Slice preparation

Solutions
ACSF: (in mM) 125 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 25 NaHCO3, 1.25 NaH2PO4, 25 glucose bubbled with 95% O2, 5% CO2, PH 7.4.
Intracellular solution: (in mM) potassium gluconate 115, KCl 20, sodium phosphocreatine 10, HEPES 10, MgATP 2, NaGTP 0.3, and 0.1% biocytin for subsequent determination of morphology.

Dissections
Adult, male Wistar rats were anesthetized with halothane and perfused through the heart with ice-cold, oxygenated, artificial cerebrospinal fluid (ACSF). Brains were removed, and 300 μm transverse hippocampal slices were made with a vibrating tissue slicer (Campden Instruments) in ice-cold ACSF. Slices were then incubated at 35C for 30 min and kept at room temperature until time of recording.

Electrophysiology

Recordings
Slices were placed on the stage of an Axioscop FS microscope (Zeiss) where they were perfused with ACSF held at 34+-2C and visualized using infrared differential interference video microscopy.

Amplifiers
Whole-cell current-clamp recordings were made using bridge amplifiers (Dagan BVC-700).

Electrodes
Patch-clamp electrodes were fabricated from thick walled borosilicate glass (EN-1, Garner Glass) and had tip resistances of 4–11 MW in saline. Bridge balance and capacitance compensation were performed for all whole-cell recordings, which were terminated if series resistance exceeded 80 MW.

Digitization
Electrophysiological traces were digitized via an ITC 16 or ITC18 digital_analog converter (Instrutech) under control of macros custom programmed in IGOR Pro (Wavemetrics). Electrophysiological records were filtered at 5 kHz and digitally sampled at 10-50 kHz.

Data

Data Acquisition
Data were acquired using Macintosh PowerPC computers using Pulse Control software (Dr. Richard Bookman, University of Miami) or our own custom macros, both written to run under Igor Pro (WaveMetrics).

Data Analysis
Analysis of electrophysiology was performed using IGOR Pro Software.

Reconstructions

Histology
Neurons were visualized using the DAB reaction (Vectastain ABC kit, Vector Laboratories Inc., Burlingham, CA) using standard procedures. No clearing or dehydration was used in order to prevent shrinkage of slices. After histology, slices were mounted in aqueous mounting medium (Moviol, Calbiochem, LaJolla, CA).

Reconstructions
The three-dimensional position and diameter of the dendritic branching pattern were reconstructed using a Zeiss inverted microscope having a 63x oil immersion objective fitted with semi-automated Neurolucida hardware and software (version 2.1, MicroBrightField Inc., Colchester, VT).

File Conversions
The Neurolucida file was then converted to a NEURON geometry file by the Neuroconvert program (version 2.0b4, D. Niedenzu and G. Klien, MPI für medizinische Forschung, Heidelberg, Germany, 1998).