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Dedicated Hippocampal Inhibitory Networks for Locomotion and Immobility

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Network activity is strongly tied to animal movement; however, hippocampal circuits selectively engaged during locomotion or immobility remain poorly characterized. Here we examined whether distinct locomotor states are encoded differentially in genetically defined classes of hippocampal interneurons. To characterize the relationship between interneuron activity and movement, we used in vivo, two-photon calcium imaging in CA1 of male and female mice, as animals performed a virtual-reality (VR) track running task. We found that activity in most somatostatin-expressing and parvalbumin-expressing interneurons positively correlated with locomotion. Strikingly, nearly one in five somatostatin or one in seven parvalbumin interneurons were inhibited during locomotion and activated during periods of immobility. Anatomically, the somata of somatostatin immobility-activated neurons were smaller than those of movement-activated neurons. Furthermore, immobility-activated interneurons were distributed across cell layers, with somatostatin-expressing cells predominantly in stratum oriens and parvalbumin-expressing cells mostly in stratum pyramidale. Importantly, each cell’s correlation between activity and movement was stable both over time and across VR environments. Our findings suggest that hippocampal interneuronal microcircuits are preferentially active during either movement or immobility periods. These inhibitory networks may regulate information flow in “labeled lines” within the hippocampus to process information during distinct behavioral states.

Key words: behavior; calcium imaging; circuits; hippocampus; interneurons; virtual reality

Significance Statement

The hippocampus is required for learning and memory. Movement controls network activity in the hippocampus but it’s unclear how hippocampal neurons encode movement state. We investigated neural circuits active during locomotion and immobility and found interneurons were selectively active during movement or stopped periods, but not both. Each cell’s response to locomotion was consistent across time and environments, suggesting there are separate dedicated circuits for processing information during locomotion and immobility. Understanding how the hippocampus switches between different network configurations may lead to therapeutic approaches to hippocampal-dependent dysfunctions, such as Alzheimer’s disease or cognitive decline.

Introduction

Neuronal networks compute information in a state-dependent manner, in which current behavior alters circuit computations. This has been clearly demonstrated in Caenorhabditis elegans and in the stomatogastric ganglion of crabs, where neuronal circuits have dramatically different output patterns, depending on the state of the animal (Bargmann and Marder, 2013). Movement and immobility are two locomotion states that have wide-ranging effects across rodent sensory systems, increasing stimulus gain in the visual cortex (Niell and Stryker, 2010; Polack et al., 2013; Reimer et al., 2014; Vinck et al., 2015), while decreasing sensory responses in the auditory cortex (Schneider et al., 2014; McGinley et al., 2015). The differential effects of movement and immobility extend into the hippocampus, where they are associated with two strikingly distinct forms of network activation (Vanderwolf, 1969). During movement, local field potential (LFP) activity is characterized by θ-frequency oscillations (7–12 Hz), and pyramidal neurons in CA3 and CA1 exhibit place-specific firing (O’Keefe and Dostrovsky, 1971; McNaughton et al., 1983; Wilson and McNaughton, 1993). In contrast, during awake immobility, LFP activity is punctuated by large, irregular activity containing periods of sharp-wave/ripple (SWR) events, during which pyramidal neurons fire in compressed temporal sequences that can replay recently experienced trajectories (Wilson and McNaughton, 1994;
Not only is movement important for controlling hippocampal network state but an internal representation of speed is a critical parameter for path integration and spatial navigation. Indeed, movement speed positively modulates firing in pyramidal neurons and in many classes of interneurons in the hippocampus, as well as in the strongly interconnected medial septum (MS) and entorhinal cortex (McNaughton et al., 1983; Ahmed and Mehta, 2012; Varga et al., 2012; Katona et al., 2014; Fuhrmann et al., 2015; Kroppf et al., 2015; Zheng et al., 2015; Himanen et al., 2016). Neurons engaged by the opposite motor state, immobility, are far less studied. Recently, researchers identified a subset of hippocampal pyramidal neurons that is preferentially active during periods of immobility and encodes the animal’s current location (Kay et al., 2016). However, little is known about interneurons that control activity during these periods of immobility. This is a critical question given the powerful role of inhibition in controlling network state (Ellender et al., 2010) and downstream behaviors. Recent studies have revealed that different subsets of interneurons are required for diverse behaviors, including fear learning (Letzkus et al., 2011; Lovett-Barron et al., 2014), sensorimotor integration (Gentet et al., 2012; Lee et al., 2013), visual system gain (Fu et al., 2014; Pakan et al., 2016), and memory formation and expression (Courtin et al., 2014; Morrison et al., 2016; Stefanelli et al., 2016).

Intriguingly, several investigators have reported putative interneurons activated by immobility (Fox and Ranck, 1975; Buzsáki et al., 1983; Colom and Bland, 1987; Mizumori et al., 1990; Csicsvari et al., 1999). However, the rarity of neurons that show this property (1.6% of all hippocampal neurons by one study; Mizumori et al., 1990), coupled with the limited information about cellular properties and anatomical localization obtained by extracellular recording, has left this population poorly described. To overcome these limitations, we used two-photon calcium imaging in awake, behaving mice. By using cre-driver lines to target our recordings to specific subtypes of interneurons (Taniguchi et al., 2011), we functionally characterized hundreds of interneurons during behavior and collected detailed anatomical information on their locations.

We found that hippocampal neurons specialize in distinct locomotion states. In the majority, activity was positively correlated with locomotion, but a distinct subset was activated specifically by immobility. These functionally defined interneurons were present in both somatostatin-expressing and parvalbumin-expressing inhibitory interneurons and were anatomically distributed across stratum oriens (SO) and stratum pyramidale (SP). Finally, each neuron’s activity correlation to movement was stable both across time and virtual-reality (VR) environments, suggesting that this cellular characteristic represents a static, rather than dynamic, function in the hippocampal network.

Materials and Methods

Animals. All experiments were approved by the Washington University Animal Care and Use Committee. Heterozygotes (+/−) from two cre-driver mice lines on a C57Bl/6f genetic background were used to label parvalbumin-expressing and somatostatin-expressing inhibitory interneurons: SSTtm1.1(cre/Zf)/Zf (SST-cre) and Pvalbhm1(cre/Zf)/HbJ (PV-cre; Jackson Labs). Wild-type (WT) mice (C57Bl/6f) were cre-negative pups from the SST-cre +/− × WT crossing (littermate controls). Both male and female mice were used.

Viral injections and hippocampal window implantation. Mice were injected with adeno-associated virus (AAV) at 2–4 months of age. Mice were anesthetized with 1–3% isoflurane and a 0.5-mm-diameter craniotomy was opened above the left cortex. Injections were made through a micropipette tip pulled to a long, thin taper and sharpened on a Sutter Instruments BV-10 beveler to an input impedance of 2–5 MΩ. Virus was micro-injected and volume (~50 nl) was estimated by visually measuring the movement of the back meniscus of virus in the pipette using an eyepiece reticle. For stereotactic injections, virus was targeted to the CA1 layer of the hippocampus at −1.6 to −1.8 mm lateral from bregma, −1.7 to −2.0 mm caudal from bregma, and −1.3 to 1.35 mm ventral from dura. For cre-dependent GCaMP6f expression, AAV1.Syn.Flex.GCaMP6f.WPRE.SV40 (Penny Vector Core, University of Pennsylvania) at a titer of 1.71 × 1013 genome copies (g.c.) was diluted 1:1–1:4 with PBS and injected in cre-driver mice. For neuronal jRGECO1a expression, AAV1.Syn.NES-jRGECO1a.WPRE.SV40 (Penny Vector Core, University of Pennsylvania) at a titer of 2.95 × 1013 g.c. was diluted 1:1 with PBS and injected into WT mice. In two somatostatin-cre mice, we made bilateral injections (300 nl) of a mixture of AAV1.Syn.Flex.GCaMP6f.WPRE.SV40 at a 1:5 dilution with rAAV8/hSyn-DIO-hm3D (Gq)-mcherry (UNC Vector Core, University of North Carolina; 5.7 × 1013 g.c.) at a 4:5 dilution to coexpress GCaMP6f and Gq-DREADD (designer receptors exclusively activated by designer drugs) in cre-expressing neurons. We did not activate DREADD receptors with the exogenous ligand CNO in any experiments presented here. GCaMP6f signaling in these mice was similar to that of mice expressing GCaMP6f only, so data from both types of animals were combined.

After virus injection, the incision was sealed with Metabond (Parkell) and a custom-cut titanium headplate (eMachineShop). Mice were water-scheduled to bring weight down to −75% of original (0.7–1 ml of water per day). After 1–3 weeks, the headplate was removed, a larger craniotomy (2.8 mm) was made, the cortex overlying the hippocampus was aspirated, and the top layers of the external capsule were removed, leaving the lower layers intact. This aspiration unilaterally removes parts of the visual, somatosensory, and parietal cortices. Previous studies found this surgery did not impair mouse behavior in numerous tasks, including VR task running (Dombeck et al., 2010; Gu et al., 2014). Anecdotally we also did not observe an obvious deficit when comparing the VR task behavior of mice implanted with an imaging cannula to mice that were only headplated.

Kwik-Sil elastomer (World Precision Instruments) was used to bond the imaging cannula [outer diameter, 2.8 mm; inner diameter, 2.36 mm; height, 1.5 mm (Microgroup); 2.5 mm round coverslip (Potomac Photonicss)] to the brain and Metabond darkened with carbon powder (Sigma-Aldrich) to prevent VR light from entering the objective was used to close the incision and attach the headplate. Cannulae were inserted at a slight angle (−7–10° down toward the lateral side) to match the angle of the lateral side of the hippocampus. During imaging, the objective was tilted a similar amount so that the long axis of the objective was perpendicular to the imaging window. Animals recovered for ~2 weeks with continued water-scheduling before beginning experiment. Animals were recorded 1–3 months after virus injection. We observed no signs of toxic overfilling of neurons (filled nuclei and long-time course transients) by calcium sensor over this time frame.

VR task running behavior. The VR system used a curved-screen monitor (Samsung S34E790C) set on lowest brightness and further dimmed using window film (Gila Glare Control, Smoke). The monitor was ~12 inches in front of the mouse and occupied 115° of horizontal (azimuth) space and −15° below to +39° above the horizon of the mouse. The mouse was head-fixed on a spherical treadmill (8-inch-diameter Styrofoam ball) floating on a column of air flowing through a custom designed, 3D-printed treadmill base. Mouse movement was tracked by monitoring ball movement using a G400 mouse (Logitech) configured in LabView (National Instruments) to read forward and yaw (rotation) ball speed. VRMEn (Virtual Reality Matlab Engine; Aronov and Tank, 2014) was used to render a closed-loop visual VR environment based on movement input from the computer mouse. The forward ball movement gain was set so that ~2.8 rotations of the ball (equal to 180 cm of distance traveled) traversed the long axis of the track. Yaw gain was set so ~12 ball rotations equaled a 360° rotation in VR in the track while in the end zones (last 10 cm of the track on either end, i.e., 0–10 and 170–180 cm) gain was set so ~2.5 ball rotations equaled a 360° rotation, which allows mice to more easily turn out of corners at the end of the track. Rewards were
controlled by a transistor–transistor logic (TTL) output from the ETL. The width of the serotonergic primary afferent axon was estimated from high-resolution images of the brainstem. The axons were 5-7 μm in diameter and ran parallel to the midline. The ETL is in the excitation light path before the galvos and does not impinge on light collection. We sequentially imaged multiple planes spanning distances of ≤180 μm in the z-axis. The focal plane was controlled by current output, which in turn was controlled by a microcontroller (Scanbox, NeuroLabware). For imaging, sections were incubated in a solution containing 2% serum, 2% BSA, 0.1% Triton in 0.5M TBS PBS at 4 °C. Sections were washed in 0.1M Tris-buffered saline (TBS) and incubated in 0.5M TBS for 2 h. For labeling, sections were incubated with the primary antibody for 2–3 weeks until they could consistently perform at >25% of baseline activity. Possible ROIs were automatically identified as contiguous regions with SD >20%. To identify pyramidal neurons identified in multiple z planes, pairs of cells with temporal activity correlation >0.7 were flagged and visually inspected. The dimmer ROI was removed when duplicates were identified.

For interneuron imaging, ROIs were selected in a semiautomated process. Possible ROIs were automatically identified as contiguous regions with SD >1.5 and an area >90 μm². ROIs that were unresponsive or had low signal-to-noise ratios were manually eliminated. The parameters for automatic detection were permissive so many puncta were identified that were clearly not cells. This was especially common in the upper imaging planes, where there is fluorescent background near the surface of the hippocampus (Fig. 1B, somatostatin interneurons, plane 1, upper right corner). Puncta that were unresponsive or had low signal-to-noise ratios were dropped from further analysis (65 of 286 for somatostatin interneurons and 109 of 234 for parvalbumin interneurons). In movies with individual planes, ROIs were automatically eliminated if the same ROI was selected multiple times and the ROI was selected on the plane where the cell was most sharply in focus. For interneuron recordings, neuropil contamination was removed by subtracting peri-stimulus fluorescence signal from an area <20 μm from the ROI border, excluding any other ROIs (F_corrected-ROI = F ROI − F_neuropil); Peron et al., 2015).

Ball-movement data, collected at 1 kHz, was binned to match imaging frame rate. Ball speed was calculated from the voltage output of the LabView program that detects ball movement. To generate a conversion factor for voltage output to ball speed, we manually spun the ball at different speeds to traverse the VR track. Since the VR track length was set to be the equivalent of 180 cm of ball distance, we could use travel in VR as a measure of real-world distance. We calculated distance over time as ball speed and generated a lookup table of ball speed versus voltage output. This function was linear for speeds that approximate mouse movement speeds. The same calibration was made for yaw ball speed. Total ball speed was calculated as the magnitude of the Euclidean x and y components of velocity (forward and yaw rotation) as sampled by the computer mouse. Acceleration was calculated as the difference between subsequent values of the calculated ball speed. Activity fields for neurons were calculated as the full width of the field (in centimeters) at half-maximal ΔF/F of peak activity. Spatial information was calculated with 5 cm bins using the following formula: SI = ΣP_i (R_i/R) log R_i/R (Markus et al., 1994) where P_i is the probability of bin occupancy, R_i is the mean ΔF/F in bin i, and R is overall mean ΔF/F. The locomotion modulation index (LMI) was calculated for individual cells as the difference between the mean fluorescence during immobile and moving periods, normalized by the sum of the mean values, LMI = μ_m − μ_i / μ_m + μ_i. Activity (ΔF/F) is displayed versus linear ball speed. Previous reports have shown activity having a linear relationship to log speed (Ahmed and Mehta, 2012; Kemere et al., 2013; Zheng et al., 2015), as well as linear speed (Kropff et al., 2015; Hinman et al., 2016). In general, activity was better fit to linear speed for somatostatin neurons, so we used linear speed throughout (112 of 192 or 59.9% better fit by linear ball speed, as measured by the sum of square residuals to the linear regression after excluding the highest 20% of speed-activity values because of activity saturation). Interestingly, there appear to be cell-type differences in the relationship between activity and speed, since parvalbumin interneurons, on the whole, were better fit to log, ball speed (76 of 125 or 60.8% better fit to log,). For morphometric analysis, ROIs were redrawn by hand over the cell soma, blind to the cell’s activity correlation with speed. Cross-sectional somatic area and eccentricity were calculated.
using the "regionprops" Matlab function. Eccentricity ranges from 0 to 1, with a perfect circle having eccentricity of 0 and more elliptical shapes toward 1.

Experimental design and statistical analysis. For pyramidal neurons, we recorded 479 cells (average ± SD: 119.8 ± 39.1 cells per animal; range: 89–177 cells) from four male mice. We recorded 192 somatostatin-cre-positive cells (average ± SD: 28.4 ± 22.3 per animal; range: 18–73 cells) from five mice (three males, two females). We recorded 125 parvalbumin-cre-positive cells (average ± SD: of 28.0 ± 11.3 per animal; range: 8–34 cells) from five mice (four males, one female).

Cell-to-cell ∆F/F and cell ∆F/F-to-speed correlations were calculated using Pearson’s correlation coefficient and significance was corrected for multiple comparisons using a Bonferroni–Holm correction. LMI significance was calculated by bootstrapping 1 s bins, sampled at ±2 s intervals (Pakan et al., 2016). Significance of differences was calculated using Wilcoxon rank-sum tests for unpaired data and Wilcoxon signed-rank tests for paired data. Multiple comparisons were Bonferroni corrected as indicated. Statistical analyses were performed in Matlab (RKID:SCR_001622).

Phase angle of cross-correlation between ∆F/F and ball speed was calculated using the Hilbert transform. The Hartigan dip test is commonly used to test for bimodality but has little power when the two populations have uneven sizes, as is the case here. To test for a diametrically bimodal population (phase angle difference of 180° between populations), we doubled the phase angle of each cell and used the Rayleigh test for uniformity on the resulting distribution (Zar, 2007). For example, if there are two population peaks at phase angles of 90° and 270°, doubling the phase angles results in one peak at 180° (90° × 2 = 180°; 270° × 2 = 540° = 180°). If the phase-doubled distribution is unimodal (by Rayleigh test), the initial distribution is significantly bimodal. The Rayleigh test was implemented from CircStat for Matlab (Berens, 2009).

There was strong correspondence between cells classified by correlation of activity to speed and phase angle. For somatostatin interneurons, 127 of 128 cells (99.2%) of positively correlated cells were classified in the positive phase while 31 of 32 (96.9%) negatively correlated cells were classified in the negative phase. There was similar correspondence for parvalbumin cells. We classified 48 of 51 cells (94.1%) of positively correlated cells in the positive phase and 10 of 11 (90.9%) negatively correlated cells in the negative phase. Throughout this paper, we use activity-to-speed correlation to characterize cells since only a subset of cells are classified by phase angle (experiments with >10 start–stop transitions: 166 of 192 somatostatin neurons and 64 of 125 parvalbumin neurons). Kernel density estimate was calculated by convolving each data point with a kernel function and summing the resulting curves to yield a probability density function. We used a Gaussian kernel with an SD (bandwidth) of 11.6°, which was the estimated optimal bandwidth for this dataset, as calculated by the Matlab function ksdensity. Stability of correlations over days was assessed via Pearson correlation coefficients by measuring the extent to which cell correlation with speed in one recording session correlated with subsequent sessions. All data are presented as ±SEM unless otherwise noted.
Results
Characterization of neuronal calcium activity in VR track running
To investigate in vivo neural network dynamics, we used two-photon imaging of neuronal calcium activity during a spatial navigation task in VR (Dombeck et al., 2010). To monitor a larger fraction of the network, as well as record from cells along the deep to superficial axis of the hippocampus, the microscope was fitted with an ETL to rapidly modulate the divergence of laser light through the objective, allowing fast z-axis focal jumps (Grewe et al., 2011). This enabled us to capture sequential imaging frames moving from dorsal to ventral at a total range of ≈180 μm (Fig. 1A). In most experiments, we imaged four separate dorsal-ventral planes at a rate of 4 Hz in each plane. Before behavioral training, mice were injected with AAV to express a genetically encoded calcium sensor targeted unilaterally to the CA1 region of the dorsal hippocampus. AAV1-Syn-jRGECO (Dana et al., 2016) was injected in WT mice to label all neurons. A mixture of AAV1-CaMKII-cre and AAV1-Syn-Flex-GCaMP6f (Chen et al., 2013) was injected in WT mice to label pyramidal neurons. AAV1-Syn-Flex-GCaMP6f was injected into somatostatin-cre+/− transgenic mice (Taniguchi et al., 2011) to label somatostatin-expressing interneurons (Fig. 1B). An imaging cannula was implanted to allow imaging into the hippocampus (Fig. 1A). Head-fixed mice ran on a floating spherical treadmill (Styrofoam ball) to control movement through the VR visual environment displayed on a curved-screen monitor in front of the animal (Fig. 1C). Ball movement was tracked with an optical computer mouse and fed into the VR engine to update the visual scene.

To compare the properties of neuronal activity in our VR setup to previous real-world and VR experiments, we used calcium transients as a proxy for spiking and examined spatial coding in pyramidal and somatostatin interneurons. We measured calcium activity from pyramidal neurons in CaMKII-cre/Syn-CMAP6f-injected (N = 2 mice, n = 281 cells) and Syn-

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**Figure 2.** Spatial activity of neurons and remapping in VR. A, Overview of VR track. Mice run to one end of the VR track and then to the opposite end to receive water rewards. Mice are restricted to the central corridor surrounded by local wall cues and distal landmarks. Arrows indicate up-direction and down-direction runs. Right, Monitor views in up and down directions.

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**B.** Data collected during VR behavior. Mouse position in VR track and timing of water rewards (transients). Ball speed during VR task. ∆F/Φ of jRGECO1a-labeled pyramidal neuron, ∆F/Φ of simultaneously recorded jRGECO1a-labeled interneuron. C, Binned distribution of ball speed for five mice during VR behavior. D, Top, Left, Heatmap of pyramidal neuron mean ∆F/Φ versus track position, specifically for up-direction runs. Each column represents the activity of an individual neuron. To generate the heatmap, ∆F/Φ in each spatial bin was summed and divided by occupancy time. Cell order is sorted by track position of peak ∆F/Φ. Reward zones at the ends of the track have been excluded. Top, Right, From a separate experiment, heatmap of somatostatin interneuron mean ∆F/Φ versus track position. Middle, Left, Heatmap of pyramidal neuron activity in down-direction runs, but with cell order set by up-direction runs. Note that spatial pattern of activity is lost, indicating direction-sensitive spatial activity of pyramidal neurons in this VR task. Middle, Right, Heatmap of somatostatin interneuron activity in down-direction runs, but with cell order set by up-direction runs. Bottom, Left, Pyramidal neuron heatmap in down-direction runs and sorted by position of peak ∆F/Φ. Bottom, Right, Somatostatin interneuron heatmap in down-direction runs and sorted by position of peak ∆F/Φ. E, Left, Distribution of spatial information from 479 pyramidal neurons in four mice. Right, Distribution of spatial information from 192 somatostatin interneurons from five mice. F, Timeline of remapping experiment. Animals spend 7 min in World 1, which is then instantaneously replaced with World 2, where they run for an additional 14 min. Data shown is after 4–5 d training in the remapping paradigm so animals are familiar with World 2. G, Top, Left, World 1 heatmap of pyramidal neuron mean ∆F/Φ versus track position, in up-direction runs. Top, Middle, World 2 heatmap for up-direction runs with same cell order as World 1. Note that spatial activity of neurons in World 1 is not maintained in World 2, indicating remapping. Top, Right, World 2 heatmap with cells sorted by World 2 spatial activity. Bottom, Left, World 1 heatmap of pyramidal neuron mean ∆F/Φ versus track position in down-direction runs. Bottom, Middle, World 2 heatmap in down-direction runs with same cell order as World 1. Bottom, Right, World 2 heatmap with cells sorted by World 2 spatial activity.
jRGECO-injected (N = 2, n = 198 cells) mice. To identify putative pyramidal neurons from pan-neuronal jRGECO1a labeling, we eliminated putative interneurons based on anatomy and calcium dynamics (see Materials and Methods). Movies were corrected for brain motion and fluorescence time-series for individual cells were extracted from ROIs selected using an automated cell-segmentation algorithm (Mukamel et al., 2009). Significant calcium transients in pyramidal neurons were detected with an algorithm designed to give <5% false-negative error rates (Dombек et al., 2009) and used for subsequent analysis. We did not extract significant calcium transients from interneuron activity traces. In contrast to hippocampal pyramidal neurons, which have a very low rate of spontaneous firing, most interneurons fire spontaneously and this basal activity is modulated up and down during behavior (Ranck, 1973; Ego-Stengel and Wilson, 2007; Royer et al., 2012; Varga et al., 2012; Katona et al., 2014). Thus, the continuous $\Delta F/F$ trace of calcium activity is more representative of interneuron activity than a thresholded version. Interneuron ROIs were identified with a semiautomated procedure and visually inspected for fluctuations in $\Delta F/F$.

Mice were water-scheduled and trained to run back and forth between ends of a 180 cm virtual track (Fig. 2A) for water rewards, while we recorded calcium activity from neurons (Fig. 2B). Mice ran in a short burst to traverse the track, stopped to drink the water reward, turned the ball to turn around in VR, and ran to the other end for another water reward. The distribution of ball speed shows a minimum at 15 cm/s, which we used as a threshold for defining stopped and moving periods. Average ball speed was $28.2 \pm 2.4$ cm/s overall, $35.3 \pm 2.3$ cm/s during movement periods, and $7.5 \pm 1.1$ cm/s during stopped periods (N = 5 mice).

For each neuron, we generated a spatial activity map by binning the track, summing the neuron’s $\Delta F/F$ in each bin, and normalizing by occupancy. Cell order for display was sorted by binning periods, and 7.5 cm/s up-direction runs, is shown for down-direction runs (Ranck, 1973; Ego-Stengel and Wilson, 2007). For each experiment, we generated a matrix of pairwise neuron–neuron activity correlation values and then plotted the distribution of all correlation $r$ values (Fig. 3C,D). After pooling the correlation $r$ values from all pairs of somatostatin interneurons into a single distribution, 1810 pairs (72.8%) were positively correlated, 329 pairs (13.3%) were negatively correlated, and 341 pairs (13.8%) had no significant correlation (Fig. 3E; N = 5 mice, n = 192 cells; $p < 0.05$, Pearson correlation coefficient with Bonferroni–Holm correction for multiple comparisons), indicating that a majority of somatostatin interneurons have correlated activity during VR track running, with a small percentage of anticorrelated neurons.

When we examined the correlation structure of pyramidal neuron population activity during VR track running, there was much lower activity correlation across cells, as can be seen in raw $\Delta F/F$ traces (Fig. 3F,G), in the correlation matrix of neuron–neuron activity correlation values, and in the distribution of correlation $r$ values (Fig. 3H,I). In the pooled distribution of correlation $r$ values for all pyramidal neurons, 3716 (21.0%) pairs were positively correlated, 5648 (14.6%) pairs were negatively correlated, and 24,888 (71.0%) pairs were not significantly correlated (Fig. 3J; N = 4 mice, n = 479 cells; $p < 0.05$, Pearson correlation coefficient with Bonferroni–Holm correction for multiple comparisons). Thus in strong contrast to the pyramidal neuron population, which shows relatively little correlated population activity, most somatostatin neurons have similar temporal patterns of activity, with a small percentage of cells whose activity is anticorrelated to the rest of the population.
Locomotion-activated and immobility-activated somatostatin interneurons

To investigate the locomotor correlates of neuronal activity, we aligned $\Delta F/F$ traces with the movement activity of the mouse, as measured by ball speed (Fig. 4A, B). Many cells were more active during movement, although some showed the opposite pattern, becoming more active at low speeds. To parametrically describe the relationship between activity and movement, we measured the correlation between $\Delta F/F$ and ball speed for each cell (Fig. 4A, B, sample cell correlograms from two mice; Fig. 4C, r values of correlation for all cells from two mice). The distribution of $r$ values from all mice (Fig. 4D; $N = 5$ mice) show consistency across animals, with activity in most cells correlated with speed, while a small percentage was uncorrelated. To quantify, we pooled the $r$ values from all somatostatin interneurons into a single distribution and found activity in 74.5% of neurons was correlated with speed, activity in 18.5% of neurons was anticorrelated with speed, and 6.8% of neurons had no correlation (Fig. 4E; $N = 5$ mice, $n = 192$ cells; $p < 0.05$, Pearson correlation coefficient with Bonferroni–Holm correction for multiple comparisons).

We further quantified the influence of locomotion on cellular activity by calculating an LMI for individual cells. In general, LMI measures were similar to the activity-to-speed correlation. Directly comparing the significance of speed/fluorescence correlation to LMI measures for all cells, we found 84.7% (150 of 177) of cells were significant in both measures and 15.3% (25 of 177) of cells were significant in LMI only. Together with Figure 1, these data show that many somatostatin interneurons have correlated activity occurring during locomotion. A smaller fraction is active during immobility and this activity is anticorrelated to activity in most of the population.

To further characterize the organization of activity by movement, we identified transitions between immobility and movement (based on the 15 cm/s speed threshold calculated from the distribution of animal movement in Fig. 2C) to align start-triggered and stop-triggered rasters of neuronal activity. A representative experiment from a behavioral session with 41 immobilized animals is shown in Figure 5A–C. Speed on every trial is shown in Figure 5A, left), whereas neuronal activity of a negatively correlated cell shows a sharp drop (Fig. 5B, left). Conversely in stop-triggered events, these data show that many somatostatin interneurons have correlated activity occurring during locomotion. A smaller fraction is active during immobility and this activity is anticorrelated to activity in most of the population.

The average fluorescence time series across all trials of the two example cells, plotted along with the average speed, shows that changes in speed are closely matched by changes in fluorescence in the positively correlated cell, while activity in the negatively correlated cell decreases with speed (Fig. 5D). Next, we looked at start-triggered and stop-triggered activity in the somatostatin population. We only included experiments where the animal had >10 start/stop episodes that crossed our speed threshold of 15 cm/s with stop duration of >1 s (166 of 192 somatostatin neurons). The average activity of these somatostatin interneurons at locomotion transitions shows that activity in most cells increases during movement while activity in a small percentage of cells is inhibited (Fig. 5E).
we analyzed the temporal relationship between activity and speed negatively correlated cells would again become active).

Figure 4. Locomotion-activated and immobility-activated somatostatin interneurons. A, ΔF/F of five somatostatin interneurons from a single imaging plane, plotted with ball speed (top), VR position, and rewards (bottom). Right, Correlation plot of ΔF/F versus ball speed (binned to match the frame rate of imaging) for cells shown in A for entire imaging session. Line is linear regression. B, Five somatostatin interneurons from a single imaging plane from another mouse. C, ΔF/F-to-speed correlations for all cells from two mice shown in A and B. Colored bars match individual cells shown in A and B. Solid bar (either dark gray or colored) is a significant relationship, either correlation or anticorrelation. D, Distribution of activity-to-speed correlation r values for neurons in individual somatostatin-cre mice. E, Pooled distribution of activity-to-speed correlation r values for all somatostatin interneurons.

Figure 5. Locomotion-activated and immobility-activated somatostatin interneurons can be identified. A–D, left; A–D, right; B, Five somatostatin interneurons from a single imaging plane, plotted with ball speed (top), VR position, and rewards, leaving open the possibility that activity is governed by a small subset; however, immobile periods mostly occur after a small subset; however, immobile periods mostly occur after

Next, we examined the temporal relationship between movement and activity by generating a cross-correlation of speed and activity signals (cross-correlation for sample cell used in Fig. 5A–D shown in Fig. 6A). We plotted the cross-correlation function as a heat map for all somatostatin cells and found that in positively correlated cells, activity followed speed with a short delay (Fig. 6B; 0.57 ± 0.08 s, 133 neurons). Activity in negatively correlated cells was much more delayed relative to speed (Fig. 6B; 4.1 ± 0.16 s, 33 neurons) and instead the nadir of activity slightly preceded speed (~0.32 ± 0.2 s). The ~4 s delay between speed and ΔF/F in negatively correlated cells reflects the average latency between movement and a subsequent immobile period (when negatively correlated cells would again become active).

These data demonstrate that the activity of individual somatostatin interneurons can exhibit a positive or negative correlation with movement. To test whether the population is bimodal, we analyzed the temporal relationship between activity and speed in start-triggered trials (Fig. 5E, left) using circular statistics by extracting the phase angle of the cross-correlation at zero time lag and plotting the distribution of cells (Fig. 6C). The resulting distribution shows a statistically significant diametrically bimodal population; one population displays activity that peaks with locomotor speed, whereas the second population is inhibited with speed (phase angle difference of 180° as tested with Rayleigh z test between the original distribution and the distribution with all angles doubled, p = 6.6 × 10⁻²⁰). To further visualize bimodality, we made a linear distribution of phase angle by “folding over” the rose plot along the line 0–180° (phase angle doubles back from 180 to 360°; Fig. 6D). The resulting histogram was smoothed by a kernel density estimate to visualize the underlying distribution by minimizing the effect of histogram bin sizes (Fig. 6D, right axis). These data show that somatostatin interneurons can be split into two populations, one whose activity peaks close to the peak of speed, and another whose activity is shifted by 180° so that the nadir of activity occurs during peak speed.

We also investigated the possibility that interneuronal calcium activity was more strongly tied to other movement-related variables than total ball speed. For each cell, we calculated goodness of fit using residual sum of squares to the linear regression of fluorescence to total ball speed, ball forward speed, ball yaw speed (total ball speed is the combination of forward and yaw, or rotational, speed), VR speed (in the long axis of the track), ball acceleration, and VR acceleration. All speed variables exhibited a better fit of linear regression to activity than acceleration variables (N = 5 mice, n = 192 cells; mean r²: total ball speed, 0.103 ± 0.008; forward ball speed, 0.092 ± 0.007; yaw ball speed, 0.091 ± 0.009; VR speed, 0.098 ± 0.007; ball acceleration, 0.007 ± 0.002; VR acceleration, 0.006 ± 0.001). Thus, total ball speed showed the best correlation with activity in somatostatin interneurons.

Locomotion is correlated to activity in most cells in the somatostatin interneuron population and anticorrelated to activity in a small subset; however, immobile periods mostly occur after rewards, leaving open the possibility that activity is governed by reward rather than movement. To distinguish between the influence of rewards and locomotion, we examined immobile periods that occurred >4 s after reward delivery, as well as immobile periods that occurred in the track rather than in the reward zones (Fig. 7A). During nonreward periods, there was still a significant increase in activity with locomotion in positively correlated cells (Fig. 7B; 82 neurons, −0.05 ΔF/F ± 0.01 ± 0.05 ± 0.01, p = 1.2 × 10⁻²º) and a decrease in negatively correlated cells (16 neurons,
0.11 ± 0.02 to 0.03 ± 0.01, $p = 6.7 \times 10^{-5}$), indicating that activity is tied to locomotion rather than reward.

**Positively and negatively correlated parvalbumin interneurons**

These data demonstrate that 18.5% of somatostatin interneurons have activity that increases during immobility. Is this functionally identified subset exclusive to somatostatin interneurons, or do other genetically specified interneuron populations also exhibit this activity? To investigate this question, we recorded from another population of interneurons, the soma-targeting parvalbumin-expressing interneurons. Similar to activity in the somatostatin interneurons, activity in most parvalbumin interneurons was positively correlated with movement, although there was a small
subset of anticorrelated cells (Fig. 8A–D). We pooled the r values from all parvalbumin interneurons into a single distribution and found that activity in 74.4% of neurons was correlated with speed, activity in 14.4% of neurons was anticorrelated with speed, and 11.2% of neurons had no correlation (Fig. 8D; N = 5 mice, n = 125 cells; p < 0.05, Pearson correlation coefficient with Bonferroni–Holm correction for multiple comparisons).

As in the somatostatin population, analysis of start-triggered and stop-triggered activity in the parvalbumin neurons revealed that some neurons increase activity during movement, while activity in a small percentage of cells is inhibited by movement (Fig. 8E). We quantified this relationship by comparing the fluorescence between immobile and moving periods. Positively correlated cells decreased activity at the transition from moving to immobile (Fig. 8F; 0.06 ∆F/F ± 0.01 to −0.08 ± 0.01, p = 7.2 × 10⁻⁹, Wilcoxon signed rank) and increased activity at the transition from immobile to moving (−0.03 ∆F/F ± 0.03 to 0.25 ± 0.05, n = 51 cells, p = 9.7 × 10⁻⁴). Conversely, negatively correlated cells increased activity when going from moving to immobile (0.25 ± 0.04 to 0.6 ± 0.03, p = 9.8 × 10⁻⁴).

Next we examined the temporal relationship between movement and activity by generating a cross-correlation of the speed and activity signals from Figure 8E. In positively correlated parvalbumin cells, activity led speed with a short delay (Fig. 8G; 0.30 ± 0.18 s), in contrast to somatostatin cells where activity slightly lagged speed (Fig. 6B, C). Activity in negatively correlated parvalbumin cells was much more delayed relative to speed.
and the separately collected z series of the imaging area. Cells in contact with SP, were scored as SP. For immunofluorescence scoring, we imaged coronal sections and omitted labeled cells in the stratum radiatum (SR) to maintain consistency with GCaMP-labeled distributions, where we did not image into SR. The distribution of GCaMP-labeled neurons and immunofluorescence labeling was similar (Fig. 9A–C; somatostatin: SO, 84.6 ± 5.5% GCaMP vs 85.0 ± 1.6% immunofluorescence, p = 1; SP, 15.4 ± 5.5% GCaMP vs 15.0 ± 1.6% immunofluorescence, p = 1; parvalbumin: SO, 10.1 ± 3.3% GCaMP vs 17.5 ± 2.7% immunofluorescence, p = 0.14, SP, 89.9 ± 3.3% GCaMP vs 82.5 ± 2.7% immunofluorescence, p = 0.08, with Bonferroni correction; total somatostatin cells: 174 immunolabeled from four mice, 192 GCaMP-labeled from five mice; total parvalbumin cells: 159 immunolabeled from three mice, 125 GCaMP-labeled from five mice).

We then compared the anatomical distribution of positively and negatively correlated cells. There was, within either the parvalbumin and somatostatin populations, no significant difference in the laminar distribution of positively and negatively correlated cells (Fig. 9D, F; somatostatin: SO, 89.5 ± 4.0% positive vs 68.5 ± 1.4% negative, p = 0.28; SP, 10.5 ± 4.0% positive vs 31.5 ± 1.4% negative, p = 0.28; parvalbumin: SO, 9.1 ± 4.5% positive vs 21.3 ± 1.1% negative, p = 1; SP, 91.0 ± 4.5% positive vs 78.8 ± 1.1% negative, p = 0.06, with Bonferroni correction). These data demonstrate that positively and negatively correlated cells exhibit approximately the same layer distribution, with somatostatin neurons predominantly in SO and parvalbumin neurons mostly in SP.

Next, we examined the spatial scale of activity correlation in interneuron populations by calculating the temporal activity correlation and spatial distance between pairs of neurons, and then plotting activity correlation as a function of distance. We did this for all somatostatin neurons, then split out positively correlated somatostatin neurons and made pairwise comparisons restricted within this class (Fig. 9G). We were unable to do the same with negatively correlated cells due to the very small number of pairs within each experiment. There was no significant relationship between activity correlation and distance [all somatostatin: Spearman’s rank correlation coefficient (\(\rho\)) = 0.02, \(p = 0.3, n = 2025\) pairs, \(N = 5\) mice; somatostatin positively correlated, \(\rho = 0.03, p = 0.3, n = 1110\) pairs]. In contrast, parvalbumin neurons (Fig. 9H) showed an inverse correlation between activity correlation and distance, when looking at all neurons, as well as within the positively correlated class (all parvalbumin, \(\rho = -0.13, p = 0.0002, n = 996\) pairs, \(N = 5\) mice; parvalbumin positively correlated, \(\rho = -0.14, p = 0.0002, n = 803\) pairs, with Bonferroni correction). These data show that the closer two parvalbumin neurons are, the more likely they are to have similar activity patterns.

Finally, we examined whether the cell morphology of locomotion-activated neurons was different from that of immobility-activated neurons. We measured the cross-sectional area and eccentricity of somata from each cell, based on the ROIs used to measure calcium activity. Soma area in negatively correlated somatostatin neurons was significantly smaller than in positively correlated neurons (Fig. 9I; 474 ± 14 \(\mu m^2\) positive vs 404 ± 21 \(\mu m^2\) negative, \(p = 0.025, n = 192\) cells, \(N = 5\) mice). There was no significant difference in eccentricity in somatostatin neurons (0.77 ± 0.03 positive vs 0.75 ± 0.01 negative, \(p = 0.44\)). We also found no significant differences in area and eccentricity between positively and negatively correlated neurons in the parvalbumin populations (area: 453 ± 11 \(\mu m^2\) positive vs 439 ± 31 \(\mu m^2\) negative, \(p = 0.30, n = 2025\) pairs, \(N = 5\) mice).
negative, \(p = 0.71\), eccentricity: 0.75 ± 0.04 positive vs 0.84 ± 0.01 negative, \(p = 0.06, n = 125\) cells, \(N = 5\) mice). These results show that there is no layer preference for positively and negatively correlated neurons (although somatostatin neurons were predominantly in SO and parvalbumin mostly in SP). Furthermore, parvalbumin interneurons show a distance-dependent scaling of activity correlation. Finally, negatively correlated somatostatin interneurons displayed smaller somata than positively correlated interneurons.

**Cellular activity-to-speed correlation is stable**

Do neuronal populations that are active or suppressed during locomotion in one environment exhibit stable identities, or are they dynamically reassigned? We examined the stability of these networks in two ways. First, each cell’s activity correlation to movement was tracked over time by recording from the same neurons as the animal performed the VR task over 5 d. Indeed, each cell’s activity correlation with locomotion was highly stable over this time period (Fig. 10A). To quantify this stability, we plotted each cell’s correlation value on different days as a scatter plot and calculated the Pearson correlation value between each day. We found a high degree of correlation of motion response between days (Fig. 10B; day 1 vs day 2, \(r = 0.83, p = 6.5 \times 10^{-65}\); day 1 vs day 3, \(r = 0.75, p = 5.4 \times 10^{-47}\); day 1 vs day 4, \(r = 0.71, p = 6.3 \times 10^{-40}\); day 1 vs day 5, \(r = 0.77, p = 5.1 \times 10^{-32}\), \(n = 170\) cells, \(N = 5\) mice), indicating that the specification of each cell to a locomotion or immobility activated network is stable over time.

Second, we examined the stability of these functional interneuron networks by transporting animals into a second VR world. In both real and virtual environments, hippocampal place fields remap in different environments (Fig. 2D,E). Is an interneuron’s activity correlation to speed similarly environment-dependent or does it remain stable across different environments? After animals were proficient in the behavioral task in one visual environment, they were remapped into a different environment (Fig. 10C). After 5 d
of training in the remapping paradigm to allow the animals to become familiar with World 2, we tested network stability by plotting each cell’s correlation between activity and locomotion between worlds as a scatter plot. Networks displayed high stability in different environments (Fig. 10C,D; \( r = 0.86, p = 2.9 \times 10^{-23}, n = 75 \) cells, \( N = 3 \) mice). These data suggest that a neuron’s relationship between activity and movement is a consistent identity. Thus, the hippocampus may contain distinct embedded inhibitory microcircuits encoding locomotor activity versus quiescence.

Discussion
In this work we applied new experimental approaches to a long-standing question in neuroscience: how is state-dependent activity organized within neural networks? In particular, are hippocampal networks generally responsive during both locomotion and immobility or are there specialized microcircuits in different behavioral states? By imaging hippocampal interneurons in awake, behaving mice, we found two functionally distinct subsets of interneurons with opposite encoding of locomotor state. Specifically, one functional subpopulation is maximally active during locomotion, whereas activity in the second population is anticorrelated with locomotion. Importantly, neither genetic identification nor spatial organization was predictive of a neuron’s functional encoding. The stability of these networks over many days and across environments indicates that they likely reflect dedicated circuits that specialize in behavior-dependent processing of hippocampal information.

The existence of two functionally identified classes of interneurons has significant implications for information processing in the hippocampus. In particular, it suggests the possibility that there are “labeled lines” for processing information when an animal is immobile or moving. A network of immobility-activated pyramidal neurons centered in CA2 and extending into CA1 and CA3 has been identified (Kay et al., 2016). One possibility is that these pyramidal neurons preferentially project to immobility-activated CA1 interneurons and drive their activity. Kay et al. identified fast-spiking interneurons whose firing was associated with immobility-activated place cells. However, the proportion of these interneurons that showed a negative correlation between activity and movement was low, suggesting no particular enhanced connectivity between immobility-activated place cells and immobility-activated interneurons.

Figure 9. Anatomical distribution and somatic morphology of positively and negatively correlated neurons. A, Left, Anti-somatostatin immunofluorescence in coronal section from WT mouse. Right, Anti-parvalbumin immunofluorescence in coronal section from WT mouse. B, Left, GCaMP-labeled neurons from somatostatin-cre mouse (coronal section in fixed tissue). Note that SP is dark, indicating the lack of GCaMP-labeled axons targeting cell somata. Right, GCaMP-labeled neurons from parvalbumin-cre mouse. Scale bar, 40 \( \mu \)m. C, Distribution of cell bodies across cell layers comparing anti-somatostatin immunofluorescence (black) to GCaMP-labeled cells (green). For comparisons in C–F, distributions of GCaMP-labeled cells were taken directly from imaging datasets, not post hoc fixed tissue. Cell location was scored by examining imaging planes to see whether cells were in contact with SP, along with detailed z series of imaged cells taken in vivo (2 steps of 3 \( \mu \)m through the imaged areas). Differences in C–F were tested by Wilcoxon rank sum, with Bonferroni correction. D, Distribution of cell bodies across cell layers comparing anti-parvalbumin immunofluorescence (black) to GCaMP-labeled cells (green). E, Distribution of cell bodies across cell layers comparing anti-parvalbumin immunofluorescence (black) to GCaMP-labeled cells (green). F, Distribution of cell bodies across cell layers from GCaMP-labeled cells in somatostatin-cre animals comparing positively correlated cells (red) to negatively correlated cells (blue). G, Plot of mean activity correlation versus distance for pairs of somatostatin neurons, either all possible pairs (All, black) or pairs restricted to the positively correlated population (Positive, red). There was a significant relationship between activity correlation and distance, tested with Spearman’s rank correlation, for either all pairs of somatostatin neurons, or just the positively correlated population. H, Plot of mean activity correlation versus distance for pairs of parvalbumin neurons, either all possible pairs (All, black) or pairs restricted to the positively correlated population (Positive, red). There was a significant relationship between activity correlation and distance, with closer cell pairs having more similar activity than distant pairs, for all pairs of parvalbumin neurons and for just the positively correlated population. *\( p = 0.0002 \). I, Left, Imaging plane of somatostatin interneurons. Right, Somatic ROIs color-coded by activity correlation to speed, with positive correlations in red and negative correlations in blue. ROIs shown here are used to calculate morphology comparisons in I,J. J, Comparison of cross-sectional area of somatostatin somata between positively correlated and negatively correlated neurons. Areas of somata of negatively correlated somatostatin interneurons were significantly smaller. *\( p = 0.025 \). All error bars are ± SEM.
terneurons. Thus, it is possible that other sources may drive the immobility-activated interneurons identified here. Finally, caution should be used when comparing results across experimental paradigms, from tetrode recording in freely moving rats to calcium imaging in head-fixed mice. In particular, the lack of vestibular input (Aronov and Tank, 2014) and nonlinear integration of calcium signals by GCaMP (Chen et al., 2013) could potentially affect the observed relationship between activity and movement.

Another possible local circuit mechanism for generating anti-correlated activity is reciprocal inhibition between the two populations of interneurons. There are inhibitory connections across classes in the hippocampus, both in the direction of parvalbumin interneuron to somatostatin interneuron (Losonczy et al., 2010; Lovett-Barron et al., 2012) and in the direction of somatostatin interneuron to parvalbumin interneuron (Leão et al., 2012; Fuhrmann et al., 2013). Another disinhibition-based local circuit mechanism for generating an immobility-activated population of interneurons is the action of a third class of interneurons, those that express VIP. This class specializes in targeting other interneuron types (Acsády et al., 1996; Tyan et al., 2014; Karnani et al., 2016). This population, if driven by running signals, could inhibit targeted populations during locomotion, and promote activity during immobile periods through disinhibition. There is significant evidence from sensory systems that VIP-expressing interneurons control other interneuron types, and downstream pyramidal neuron activity, in a task-specific manner (Lee et al., 2013; Pi et al., 2013; Fu et al., 2014; Kuchibhotla et al., 2017).

The source of movement and immobility information can also originate from outside the hippocampus. One critical source

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**Figure 10.** Cellular correlation between activity and locomotion is stable. A, Top, Imaging planes of same somatostatin cells imaged over 5 consecutive days. Scale bar, 20 μm. Middle, Somata of cells above, color-coded by activity correlation to ball speed over 5 d. Red is positive correlation; blue is negative. Bottom, ΔF/ΔF correlation to speed for all cells in sample experiment, over 5 d. Cell order is the same across all days. B, Scatter plot of ΔF/ΔF correlation to speed for all cells comparing day 1 to all other days. Inset, Histogram shows distribution of differences between each cell’s correlation value between days. Bin size, 0.05. C, Left, Schematics of World 1 and World 2. Middle, Somata of cells color-coded by activity correlation to ball speed in World 1 and World 2. Red is positive correlation, blue is negative. Scale bar, 20 μm. Right, ΔF/ΔF correlation to speed for all cells in sample experiment, in World 1 and World 2. Cell order is the same across worlds. D, Scatter plot of ΔF/ΔF correlation to speed for all cells comparing World 1 to World 2. Inset, Histogram shows distribution of differences between each cell’s correlation value between worlds. Bin size, 0.05.
for speed information to the hippocampus is the MS, which is required for running-related θ oscillations in the hippocampus (Winson, 1978). The MS sends three types of afferent to the hippocampus: glutamatergic, GABAergic, and cholinergic (Frotscher and Léranth, 1985; Freund and Antal, 1988). Glutamatergic input from the MS targets somatostatin-expressing oriens lacunosum-moleculare interneurons and drives speed-correlated firing of those interneurons (Fuhrmann et al., 2015). GABAergic input from the MS exclusively targets hippocampal interneurons (Freund and Antal, 1988; Unal et al., 2015) and this connection, which is at least partly active during locomotion (Kaufosh et al., 2013), could serve to inhibit immobility-active neurons during movement. Indeed, this scenario with opposite regulation of activity in two populations of interneuron activity driven by opposing signals from the MS has been hypothesized (Colom and Bland, 1987; Mizumori et al., 1990). Finally, acetylcholine release from MS afferents increases with locomotion and has divergent effects on interneurons, exciting some and inhibiting others (McQuiston and Madison, 1999a,b; Lawrence et al., 2006; Leão et al., 2012; Chittajallu et al., 2013). None of the mechanisms discussed are mutually exclusive; in fact, it is likely that multiple-circuit and neuromodulatory systems are engaged to robustly enforce state-dependent network changes on multiple timescales. We note that whatever mechanisms generate these two classes of interneuron require considerable synaptic or cellular specificity. It remains to be seen whether the downstream targets of these interneurons are targeted so precisely.

Our finding of immobility-activated interneurons confirms and expands on earlier work looking for behavioral correlates of hippocampal units using in vivo electrode recordings in freely moving rats. In these studies, occasional “anti-θ” or θ-off units were identified that increased firing when the animals stopped moving. Since such units were infrequently seen, little was known about them other than they were putatively identified as interneurons (Fox and Ranck, 1975; Buzsáki et al., 1983; Colom and Bland, 1987; Mizumori et al., 1990; Csicsvari et al., 1999). Although we cannot directly confirm that the interneurons we have identified are θ-off units, they share the same functional properties. Through genetic identification, we confirm that these neurons are interneurons and, furthermore, immobility-activated neurons exist in multiple genetically defined interneuronal classes. The fact that this network draws from multiple classes of interneurons with distinct postsynaptic targets suggests a comprehensive circuit for regulating activity during immobile periods. The morphological distinction of smaller somata in immobility-activated somatostatin neurons hints that these functionally defined cell types may correspond to cell classifications based on anatomy or gene expression, although further studies are required to strongly link function to anatomy.

What could be the functional role of these immobility-activated interneurons? One possibility is that they have a causative role in switching the network from θ oscillations during movement to large irregular activity during stops, as suggested by studies in urethane-anesthetized rats showing that θ-off units (putative immobility-activated cells) were the first type of unit recruited during LFP transitions (Bland et al., 1999). In addition to possible roles in switching network state, these neurons likely contribute to regulating pyramidal networks. During immobility, there are two well-characterized network activities. First are the aforementioned hippocampal pyramidal neurons that code for place during stops. Second are SWRs where firing sequences of pyramidal neurons are reactivated. It is unclear whether these immobility-activated interneurons regulate SWRs since it is unknown whether, or when, SWRs occur during our VR task (although SWRs are associated with consummatory behaviors, such as licking). However, in freely moving animals, SWRs are fast events (100–200 ms) and occupy <10% of time during immobile periods (Kay et al., 2016), whereas the activity of immobility-activated interneurons appears tonic (we note that the temporal resolution of calcium imaging makes it difficult to distinguish tonic activity from periodic phasic activation). Simultaneous imaging and LFP recording may clarify the relationship between immobility-activated interneurons and SWRs. Finally, it is also possible that these interneurons regulate other unknown network activities during immobility.

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