Table S1, related to Figure 4. Passive and active electrophysiological parameters for model cells.

Measured properties	dPC	sPC	PVBC
Model R_{input} (MΩ)	126.5	124.9	39.3
Experimental R_{input} (MΩ)	124	117	--
Model Resting Mem. Pot. (mV)†	-79.4	-80.7	-60.1
Experimental Resting Mem. Pot. (mV)†	-86.6	-80.9	--
Model Rheobase (pA)[#]	+200	+200	+400
Experimental Rheobase (pA)[#]	+150	+150	--
Max. conductance of channels at soma (S/cm^2)			
$K_{Ca,S}$	0.07	0.07	0.000002
$K_{v,Ca,B}$	0.03	0.01	0.0002
$Ca_{v,L}$	0.01125	0.0075	0.005
$Ca_{v,N}$	0	0	0.0008
$Ca_{v,T}$	0.05	0.05	0
HCN	0.000038	0.00005	0
$K_{v,A}$	0.0108	0.006	0.00015
$K_{dr,slow}$	0.05	0.001	0
K_{dr}	0.09	0.03	0.013
Na_v	0.3375	0.264	0.12
Other properties			
Specific membrane capacitance ($\mu F/cm^2$)	1	1	1.4
Specific membrane resistance ohm*cm^2	24,150	32,900	3,333
Specific axial resistance ohm*cm	295	295	100

† These membrane potentials are corrected for junction potential. Model cells require no junction potential correction; experimental conditions produced a junction potential of +14.6 mV (calculated using pClamp's Junction Potential Calculator). As indicated in the AxoClamp instruction manual for whole cell patch configuration, the junction potential was subtracted from the measured potential to determine the actual membrane potential (Axon Manual).

The rheobase does not include a tonic current injection applied to the cells throughout the characterization. In the case of the model cells, +18.5 pA was applied to the dPC and -61 pA was applied to the sPC so that they both rested at -84.6 mV, and no current was applied to the PV. In the biological PCs, the level of tonic current was not recorded (the amount given was that needed to achieve a resting membrane potential of -84.6 mV, junction potential corrected). Note that the current sweeps were performed at steps of 50 pA, limiting the resolution of the rheobase.

Supplementary Experimental Procedures

1. Mice

To target PV expressing interneurons for patch-clamp recordings, we crossed a commercially available PV-Cre line [B6;129P2-Pvalbtm1(cre)Arbr/J; The Jackson Laboratory stock #008069] (Hippenmeyer et al., 2005) with a commercially available red reporter line [B6;129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J; The Jackson Laboratory stock #007905] (Madisen et al., 2010), to produce mice expressing the red fluorescent protein tdTomato in PV expressing cells. These offspring are referred to as "PV-TOM" in this manuscript. Selective expression of excitatory channelrhodopsin (ChR2) in PV^+ interneurons was achieved by crossing PV-Cre line with floxed-STOP ChR line (Ai32; Rosa-CAG-LSLChR2H134R-EYFP-deltaNeo generated by Hongkui Zeng, obtained from the Allen Institute; Ai32D is now available from The Jackson Laboratory, stock #012569) (Madisen et al., 2012). These offspring are referred to as "PV-ChR2" in this manuscript. Ospin-negative littermates were used as controls. In a subset of experiments, C57BL/6J mice were used (these included: paired recordings; Figure 5; mapping of retrogradely labeled PCs, Figures S5A to S5C, 6A to 6C; triple immunocytochemical experiments using antibodies against PV and ankyrinG and VGAT, Figure S2; immunocytochemical analysis of the PV^+ or $CB1R^+$ boutons on sPCs and dPCs, Figures 1L, S1K, S4; and *in vivo* filling of PVBCs with biocytin, Figures 2G to 2I; when applicable, since the results were not different between the two groups, data were combined from the C57BL/6J and PV-TOM mice). For the *in vivo* imaging, PV-TOM mice were used.

2. *In vitro* electrophysiology

Coronal hippocampal slices (300μm) were prepared from 2-3 month-old PV-TOM mice, PV-ChR2 mice, and C57BL/6J mice of either sex. The septal and temporal hippocampal slices were from the following coordinates: septal slices, anterior-posterior: -1.9 to 2.5 mm from Bregma; temporal slices, anterior-posterior: -3.1 to -3.7 mm from Bregma. Slices were incubated in sucrose-containing artificial CSF (ACSF) for an hour (85 mM NaCl, 75 mM sucrose, 2.5 mM KCl, 25 mM glucose, 1.25 mM NaH_2PO_4, 4 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 24 mM $NaHCO_3$). After the initial incubation period, slices were transferred in the same ACSF solution used for recordings (126 mM NaCl, 2.5 mM KCl, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.25 mM

NaH_2PO_4, and 10 mM glucose). Slices were visualized with an upright microscope (Olympus; BX61WI or Nikon; Eclipse FN-1) with infrared–differential interference contrast (IR-DIC) optics. These microscopes were additionally equipped with a mercury or xenon lamp light source for epifluorescence. All electrophysiological recordings were made at 33°C. MultiClamp700B amplifiers (Molecular Devices) was used for *in vitro* recordings. Signals were filtered at 3 kHz using a Bessel filter and digitized at 10 kHz with a Digidata 1440A analog–digital interface (Molecular Devices). Series resistances were carefully monitored, and recordings were discarded if the series resistance changed >20% or reached 20 MΩ. The recorded traces were analyzed using Clampfit 10.2 (Molecular Devices).

A. *Paired recordings*: All interneurons were identified post-hoc as PVBCs or CCKBCs (for detailed information, see section 3. Cell type identification). For paired recordings of interneuron-to-PC connections, whole-cell recordings in current-clamp configuration were obtained from the interneurons (PVBCs and CCKBCs; holding potential was -60 mV) with patch pipettes (3–5 MΩ) filled with internal solution containing 126 mM K-gluconate, 4 mM KCl, 10 mM HEPES, 4 mM ATP-Mg, 0.3 mM GTP-Na, 10 mM phosphocreatine, and 0.2% biocytin, pH 7.2, 285-290 mOsm. The postsynaptic pyramidal cells were recorded with one of two internal solutions: 1) 40 mM CsCl, 90 mM K-gluconate, 1.8 mM NaCl, 1.7 mM $MgCl_2$, 3.5 mM KCl, 0.05 mM EGTA, 10 mM HEPES, 2 mM MgATP, 0.4 mM Na_2GTP, and 10 mM phosphocreatine, 0.2% biocytin, pH 7.2, 285-290 mOsm (voltage clamp configuration, the holding potential was -70mV); 2) 126 mM K-gluconate, 4 mM KCl, 10 mM HEPES, 4 mM ATP-Mg, 0.3 mM GTP-Na, 10 mM phosphocreatine, and 0.2% biocytin, pH 7.2, 285-290 mOsm (current clamp configuration for Figures 4A and 4B). The paired recording event amplitudes indicated as effective unitary IPSCs (euIPSCs) or effective unitary EPSCs (euEPSCs) include both successful events and failures (Neu et al., 2007). For more detailed analysis of presynaptic properties, coefficient of variation (CV) of unitary IPSCs in PCs evoked by PVBCs was measured from 50 events per pair including failures. To measure short-term depression (paired pulse ratio) of GABA or glutamate release from PVBCs or CA1 PCs, we evoked PVBC-derived IPSCs or PC-derived EPSCs by suprathreshold stimulation of PVBCs or CA1 PCs at 10Hz and calculated the ratio of the 2nd response to the 1st response in each pair.

B. *Single cell recordings*: We recorded CA1 PCs with 126 mM K-gluconate, 4 mM KCl, 10 mM HEPES, 4 mM ATP-Mg, 0.3 mM GTP-Na, 10 mM phosphocreatine, and 0.2% biocytin, pH 7.2,

285-290 mOsm (current clamp configuration for Figures S1E to S1H, S3B to S3D). For I-F curves of CA1 PCs, the PCs were held at -70mV and action potential discharges were evoked by current injections (1s-long step currents, from 0 to +550pA with 50pA increments, Figure S1E). To determine input resistance of CA1 PCs, the PCs were held at -70mV and voltage responses to small current pulses (1s-long current steps from -100pA to +100pA with 50pA increments) were measured at steady state (0.8s -1.0s from the start of 1s-long current steps). All voltage measurements remain uncorrected for liquid junction potential error in the experimental part of the study. Calculated junction potentials using Axoclamp's Junction potential calculator were +14.6mV and +10.2mV for 4mM chloride and 48.7mM chloride pipette solution, respectively.

C. *Optogenetic experiments*: The general procedures used in the optogenetic experiments were similar to our previous study (Krook-Magnuson et al., 2013). Briefly, CA1 PCs were recorded from slices from PV-ChR2 or opsin-negative control mice (intracellular solution: 40 mM CsCl, 90 mM K-gluconate, 1.8 mM NaCl, 1.7 mM $MgCl_2$, 3.5 mM KCl, 0.05 mM EGTA, 10 mM HEPES, 2 mM MgATP, 0.4 mM Na_2GTP, and 10 mM phosphocreatine, 0.2% biocytin, pH 7.2, 285-290 mOsm; voltage clamp configuration, holding potential: -70mV). Blue light was delivered through the epifluorscence port of a Nikon Eclipse FN-1, using a Lambda DG-4 with smart shutter and LAMDA SC controller (Shutter Instruments), and TTL input from a Digidata 1322A (Axon Instruments).

3. Cell type identification

After *in vitro* recording with pipettes containing biocytin, slices were transferred into a fixative solution containing 4% paraformaldehyde and 0.2% picric acid in 0.1 mM phosphate buffer. Slices were resectioned into 70 μm thin sections and tested for CB1R immunoreactivity (Frontiers Science Co, CB1-GP-Af530-1; 1:5000, guinea pig) (Figure 5A). For calbindin immunoreactivity, mouse brains after fixation were sectioned into 70 μm thin coronal sections and tested for calbindin (Swant, anti-Calbindin D-28k; 1:500, rabbit) (Figure 1A). Secondary antibodies conjugated to Alexa 488/649, raised in donkey against rabbit/guinea pig (Invitrogen) were used to detect the location of the primary antibodies, and streptavidin conjugated to Alexa 350 (Invitrogen; 1:500) or DyLight 594 (Jackson Immunoresearch; 1:500) were used to detect biocytin. All primary and secondary antibodies were diluted in Tris-buffered saline containing 2% normal goat serum. The sections were then mounted in Vectashield (Vector Laboratories)

and analyzed with a fluorescent microscope. To reveal the axonal and dendritic arbors, the biocytin-filled cells were subsequently visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB, 0.015%) using a standard ABC kit (Vector).

For a subset of experiments (Figures 2G, 2H, 2I), PVBCs were filled with Neurobiotin (1.5-2.0%) using juxtacellular recordings *in vivo* in the septal (dorsal) hippocampus as described in Varga et al. (2012). Two to four hours after the recorded cells were labeled, mice were deeply anesthetized with Nembutal and transcardially perfused with saline followed by a fixative solution (4% paraformaldehide, 0.01% glutare-aldehide and 15% saturated picric acid in 0.1M phosphate buffer). The brains were cut into 60 μm thin sections with a vibratome (Leica) and the cells were visualized with Alexa488 conjugated streptavidin (Invitrogen) and selected sections containing axonal/dendritic arbors were tested for PV immunoreactivity (Swant, PV25; 1:5000, rabbit). The primary antibody was visualized by DyLight 649-conjugated affinity-purified secondary antibodies raised in donkey against rabbit (1:1,000) (Jackson ImmunoResearch Laboratories). The sections were then mounted in Vectashield (Vector Laboratories) and analyzed with a fluorescent microscope. To reveal the axonal and dendritic arbors, the Neurobiotin-labeled cells were visualized with DAB (0.015%) using a standard ABC kit (Vector).

All the interneurons were identified post-hoc as PVBCs or CCKBCs depending on their distinct axonal morphology (axons ramifying predominantly in the pyramidale layers), CB1R immunopositivity for CCKBCs (note that CB1R is an excellent marker for CCKBCs) and PVTOM (for *in vitro* filling with biocytin) or PV (for *in vivo* filling with Neurobiotin) positivity to PVBCs (Katona et al., 1999; Lee et al., 2010; Krook-Magnuson et al., 2011; Varga et al., 2012). All CA1 PCs were also identified post-hoc and divided into sPCs and dPCs (for definition of CA1 PC sublayers, see section 4 below) or classified based on their long-distance targets (for detailed information, see section 6. Retrograde tracer injections). Recovered neurons were randomly chosen and 3-dimensionally reconstructed using the Neurolucida system (Micro Bright Field Bioscience) on Zeiss Axioplan 2 at 63× magnification for detailed morphological analysis (Figures 2, S1, S3, and S5). In some cases, recovered neurons were morphologically reconstructed with a drawing tube at 60× magnification mounted on a Zeiss microscope Axioskop 2 plus (Figures 1B, 1J, 1K, 5A, and 5G).

4. Definition of CA1 PC sublayers

The definitions of the superficial and deep sublayers used in this paper were as follows: in the septal (dorsal) CA1: superficial sublayer: 0-20μm; Deep sublayer: 20-40μm; in the temporal (ventral) CA1: superficial sublayer: 0-50μm; Deep sublayer: 50-200μm). The rationale for the latter subdivisions is as follows. Somatic locations of CA1 PCs have been shown to divide CA1 PCs into morphologically, physiologically, and functionally distinct superficial PCs [closer to the stratum pyramidale/ stratum radiatum (Pyr/Rad) border] and deep PCs (closer to stratum oriens) (Bannister and Larkman, 1995; Jarsky et al., 2008; Mizuseki et al., 2011; Slomianka et al., 2011). In the *septal* (dorsal) CA1, calbindin is expressed in many of the PCs within the first one to two cell rows adjacent to the Pyr/Rad border, but typically not in the deeper cells (Baimbridge and Miller, 1982; Figure 1A, top, right); these calbindin expressing PCs comprise about half (20μm) of the total width of PC layer (~40μm) in the septal (dorsal) hippocampus. Therefore, we defined the superficial sublayer as being within 20μm from the Pyr/Rad border; the deep sublayer is thus defined as being 20μm to 40μm from the Pyr/Rad border. For the *temporal* (ventral) hippocampus, a different definition needs to employed, because the width of the CA1 PC layer increases from the septal (width: 40μm) to the temporal CA1 (200μm) (see Figure 1A). In addition, in the temporal part, calbindin cannot be used for demarcating the division of the superficial/deep sublayers, since calbindin is not exclusively expressed in the PCs closest to Pyr/Rad border in the temporal CA1. However, in the temporal (ventral) CA1, the definition of the superficial and deep sublayers can be based on the degree of compactness of the cell bodies within the PC layer (e.g., see Figures 1, 2, 6 in Slomianka et al, 2011). Specifically, the superficial sublayer consists of a 50μm-wide layer containing densely packed PC somata situated closer to the Pyr/Rad border, while the deep sublayer is comprised of losely packed cell bodies located 50μm to 200μm from the Pyr/Rad border (Figure 1A). It is important to note here that the meaningful nature of these superficial-deep sublayer definitions was also indicated by the fact that, in agreement with previous morphological and physiological studies (Bannister and Larkman, 1995; Jarsky et al., 2008), we found significant morphological (i.e., soma size and dendritic length) and physiological differences (i.e., sag amplitude) between PCs in the superficial and deep sublayers (Figures S1A to S1H).

5. Morphological analysis

A. The contribution of axo-axonic (chandelier) cells to *in vivo* calcium imaging: Mice (n=3) were deeply anesthetized with Nembutal and transcardially perfused with saline followed by a fixative (4% paraformaldehide, and 15% saturated picric acid in 0.1M phosphate buffer). The brains were sectioned into 60 μm thin sections and incubated in primary antibody mixtures: rabbit anti-parvalbumin (1:5,000) (PV25; Swant), mouse anti-ankyrinG (1:5,000) (AnkG; Santa Cruz Biotechnology), and guinea pig anti-VGAT (1:5,000) (VGAT; Synaptic Systems). Secondary antibodies conjugated to DayLight 488/594/649, raised in donkey against rabbit/mouse/guinea pig (Jackson ImmunoResearch Laboratories) were used to detect the location of the primary antibodies. All antibody cocktails contained 0.3% Triton-X100 and 5% normal horse serum dissolved in 0.1M PB. The sections containing the dorsal hippocampus (where *in vivo* calcium imaging was performed) were then mounted in Vectashield (Vector Laboratories) and analyzed from merged projection image of 5 Z-planes (0.25μm Z step; Zeiss LSM700 microscope; objective: Plan Apochromat 63×/1.40). $PV^+/VGAT^+$ boutons within 0.5μm from $ankyrinG^+$ profiles were considered initial segment-targeting boutons; all other $PV^+/VGAT^+$ boutons within the stratum pyramidale were classified as somatic/proximal dendritic boutons.

B. Counting PV^+ or $CB1R^+$ boutons around the somata of individual sPCs and dPCs: After filling of sPCs or dPCs in slices with biocytin, the slices were transferred into a fixative solution (4% paraformaldehyde, 0.2% picric acid in 0.1 mM phosphate buffer). Slices were cut into 70 μm thin sections and incubated in rabbit anti-parvalbumin (1:5000) (PV25; Swant) or guinea pig anti-CB1R (1:5000) (CB1-GP-Af530-1; Frontiers Science Co). Secondary antibody conjugated to Alexa 488 raised in donkey against guinea pig (Invitrogen) or Alexa 488 raised in goat against rabbit (Invitrogen) was used to detect the location of the primary antibodies, and streptavidin conjugated to Alexa 350 (Invitrogen; 1:500) or DyLight 594 (Jackson Immunoresearch; 1:500) were used to detect biocytin. All primary and secondary antibodies were diluted in Tris-buffered saline containing 2% normal goat serum. The sections were then mounted in Vectashield (Vector Laboratories). PV^+ or $CB1R^+$ bouton-like profiles in close apposition with the somata of individual biocytin-filled sPCs and dPCs were counted from the images along the entire height of PCs (0.75μm Z step; Zeiss LSM700 microscope; objective: Plan Apochromat 63×/1.40). In separate experiments, we examined whether PV^+ or $CB1R^+$ bouton-like profiles show immunopositivity for VGAT. Slices were incubated in rabbit anti-parvalbumin (1:5000) (PV25;

Swant) and guinea pig anti-VGAT (1:5000) (VGAT; Synaptic Systems) or guinea pig anti-CB1R (1:5000) (CB1-GP-Af530-1; Frontiers Science Co) and mouse-anti-VGAT (1:1000) (VGAT; Synaptic Systems). Secondary antibodies similar to those described above were used to detect the location of the primary antibodies. The sections were then mounted in Vectashield (Vector Laboratories) and analyzed from merged projection image of 5 Z-planes (0.25μm Z step; Zeiss LSM700 microscope; objective: Plan Apochromat 63×/1.40). We found that 295 of 300 PV^+ bouton-like profiles show immunopositivity for VGAT and 292 of 300 $CB1R^+$ bouton-like profiles show immunopositivity for VGAT, indicating that most counted PV^+ or $CB1R^+$ bouton-like profiles belong to presynaptic terminals, not postsynaptic compartments.

C. Quantification of 3D-reconstructed PVBCs (to obtain information on axonal length, bouton numbers, and inter-bouton distance): PVBCs were filled with biocytin or Neurobiotin in either *in vitro* (using whole cell recordings, 7 PVBCs) or *in vivo* (using juxtacellular recordings, 4 PVBCs) and were visualized with DAB (0.015%) using a standard ABC kit (Vector). We reconstructed randomly chosen sections of PVBCs (two 60 μm thin sections/cell for *in vivo* filled PVBCs; one 70 μm thin section/cell for *in vitro* filled PVBCs) using the Neurolucida system (Micro Bright Field Bioscience) on Zeiss Axioplan 2 at 63× magnification.

D. Other analyses: For counting of putative axon terminals for the experiments in Figures 1J and 5G and in long-distance target dependent PCs, the potential contact sites between the paired recorded and filled pre- (PVBCs or CB1RBCs) and postsynaptic cells (sPCs or dPCs or $_{mPFC}$PCs or $_{AMG}$PCs) were closely examined under a 100× oil immersion objective, and the number of closely juxtaposed putative presynaptic terminals was counted on the soma or proximal dendrites. For assessment of recovery rate after recording, we examined the soma, basal dendrites, and apical dendrites of the biocytin-filled PCs (Figures S1J and S5K). PC dendrites (for dendritic length) were analyzed using the Neurolucida explorer (Micro Bright Field Bioscience). For soma surface area of PCs, after visualization of biocytin filled CA1 PCs by streptavidin conjugated to DyLight 594 (Jackson Immunoresearch; 1:500), the PCs were analyzed from Z-stacked images (Zeiss LSM700 microscope; objective: Plan Apochromat 20×/0.8). The soma surface areas were measured using Volocity (PerkinElmer).

6. Retrograde tracer injections and mapping of retrogradely labeled PCs

Mice were deeply anesthetized with isoflurane during the whole procedure and maintained on a heating pad. To retrogradely label CA1 PCs, we performed a small craniotomy and then made a stereotaxic injection of 100 nl of red or green fluorescent latex microspheres (Lumafluor) using a syringe (Hamilton Company) into the AMG (lateral: -2.5mm, anterior-posterior: -1.9mm, ventral: -5.3mm from Bregma), mPFC (lateral: -0.5mm, anterior-poster: -1.8mm, ventral: -2.5mm from Bregma), and MEC (lateral: -3.5mm, anterior-poster: -4.9mm, ventral:-4.0mm from Bregma) in PV-TOM mice or C57BL/6J mice (2-3 month-old). For the AMG injection, a lateral oblique approach at an angle of 24° to the vertical was used to avoid involvement of the fornix system. Only those retrogradely labeled cells where tracer injections were subsequently determined as correctly placed in the intended target areas were included in the current study. For mapping of retrogradely labeled PCs (Figures 6A and 6B), C57BL/6J mouse brains were transferred into a fixative solution containing 4% paraformaldehyde and 0.2% picric acid in 0.1 mM phosphate buffer. Brains were sectioned into 80 μm thin coronal sections and the sections were stained with blue Nissl (NeuroTrace 435/455, Molecular Probes). The sections were then mounted in Vectashield (Vector Laboratories) and retrogradely labeled CA1 PCs were examined and mapped from Z-stacked images (Zeiss LSM700 microscope; objective: Plan Apochromat 40×/1.3). Retrogradely labeled CA1 PCs were mapped using Volocity (PerkinElmer).

The PCs that project to AMG or mPFC or MEC were found in the temporal part of the hippocampus. Almost no mPFC-projecting PC or AMG-projecting PCs were found in the septal part, while MEC-projecting PCs were found throughout the septotemporal axis. Thus, we focused our studies related to Figures 6 and S5 on the temporal (ventral) CA1. All data in the paper originating from the temporal hippocampus were from a restricted area centered at the level of the rhinal fissure (anterior-posterior: -3.1 to -3.7mm from Bregma; the sampled PCs were located within 480 μm in the dorso-ventral direction from the level of the rhinal fissure).

7. *In vivo* calcium imaging

A. Imaging: PV-TOM mice (2-3 month-old, n=5) were injected with rAAV1/2(*Synapsin-GCaMP5*)^{cre} into the left dorsal CA1 (lateral: -1.5mm, anterior-posterior: -1.5mm, ventral: -1.0mm from Bregma; 0.1mm steps, 50nL each step). Mice two weeks post-injection were implanted with a chronic hippocampal imaging window above the left dorsal CA1 by aspirating

off cortical tissue but leaving the most ventral layer of the external capsule intact to prevent direct contact with hippocampal tissue. We cemented the upper lip of this window to the skull along with a stainless-steel head-post for head-fixation on a miniaturized treadmill (Kaifosh et al., 2013). After implantation, mice recovered for at least a week before habituation to the experimenter's hand, the treadmill, and eventually head fixation on the circular treadmill. After habituation mice underwent 2-3 days of awake imaging experiments (n=5) and one day of anatomical imaging under ketamine (80mg per kg)/xylazine (10mg per kg) anesthesia (n=3 of 5), spread out over at least a week. In order to track running activity during image acquisition using this arrangement, we equipped the treadmill wheel with spokes that pass between an infrared LED and photo-transistor, the voltage across which is recorded on an Arduino micro-controller (Arduino). By aligning the times of infrared beam-breaks with imaging frames, it is possible to determine whether or not running activity was occurring during frame acquisition. For the *in vivo* calcium imaging, the fluorescence measurements were from two PC layers located at 0-10μm and 20-30μm from the radiatum border (see section 4. definition of CA1 PC sublayers).

B. Analysis: *In vivo* GCaMP Ca^{2+} imaging data were motion corrected off-line both across frames and across lines within frames (Kaifosh et al., 2013). This was achieved using in-house software written in MATLAB (Mathworks). Briefly, frames are first coarsely aligned based on a spatial cross-correlation analysis. The resulting displacements are used as parameters for a Hidden Markov Model that estimates line-by-line displacements with a frame in an approach similar to that presented by Dombeck et al. (2007). Regions of interest (ROIs) were drawn manually in ImageJ (NIH). Full-field ROIs were drawn to include boutons and axon segments alone; no PV^{+} somata were included in these ROIs. For each pair of superficial-deep fields of view (FOV), the same full-field ROIs were applied, controlling for area and relative position of the ROI within the FOV. Bouton ROIs were drawn based upon anatomical identification of axonal varicosities adjacent to an unlabeled somatic profile from the stationary red channel (tdTomato fluorescence). For full-field ROI analysis, each fluorescence time series was computed as the unweighted mean across all within-frame pixels in the ROI at each frame. For the bouton ROI analysis, fluorescence time-series extraction required consideration of the fact that these small regions could move out-of-frame during running episodes. Consequently, we implemented a Kalman-filter model to estimate the signal intensity. Each individual pixel fluorescence time-series was regressed against the raw ROI fluorescence signal (the mean

intensity across in-frame pixels over time) to provide an expectation value for each pixel. For both the full-field and single-bouton ROIs, fractional change in fluorescence was calculated using the method similar to that described by Jia et al. (2011). The fluorescence time series was first smoothed using an 8-frame centered sliding window. A time-varying baseline was then calculated as the 5th percentile of the preceding 400 imaging frames, and fractional change in fluorescence at each time point was calculated relative to this baseline. An exponentially weighted moving average was then applied for noise-filtering using a time-constant of 2 frames. For full-field ROI analysis, the mean fractional change in fluorescence was calculated for each ROI during each running-interval. Running intervals are defined as a collection of imaging frames in which no longer than 2 seconds passes without running activity. We averaged across running intervals to obtain a mean running-related fractional change in fluorescence for each ROI and then averaged across ROIs in the field of view to obtain a mean value for the FOV. Two superficial-deep FOV pairs were obtained from each of five mice, yielding 10 pairs of superficial-deep FOVs. For the bouton ROI analysis, the mean fractional change in fluorescence was calculated for bouton during each running interval. Averaging across running intervals yielded the mean running-related change in fluorescence for bouton, which we then pooled within FOVs to allow comparison between superficial-deep FOV pairs. In addition, the calcium signal sometimes appeared to precede the onset of the running (e.g., in Figure 2B) associated with the fact that running episodes on the treadmill were in some case preceded by initial preparatory periods with elevated PV+ bouton GCaMP signal. The precise detection of these preparatory periods would have been difficult in our experimental system, as these initial movements were not necessarily associated with continuous locomotion of the animal on the treadmill. Therefore, we excluded these initial periods from the analysis of the running-related GCaMP calcium signal, and marked and analyzed only part of continuous bouts of locomotion with longer than 2s of duration with at least one beam break between successive imaging frames as 'running' episodes.

8. Computational modeling

A. *Single cell templates*: We matched the dendritic morphology, including length and total surface area, as well as the total somatic surface area for the PC models (Figure S3A). We also matched the key electrophysiological features of the biological cells (Table S1). We tuned the

cell parameters and maximum ion channel conductances (Table S1) to achieve realistic electrophysiological properties of our cells. Detailed ion channel characterizations will be made available online with the model code (see the following section I, Model accessibility). We matched the experimental current sweep protocol by applying a tonic current to cause each cell to rest at -84.6 mV, in line with the experimental preparation (junction potential corrected), then applying all current steps from -400 pA to +550 pA in steps of +50 pA (in addition to that baseline current level) while measuring the membrane potential at the soma. We closely matched the steady state potential of the cell when subthreshold currents were applied (Figure S3B), the sag amplitude in hyperpolarized current injections (Figure S3C), the rheobase (current injection threshold required to induce firing, Figure S3D) and the curve of the firing frequency versus current injection (Figure S3D). Voltage traces of sPC model (top) and dPC model (bottom) in response to current steps are shown in Figure S3E. To further match experimental conditions, we used the value of E_K=-104 mV as calculated for the experimental solutions. We also used E_{HCN}=-30 mV, E_{Na}= +55 mV, E_{Ca}= +130 mV, $[Ca^{2+}]_i$ of 50e-6 mM for PCs, 5e-6 mM for PVBCs, and $[Ca^{2+}]_o$ of 2 mM.

B. *Connection strengths and kinetics*: For both inhibitory and excitatory synapses, we employed double exponential synapses that allowed specification of a rise time constant, a decay time constant, a reversal potential, and a maximum conductance. We replicated the experimental conditions under which the paired recordings were performed in order to match our model synapses to the experimental data. For IPSC measurements of PVBC synapses onto PCs, the postsynaptic PC was held at -80.2 mV (junction potential corrected; holding potential of -70 mV, calculated junction potential of +10.2 mV) and the reversal potential of the synapse E_{GABA} was calculated as -26 mV. Under these conditions, we matched the experimentally determined data with an amplitude of -65 pA for IPSCs onto dPCs and -21 pA for IPSCs onto sPCs (Figure S3G). We adjusted IPSC kinetics so that both cells had similar IPSC kinetics at the PVBC to PC synapses, with a 10%-90% rise time of 0.63 ms and a decay tau of about 5.45 ms (Földy et al, 2007). To replicate the *in vivo* conditions during network simulations, all parameters of the synapse remained the same except that the reversal potential $E_{GABA(A)}$ was set to -75 mV. For EPSC measurements of PC inputs onto PVBC, the postsynaptic PVBC was held at -84.6 mV (junction potential corrected; holding potential of -70 mV, calculated junction potential of +14.6 mV) and the reversal potential of the synapse E_{AMPA} was set to 0 mV. Under these conditions, we

match the experimental data with an amplitude of -34 pA for EPSCs from dPCs onto PVBCs and -45 pA for EPSCs from sPCs onto PVBCs (Figure S3H). EPSC kinetics were set to be similar between the two connections, with 10-90% rise times of 1.01 (dPC) and 1.03 (sPC) ms and decay time constants of 4.12 ms for both cell type inputs. All synapse parameters including E_{AMPA} remained the same for the network simulations. For EPSCs from afferents, single PC synapses were fit to the amplitudes reported in Magee and Cook (2000). While PCs were held at -70 mV, EPSCs of amplitude -5.38 pA (dPC) and -5.37 pA (sPC) were obtained with a reversal potential of 0 mV (Figure S3I). We made the rise time and decay tau of the afferent synapses similar, at 1.33 ms (dPC) and 1.27 ms (sPC) 10-90% rise time and 4.15 ms (dPC) and 4.47 ms (sPC) decay time constants. Afferent synapses onto PVBCs (held at -60 mV) were set to an amplitude of 5.39 pA, with a 10-90% rise time of 0.80 ms and decay time constant of 3.60 ms (Figure S3I).

C. *Network Numbers*: We used the ratio of PCs to PVBCs that was calculated in our quantitative assessment of the CA1 (Bezaire and Soltesz, 2013). This ratio is approximately 2000:36, so that we included in our network 1000 dPCs, 1000 sPCs, and 36 PVBCs. We also included a large number of afferent spike train generators (20,000) to ensure each cell in our model received afferent excitation from a unique combination of afferents.

D. Network Connectivity: We used connectivity data specified by the experimental observations made in this paper as well as those published in Bezaire and Soltesz (2013). Excitatory synapses made from dPCs or sPCs onto PVBCs consisted of 3 synapses per each single connection (and the synapse tuning for this connection type described above was based on connections comprising 3 synapses). The ratio of synapses from deep:superficial PCs onto each PVBC was 1:3. The total number of synapses from all PCs onto each PVBC was 2,460. While this number may be lower than the total number of PC synapses expected on a single PVBC (Bezaire and Soltesz, 2013), we believe the total number of excitatory events experienced by the PVBC from PCs is similar to what would be experienced *in vivo*, as only a fraction of the PCs would be active at any time *in vivo*, compared to 100% of the PCs in our network under the control condition. For the excitatory afferents onto PVBCs, 1 synapse was present per connection from each independent spike train, for a total of 750 synapses (750 connections) from excitatory afferents. Inhibitory synapses made from PVBCs onto PCs comprised 9 synapses for connections to dPCs and 4 synapses for connections onto sPCs. The synaptic parameter tuning

described above was based on paired connections with these numbers of synapses. Each PC is expected to receive inputs from approximately 17 distinct PVBCs (Bezaire and Soltesz, 2013); therefore the PCs in this model each received 17 connections from PVBCs (for a total of 153 synapses onto the perisomatic region of dPCs and 68 synapses onto the perisomatic region of sPCs). The excitatory afferent connections contained 1 synapse each. In the control condition, each pyramidal cell received 5,000 connections from afferent spike train sources, for a total of 5,000 excitatory afferent synapses.

E. Statistically independent trials: Three distinct networks were run, to simulate data "recorded" from three separate mice. Therefore, the networks differed in the specific cells that were connected to each other, as well as the locations of the synapses (though on average all synapse numbers and distribution of synapses across locations is the same across the networks). The networks also received unique spike train inputs, though all the statistics and properties associated with the spike trains remained the same across networks. For each network, the connections and the specific spike times and patterns of the afferent spike trains remained the same across each excitation condition. Data from all three trials were included in the statistical analysis, with the means and standard error of each condition reported in the results.

F. *Stimulation*: In the control conditions for the networks, each PC received excitation from 5,000 afferents, while each PVBC received input from 750 afferents. The specific afferent fibers that connected to each cell were randomly chosen from a pool of 20,000 afferents. Each afferent was an independent spike train whose spikes were generated at random times according to a Poisson distribution with a mean firing frequency of 1.81 Hz. This level of excitation was chosen to achieve similar firing rates of the sPCs and dPCs, with physiological firing rates for all three cell types, in the control condition (Figure S3J). The various excitation conditions were achieved by connecting an addition number of afferents to the cell type of interest. For example, in the "+5% to sPC" condition, sPCs received a total of 5,250 afferent connections while dPCs still only received the control level of 5,000 afferents connections.

G. *Simulation parameters and hardware*: The temperature of the simulations was 34º Celsius. Each simulation was run for 5,000 ms with a fixed time step of 0.05 ms. The network simulations were run on three supercomputers and the component characterizations (cells and synapses) were run on a personal computer with the Windows 8.1 operating system. The supercomputers used include the University of California at Irvine's High Performance

Computer (with over 50 nodes containing 64 cores each), the San Diego Supercomputing Center's Trestles computer (both via an XSEDE allocation funded by NSF and via the Neuroscience Gateway also funded by the NSF), and the University of Texas' Stampede computer (via an XSEDE allocation funded by the NSF). Simulations were executed in parallel on 64 – 256 processors at a time.

H. *Model Analysis*: The model simulations were organized and the results analyzed using the SimTracker, our custom MATLAB tool (The Mathworks).

I. *Model Accessibility*: We have uploaded the NEURON model code used to generate these simulations to ModelDB (#153280), available at: http://senselab.med.yale.edu/ModelDB/ShowModel.asp?model=153280. We have also uploaded the latest development version of the code that is compatible with NeuroConstruct, along with NeuroML definitions for use in implementing the code in an alternate simulator such as GENESIS, to the Open Source Brain website: http://www.opensourcebrain.org/projects/nc_superdeep. If the reader wishes to re-analyze the code results or reproduce the figures, or to run the simulations with different parameters, we recommend using our SimTracker tool, available at: http://senselab.med.yale.edu/SimToolDB/ShowTool.asp?Tool=153281. In addition, the reader can download all the raw results data from our simulations and also examine the model components via an interactive graphic on our lab website at: http://www.ivansolteszlab.org/models/superdeep.html.

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