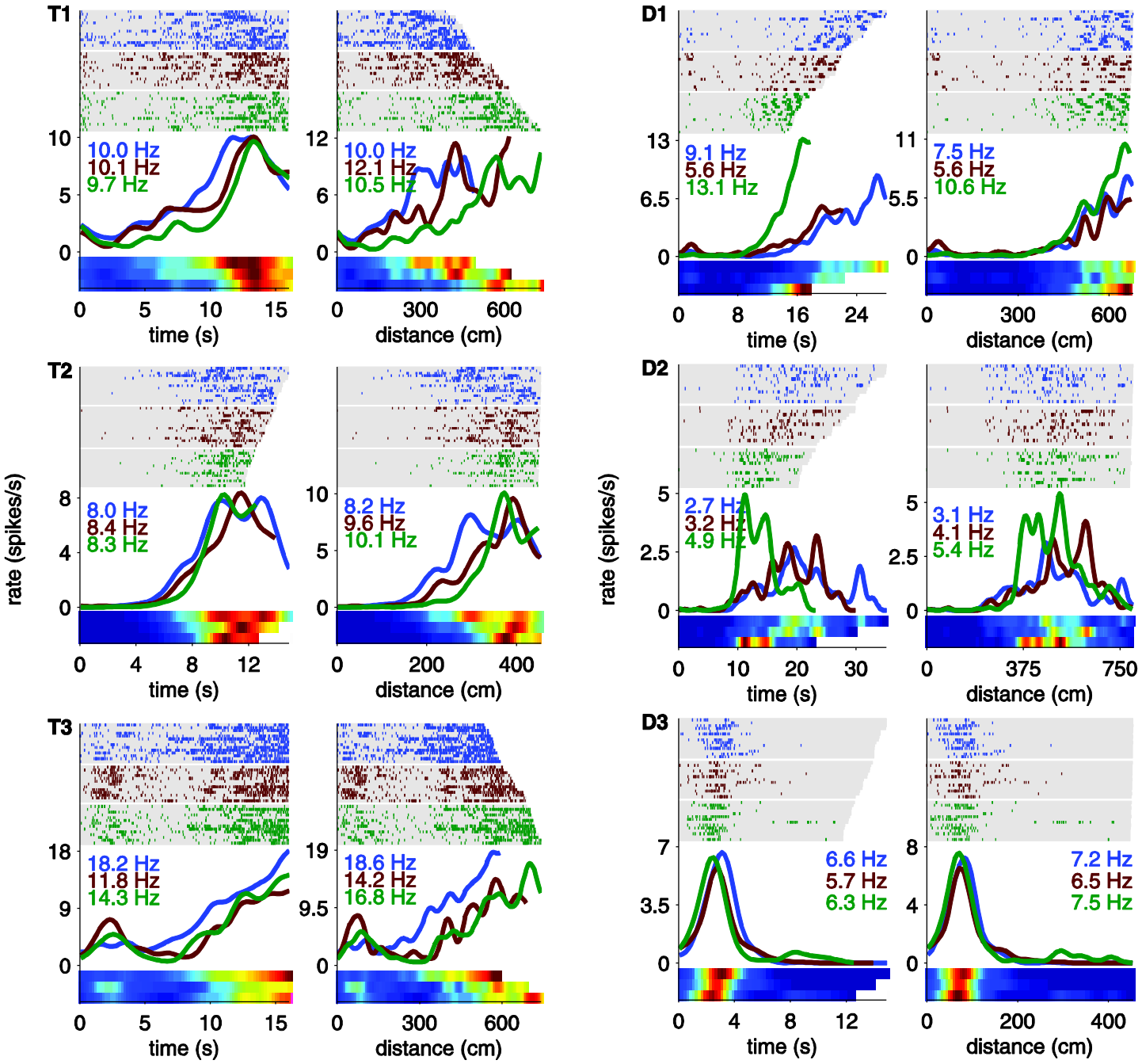

Figure S2: Hippocampal coding for time and distance (additional examples)

Additional examples of three cells that more accurately encode time (T1, T2, and T3) and three cells that more accurately encode distance (D1, D2, and D3), plotted as shown in Figure 7. For each neuron, the activity is plotted both as a function of time since the treadmill started

(left panels) and distance traveled on the treadmill (right panels). Blue, brown, and green ticks (and tuning curves) represent the slowest 1/3 of runs, middle 1/3 of runs, and fastest 1/3 of runs respectively. Numbers in blue, brown, and green indicate the peak firing rate in spikes per second (Hz) of the corresponding group of runs. The rows in the raster plots represent treadmill runs sorted in order of slowest speed (on top) to fastest speed (on bottom). The activity of each of these neurons on the remainder of the maze can be seen in Figure S3.

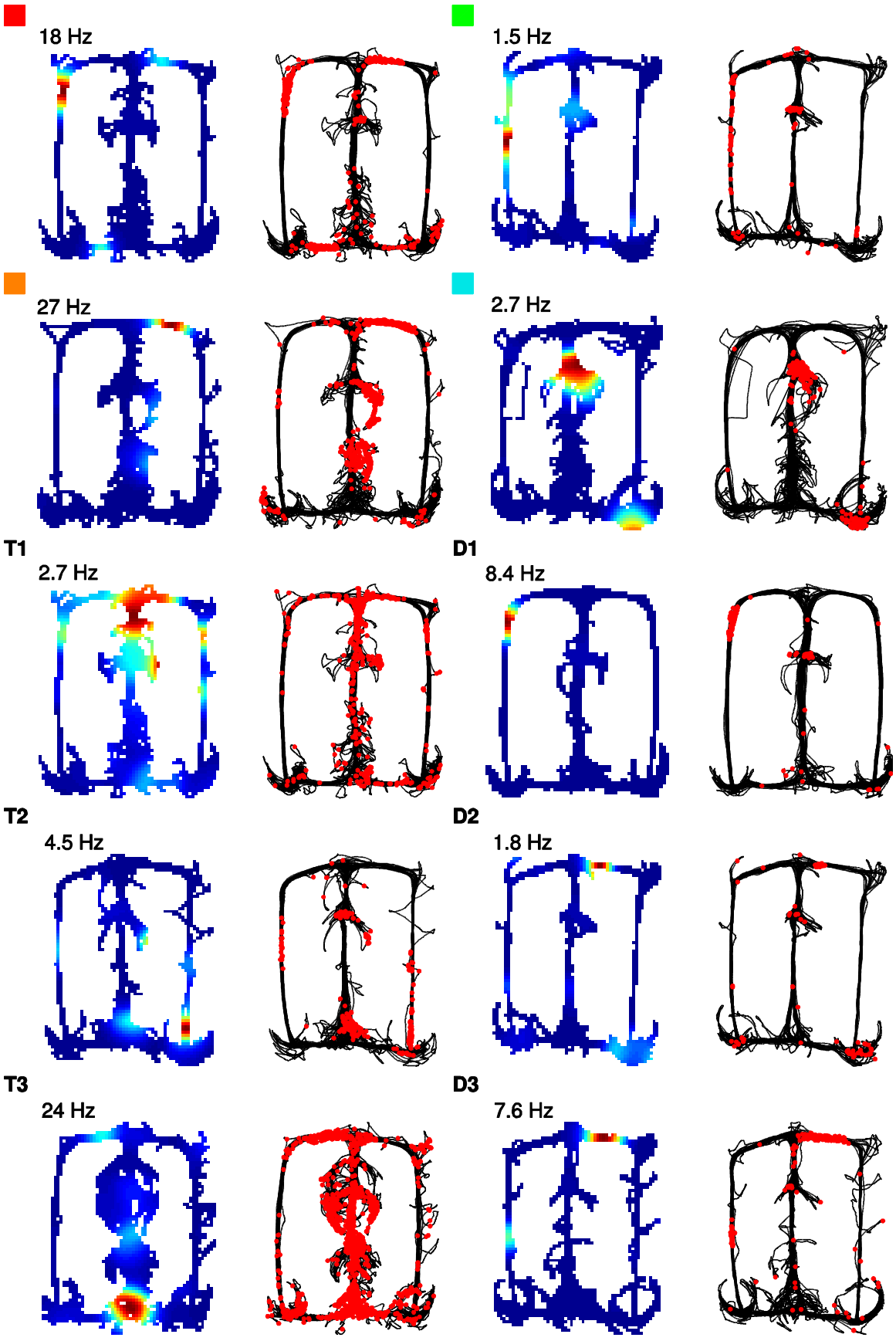
18 Hz
1.5 Hz
27 Hz
2.7 Hz
T1
D1
2.7 Hz
8.4 Hz
T2
D2
4.5 Hz
1.8 Hz
T3
D3
24 Hz
7.6 Hz

Figure S3: Neural activity on the maze

Firing activity of ten example neurons during periods when the rat was traversing the maze (excluding periods when the treadmill was active). The cells show clear place fields away from the treadmill. The top four examples correspond to the example neurons shown in Figure 7, as indicated by matching color-coded squares next to each neuron. The remaining six correspond to the example neurons shown in Figure S2, and have matching labels. Each neuron consists of two panels. The left panel shows the spatial tuning curve of the neuron during times when the treadmill was off, using 2 cm × 2 cm bins. In each rate map: white represents areas that were not visited by the rat; blue represents no firing (zero spikes per second) in a visited location; red represents peak firing for that particular neuron. The number in the upper-left corner indicates peak firing rate in spikes per second (Hz). The right panel shows the trajectory of the rat (in black) during times when the treadmill was off, with red dots overlaid indicating the location of the rat each time the neuron fired.

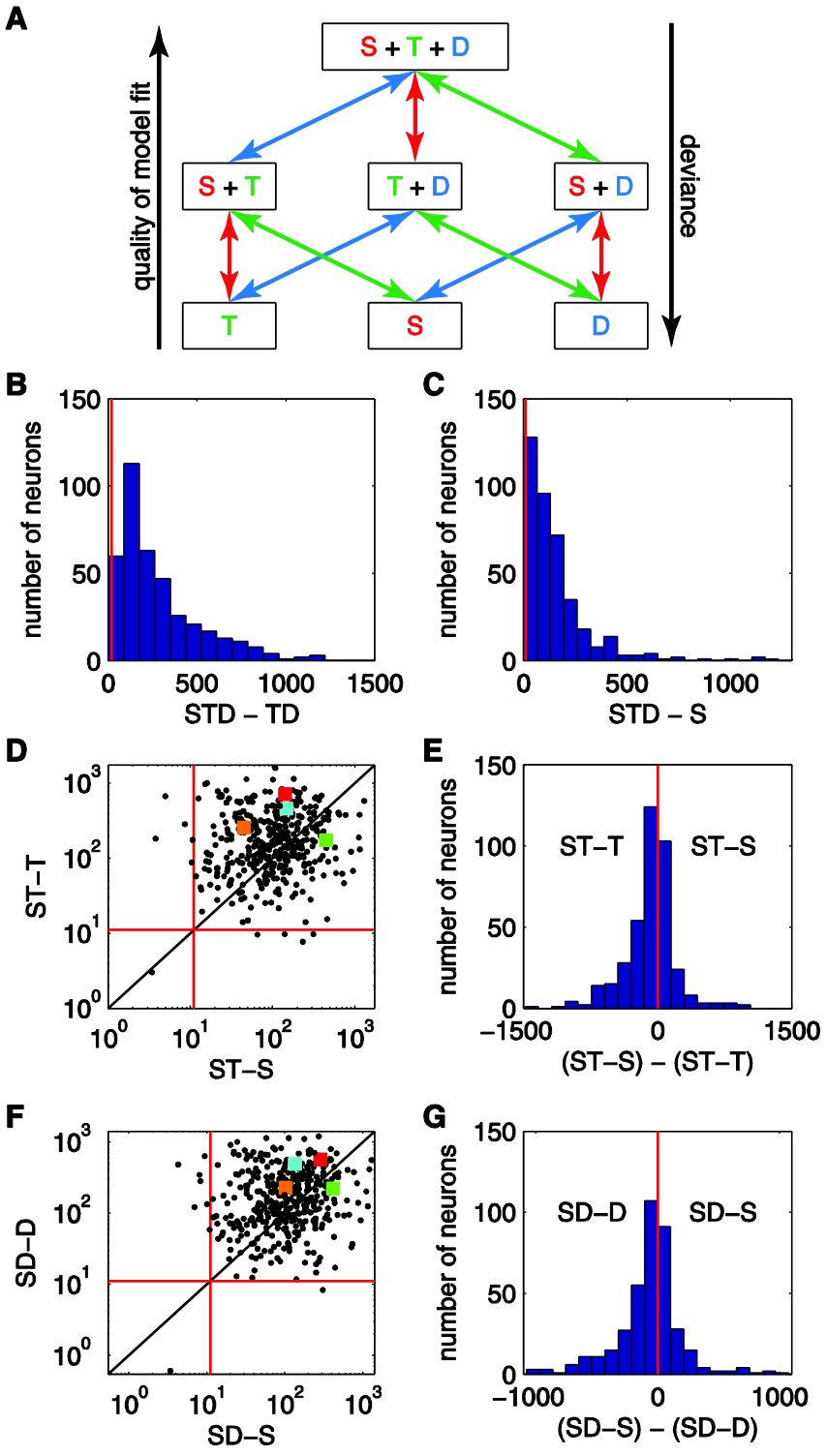
A
S + T + D
S + T
T + D
S + D
T
S
D
quality of model fit
deviance
B
number of neurons
STD – TD
C
number of neurons
STD – S
D
ST–T
ST–S
E
number of neurons
ST–T
ST–S
(ST–S) – (ST–T)
F
SD–D
SD–S
G
number of neurons
SD–D
SD–S
(SD–S) – (SD–D)

Figure S4: GLM comparison of space, time, and distance.

(A) Schematic diagram of the seven different models used for GLM analysis, including the full model with space, time, and distance ("S+T+D"), as well as six nested models. The letters indicate whether space ("S"), time ("T"), or distance ("D") were included in that nested model. Colored arrows indicate removal (or addition) of the corresponding colored component. The full model at the top of the schematic has the highest quality model fit. As you remove components from the model (either space, time, or distance), the quality of the model fit decreases, and consequently the deviance from the full model increases. The deviance quantifies the importance of the component removed to the overall quality of the model fit. (B) Histogram of deviances of the space ("S") model from the full ("S+T+D") model, resulting from the removal of both time ("T") and distance ("D") (hence the label "STD-TD"). Larger values indicate a more significant combined contribution from time and distance. (C) Histogram of deviances of the time and distance ("T+D") model from the full ("S+T+D") model, resulting from the removal of space ("S") (hence the label "STD-S"). Larger values indicate a more significant contribution due to space. Red lines in both (B) and (C) indicate minimum threshold for significance. (D) Deviances of the space ("S") model from the space and time ("S+T") model, compared to deviances of the time ("T") model from the space and time ("S+T") model. Larger x-value indicates more significant contribution from space; larger y-value indicates more significant contribution from time. Each point represents a single neuron. The clustering of points above the diagonal reflects a stronger influence of time than space for the majority of neurons. (E) Histogram of the y-values from (D) subtracted from the x-values from (D). More positive values indicate a larger contribution from space; more negative values indicate a larger

contribution from time. (F and G) Similar comparison of space and distance (“S+D”) model to the space (“S”) and distance (“D”) nested models. As with (D) the clustering of points above the diagonal reflects a stronger influence of distance than space for the majority of neurons. Red lines in both (D) and (F) indicate minimum thresholds for significance. Red lines in both (E) and (G) are at 0 (equal contribution from space and either time or distance). See Figure 8 for a GLM comparison of time vs. distance, as well as a more detailed discussion of how to interpret the figures. Colored squares in both (D) and (F) correspond to the example neurons shown in Figure 7.

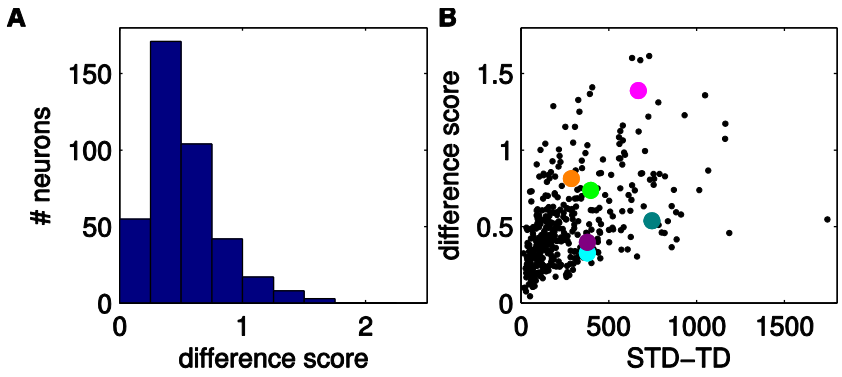

Figure S5: GLM model results correlate with "difference score"

The area between the empirical and model temporal tuning curves was calculated to give an estimate of the degree to which space alone could account for the temporal tuning. This "difference score" could range from 0 (space completely accounted for temporal tuning) to 2 (temporal tuning completely contradicted the firing predicted by space). (A) A histogram showing the distribution of difference scores among the entire population. (B)The generalized linear model was used to predict firing using either space alone ("S" model) or space, time, and distance ("S+T+D"). A large deviance of the "S" model from the "S+T+D" model ("STD-TD") suggests that space played a significant role in predicting firing activity. Panel (B) shows that the results from these distinct approaches were significantly correlated (Pearson's linear correlation coefficient: 0.49; $p = 2 \times 10^{-24}$). The colored circles correspond to the example neurons shown in Figure 6.

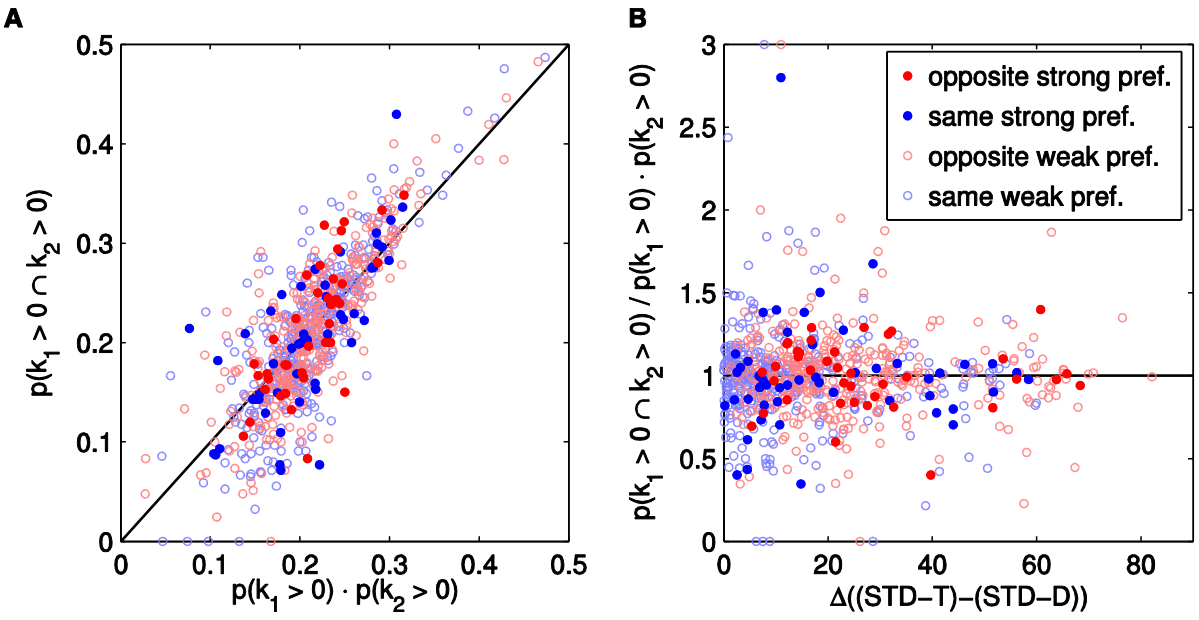

Figure S6: Hippocampus represents both time and distance simultaneously

To determine whether neurons responding significantly to only time were active concurrently with neurons responding significantly to only distance, the data recorded during treadmill running were segmented into "theta bins" defined relative to the phase of the theta signal with the least firing (as in Jezek et al., 2011). For each pair of cells, theta bins were selected in which both cells were active in neighboring theta bins (see Supplemental Experimental Procedures). Among this subset of theta bins, the expected probability of both cells firing within the same theta bin was calculated and compared to the actual frequency. (A) Data from one representative session showing comparisons between all pairs of cells active on the treadmill during that session. Plotted on the x-axis is the probability that the number of spikes from cell 1 (k_1) in each theta bin is greater than zero ($p(k_1 > 0)$) multiplied by the probability that the number of spikes from cell 2 (k_2) in each theta bin is greater than zero ($p(k_2 > 0)$). If the firing of the two cells is independent, then the expected probability of both cells firing in the same theta bins is given by $p(k_1 > 0 \cap k_2 > 0) = p(k_1 > 0) \cdot p(k_2 > 0)$. The

actual frequency of both cells firing in the same theta cycle ($p(k_1 > 0 \cap k_2 > 0)$) is plotted on the y-axis. Each cell was categorized based on the GLM analysis as having either a "strong preference" for time or distance (only time or distance informative, but not both), or "weak preference" for time or distance (both time and distance informative) (see Figure 8A). Comparisons between two cells of the same type (two cells preferring distance or two cells preferring time) are colored in blue. Comparisons between two cells of opposite type (cells preferring time with cells preferring distance) are colored in red. Comparisons involving two cells with "strong preferences" (at the extremes of the distribution shown in Figure 8) use solid circles, while the remaining ("weak preference") comparisons use open circles. If neurons at opposite extremes of the distribution were only active during different theta cycles, then a gradient would be expected with solid blue circles falling on or above the 45° diagonal (indicating that two cells of the same type tend to fire together in the same cycle more often than by chance) and solid red circles lying along the x-axis (indicating that two cells of opposite types never fire together in the same theta cycle). In contrast, the majority of comparisons (including comparisons of opposite types) fall near the 45° diagonal (black line), indicating that both types of cells are regularly firing within the same theta cycles. (B) Data from the same session as in panel (A). For each pair of cells, the ratio of the y-value to the x-value from panel (A) ($p(k_1 > 0 \cap k_2 > 0)/p(k_1 > 0) \cdot p(k_2 > 0)$) was plotted against relative preference for time versus distance ($\Delta((STD - T) - (STD - D))$) (Figure 8B). For reference, a black line is drawn at a value of 1. If cells with very different preference for time or distance were only active during different theta cycles, then as the value on the x-axis increased (indicating a larger difference in preference for time or distance), then the probability of both cells firing in the

same bin (y-axis) should decrease to zero. In contrast, no correlation was seen between these two variables (Pearson's linear correlation coefficient, $p >> 0.05$).

Supplemental Experimental Procedures

Apparatus Control and Treadmill Calibration

The rat's behavior was recorded throughout testing at 30 frames per second and synchronized to the neural data by CinePlex Video Tracker (Plexon Inc). The rat's position in each frame was tracked using two LEDs located on either the recording headstage or microdrive. The electrical signals activating each water port, and sending start/stop commands to the treadmill, were time-stamped and synchronized to the neural data by the MAP.

The behavioral apparatus was controlled using custom scripts written in MATLAB (The Mathworks, Inc., Natick, MA), interfacing with the water ports and treadmill using the Plexon Break-Out Board (PBOB) and custom built circuitry. During training, instructions were sent to MATLAB by an experimenter using a generic commercial gamepad. During testing, the rat's location, as detected by CinePlex, was used to automatically control the task.

The treadmill speed was controlled using a 0–5 V analog speed control signal. To measure the treadmill speed, alternating white and black lines were painted on the inside of the treadmill belt with a period of 4 cm. Two light sensors located inside the treadmill box emitted a TTL pulse every time a black line passed the sensor. These TTL pulses were time-stamped and synchronized to the neural data by the MAP. The instantaneous speed of the treadmill was determined by dividing 4 cm by the time between subsequent pulses, and then fitting the function $v(t) = v_{max}(1 - e^{-t/\tau})$ to the resulting values (t is the time since the start signal, τ is a rate constant indicating the acceleration of the treadmill, v_{max} is the final speed of the treadmill, and $v(t)$ is the treadmill speed at time t. The fitted value of v_{max} was then used to calibrate the 0–5 V speed control signal.

Hippocampus Represents Both Time and Distance Simultaneously

To determine whether neurons responding significantly to only time were active within the same theta cycles as neurons responding significantly to only distance, the data recorded during treadmill running were segmented into "theta bins" defined relative to the theta phase with the least firing (as in Jezek et al., 2011). The theta signal was calculated by band-pass filtering the local field potential between 6 and 10 Hz using a zero-phase Butterworth filter. The instantaneous phase of the theta signal was then calculated as the angle of the Hilbert transform. All spikes recorded from the session were then binned based on the phase of the theta signal (5° bins) to determine the phase with the lowest mean firing rate. Once the phase with the lowest mean firing rate was determined, the entire time spent on the treadmill was then segmented into 360° "theta bins" to create one bin per theta cycle, with the border between bins defined as the theta phase with the lowest mean firing rate. In a subset of sessions lacking a recording of the local field potential, a count of all recorded "spikes" (including all threshold crossings across all active tetrodes, both sorted and unsorted) was binned into 15.6 ms bins and used as a surrogate for the local field potential. This signal was then band-pass filtered as before to obtain a theta signal. This method was validated and found to be an accurate estimate of the theta signal by comparing the signal derived from all threshold crossings to the actual theta signal in sessions with a local field potential recording.

For each theta bin, a population vector was formed indicating with either 0 or 1 whether each cell fired at least once within that theta bin. The result was an *m* by *n* matrix (*m* theta bins by *n* cells). This matrix was convolved with a square wave three theta bins (1080° or three full

theta cycles) wide (3 by 1) to obtain a new matrix indicating whether each unit fired within a three theta bin wide sliding window of time surrounding each individual theta bin.

For each pair of cells, individual theta bins were selected in which both cells were active within the three theta bin wide sliding window surrounding that theta bin. This filtering was done to limit the analysis to only those units whose firing fields overlapped at some point during treadmill running, and to analyze only those theta bins that occurred while the rat was located within the overlapping firing fields for each cell in the pair. If theta-paced "flickering" is occurring between two different representations (Jezek et al., 2011), the three theta bin wide sliding window should smooth across multiple representations.

Among this subset of theta bins (selected for each pair of cells), the expected probability of both cells firing within the same theta bin (assuming independence) was calculated. The probability of cell i having at least one spike ($p(k_i > 0)$) was calculated by counting the theta bins in which cell i fired and dividing by the number of theta bins. The probability ($p(k_1 > 0) \cdot p(k_2 > 0)$) of both cells firing (assuming independent firing within this subset of theta bins) was then calculated, and compared to the actual fraction of theta bins in which both cells fired ($p(k_1 > 0 \cap k_2 > 0)$). The results are plotted in Figure S6A.

Through the GLM analysis, each neuron was assigned a number ($\Delta \mathrm{D}_{\mathrm{T-D}}$) indicating that neurons preference for time versus distance (See "Generalized Linear Model" in the Experimental Procedures). For each pair of neurons, the difference between these numbers was calculated ($\left|\Delta \mathrm{D}_{\mathrm{T-D},1} - \Delta \mathrm{D}_{\mathrm{T-D},2}\right|$). A small value indicates that both cells had similar preference for either time or distance. A large value indicates that each cell had a different preference for either time or distance. This number is indicated by $\Delta((STD - T) - (STD - D))$

in Figure S6B. The ratio $p(k_1 > 0 \cap k_2 > 0)/p(k_1 > 0) \cdot p(k_2 > 0)$ was then plotted against this value (Figure S6B), and the Pearson's linear correlation coefficient was calculated. Further discussion of this comparison is provided in the figure legend for Figure S6.