Anatomical and Electrophysiological Comparison of CA1 Pyramidal Neurons of the Rat and Mouse

Brandy N. Routh, Daniel Johnston, Kristen Harris, and Raymond A. Chitwood

Center for Learning and Memory, University of Texas at Austin, Austin, Texas

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INTRODUCTION

The mammalian hippocampus is a region of the medial temporal lobe that has been implicated in various aspects of memory. Rodents with lesions to the hippocampus show impaired trace eyelid conditioning, contextual fear conditioning, and spatial learning (Logue et al. 1997; Morris et al. 1982; Sutherland et al. 1982). Neurons of this region demonstrate functional plasticity, which is a presumed substrate for information storage (Morris et al. 1990). In addition, pharmacological or genetic manipulation of protein function in the hippocampus can disrupt both long-term cellular plasticity and certain forms of learning and memory (Chen et al. 2006; Nolan et al. 2004; Zeng et al. 2001).

Anatomical, physiological, and theoretical studies of CA1 pyramidal neurons, the primary output of the hippocampus proper (Witter and Amaral 2004), have predominantly been performed in rats (Golding et al. 2005; Harris and Stevens 1989; Megias et al. 2001; Poirazi et al. 2003). The development of genetic methods that alter protein expression in specific neurons, however, most often uses mouse models and has resulted in numerous studies related to learning and memory being carried out in mice (Chen et al. 2006; Nolan et al. 2004; Picciotto and Wickman 1988). Although mice are closely related to rats, there are important differences that complicate direct comparisons between species. Behavioral studies suggest that mice have different strategies for learning spatial information. For example, on the Morris water maze, a spatial memory task in which rodents use spatial cues to navigate through a circular pool to a hidden platform (Morris 1984), mice do not perform as well as rats (Frzik et al. 2000; Whishaw 1995) and use simpler strategies than rats do to locate the platform (Whishaw et al. 2001).

The behavioral disparities between rats and mice could stem from anatomical and/or physiological differences in CA1 pyramidal neurons. At the anatomical level, these differences may include: neuronal size reflected by total dendritic length, surface area, or volume; spatial distribution or branching of the dendritic arbor; or dendritic spine size or density. At the physiological level, distinctions could include: passive membrane properties such as resting membrane potential ($V_m$), input resistance ($R_{in}$), and membrane time constant ($\tau$); and active membrane properties such as those contributing to action potential generation as well as subthreshold membrane resonance.

Individual studies have reported passive physiological properties of mouse or rat CA1 neurons (Biscoe and Duchen 1985; Staff et al. 2000), but the combined morphological and physiological properties of CA1 pyramidal neurons have not been systematically compared between rats and mice. Observations from rats and single-cell models created from rat neuron morphologies should not be generalized to explain data obtained from mice without evidence that the CA1 pyramidal neurons of both species are similar in structure and function. In this study, we performed a systematic analysis of rats and two common strains of mice used for genetic manipulations, C57BL/6 and 129/SvEv, comparing gross hippocampal anatomy, dendrite and spine morphology, passive and active membrane properties, and membrane potential resonance. In addition, we created animal-specific, single-neuron models that incorporate the unique physiological and morphological properties of the CA1 pyramidal cells from the different species and strains. This study reveals significant differences between mouse strains, as well as between mice and rats. A major difference between the two species is that mice have less $I_h$ active at resting membrane potentials than rats.

METHODS

Slice preparation

All animals used in this study were males aged 5–7 wk. This range was chosen to facilitate comparison with other physiological studies...
in both rat (Magee 1999; Pooles et al. 2002; Staff et al. 2000) and mouse species (Chen et al. 2006; Nguyen et al 2000a,b; Nolan et al. 2004; Tsay et al. 2007), as well as a behavioral study comparing hippocampal-dependent learning in rats and mice (Whishaw and Tomie 1996). Sprague–Dawley rats, C57BL/6Jax mice, and 129/SvEvTac mice were anesthetized with a ketamine/xylazine mixture (90/10 mg/ml) and intracardially perfused with ice-cold artificial cerebral spinal fluid (aCSF) consisting of the following (in mM): 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 0.5 CaCl2, 7 MgCl2, 7 dextrose, and 205 sucrose, bubbled with 95% O2-5% CO2 to maintain a pH of about 7.4. The brain was removed, the cerebellum was cut away, and a cut was made longitudinally down the center fisure to separate the hemispheres. A blocking cut was made along the dorsal surface of each hemisphere at an angle of about 75° referenced to vertical to maximize the dendritic projections within the plane of the slice. Each section was mounted on its dorsal surface, rostral end toward the blade, and sliced on a tissue slicer (Vibratome 3000, Bratome, St. Louis, MO). Because rats have larger brains and hippocampi relative to those of mice (Kalisch et al. 2006; Kovacevic et al. 2005; Ma et al. 2005; Sahin et al. 2001), horizontal slices were cut 350 μm thick in rats and 300 μm thick in mice to obtain approximately the same number of slices across the dorsoventral axis from each species. Typically four to six sections were harvested from the middle of the hippocampus relative to the dorsoventral axis. Slices were held for about 45 min at 37°C in a holding chamber of aCSF solution containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 2 MgCl2, 10–25 dextrose, 1.3 ascorbic acid, and 3 sodium pyruvate, bubbled with 95% O2-5% CO2. Incubation beyond 45 min was at room temperature (±2°C). All methods were approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

Whole cell recordings

Whole cell current-clamp recordings were performed on slices submerged in a chamber filled with aCSF heated to 32–34°C, flowing at a rate of 1 to 2 ml/min (same as holding aCSF, minus pyruvate and ascorbate). Neurons were visualized using an Olympus BX51 (Model BX51WD) fitted with differential interference contrast optics using infrared illumination (Stuart et al. 1993). Patch pipettes (4–7 MΩ) were pulled from capillary glass of external diameter 1.65 mm (World Precision Instruments), using a Flaming/Brown micropipette puller (Model P-97, Sutter Instruments), and filled with an internal solution containing the following (in mM): 120 K-glucuronate, 20 KCl, 10 HEPES, 4 NaCl, 7–14 Tris-phosphocreatine, 0.3 Tris-GTP, and 4 Mg-ATP. Neurobiotin (Vector Labs) was included (0.1–0.2%) for subsequent histological processing. Data were acquired with a Multiclamp 700B amplifier (Molecular Devices) and digitized at 20–50 kHz with an ITC-12 (Instrutech) interface and Axograph 6.0 (AxoGraph Scientific) acquisition software. Membrane properties were measured on each cell in the presence of a synaptic blocker (50 μM 2-amino-5-phosphonovaleric acid [APV], 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione [CNQX], 10 μM bicuculline, and 10 μM picROTOXIN) and 2 synaptic blockers plus an h channel blocker (5 mM CsCl or 20 mM 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyridinium chloride [ZD7288]). All drugs were made up as stock solutions in water, DMSO, NaOH, or ethanol (final concentration of each solvent <0.1%). At least 5 to 10 min were allowed for solutions to wash in before taking measurements. CsCl and ZD7288 caused a change in Vm, so a holding current was applied to hold the cells at the initial Vm measured in synaptic blockers. Because many of our measurements are critically dependent on minimization of recording artifacts, pipette capacitance was compensated for, and the bridge was balanced manually prior to each recording run. Injected currents used for this purpose were typically 50% larger than those used for data collection to further minimize errors. Series resistance was 8–25 MΩ for all recordings and experiments were terminated if this range was exceeded.

Input resistance was calculated as the slope of the linear fit of the voltage–current plot between −40 and +40-pA current injections for rats and C57BL/6 mice. In 129/SvEv mice, the line was fit between −60 and +20 pA because neurons had larger input resistances and were more likely to fire action potentials with current injections above +20 pA. Membrane time constant traces were analyzed using Igor (WaveMetrics), and slow and fast time constants were calculated from a double-exponential fit of the averaged voltage decay resulting from 100 trials of identical 1-ms, −400-pA current injections. Active membrane properties were calculated as the average spike threshold, maximum dV/dt, peak, amplitude, and half-width of five individual action potentials. Threshold was defined as the voltage at the time corresponding to the first maximum of the third derivative of the voltage response. Action potential amplitude was measured from threshold to peak, with the half-width measured at half this distance. The sag ratio was calculated as the proportion of steady-state to maximum voltage drop of the hyperpolarizing current injections. Resonance was measured using a sinusoidal current injection of constant amplitude and linearly spanning 0–15 Hz in 15 s, and the impedance amplitude profile was determined by taking the ratio of the Fourier transform of the voltage response to the Fourier transform of the current stimulus (Narayanand and Johnston 2007; Puil et al. 1986). Resonance frequency was measured as the frequency of the peak impedance, and resonance strength was the ratio of the peak impedance to the steady-state impedance at a frequency of 1 Hz.

Cellular morphology

Slices were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and stored at 4°C for ≤3 mo. They were processed using an avidin–horseradish peroxidase system activated by dianisobenzidine (DAB, Vector Labs). DAB-processed slices were mounted on slides in glycerol and viewed with a compound (Leitz Diaplan) microscope. Dimensions of a sample of slices were measured in vitro, prior to fixation, and again after DAB processing, and no significant shrinkage of tissue was observed (<1%). The length of the hippocampus in a slice was measured as the maximum distance from the apex of CA3 to the angular bundle, and the width was measured across the extent of the granule cell layer from each of the blades of the dentate gyrus and across CA1 to the alveus. The width of the dentate gyrus was measured as the distance between the ends of the granule cell body layer. To account for differences in hippocampal geometry due to slicing across the dorsoventral axis, processed slices were ranked from 1 to 11 (ventrodorsal), according to their shape, to represent their position along this axis. The average location of slices was in the middle of the dorsoventral axis (ranked 6) and was not different among the three groups.

For illustrative purposes, images of the cells were created from z-stacks of photos taken at ×10 or ×100 and processed using Helicon Focus Pro software (Helicon Soft). Neurons were reconstructed using a ×40 objective with a computer-controlled indexing system running Neuronlucida 6.0 imaging software (MicroBrightField). Whole cell, three-dimensional (3D) reconstructions included the soma and dendritic shafts, but not dendritic spines. To quantitatively analyze dendritic anatomy, all morphological measurements were done in Neuroulicuda Explorer (MicroBrightField). Total dendritic length, membrane surface area, and cell volume included the sum of the lengths, surface areas, and volumes of all dendritic segments. Dendritic branching patterns were analyzed using Sholl (1953) analyses, in which a set of nested concentric spheres of linearly increasing radius were centered at the cell body, and noncumulative counts of intersections and dendritic lengths were measured for each sphere. The number of intersections refers to the number of times the processes intersect an individual Sholl sphere and the length refers to the total length of all processes
within a sphere, not including length in smaller spheres. Due to variation in total dendritic length, the radius of the smallest Sholl sphere that encompassed all dendritic processes ranged from 480 to 740 μm (to the nearest 20 μm) for different cells. To compare Sholl analyses across this large range of lengths, we used a different set of radii for each cell, scaled to give a total of 20 Sholl spheres for each cell. For example: a cell extending ≤600 μm from the soma would have 20 spheres of radii incrementing by 30 μm, whereas a cell extending 480 μm would have 20 spheres with 24 μm increments to the radii.

To compare spine densities among groups, samples of dendritic segments including spines were reconstructed at 100. These segments were randomly chosen but met the following criteria: 1) having a dark stain and clearly discernible spines, 2) being 25 to 50 μm long, 3) including no branch points, and 4) the dendrite being planar along the horizontal axis. Because spine density varies with location (Megías et al. 2001), we looked at dendritic segments from six of the seven spiny regions of the CA1 neurons, described in Megías et al. (2001): lacunosum-moleculare thin (LMt) and medium (LMM), radiatum (RTt), radiatum thick distal (RTd) and medial (RTm), and oriens distal (OD). The lacunosum-moleculare thick spiny segments described by Megías et al. (2001) were not included because their small length (1 μm) made their location difficult to determine. The average distance from the soma of each reconstructed segment was measured by linear distance. Each spine was reconstructed as a cylinder, with its length defined as the distance from the edge of the dendritic shaft to the end of the spine and its diameter defined as the diameter of the spine head. More accurate measures of spine length and head diameter were obtained by serial-section electron microscopy (Harris and Stevens 1989) for comparison. The light microscope reconstructions enabled us to analyze a large number of dendritic segments. Spine densities were calculated as the number of spines divided by the length of the segment. To account for spines obscured from view by the dendritic shaft, spine densities were corrected using the geometric equation derived in Feldman and Peters (1979)

\[ N = \frac{n\pi[(Dr + Sl)^2 - (Dr + Sd)^2]}{[0/\pi90\cdot(Dr + Sl)^2 - 2(\pi/\pi90\cdot\sin \theta (Dr + Sd))]} \]

where \( N \) is the estimated spine density; \( n \) is the raw spine density, before accounting for hidden spines; \( Dr \) is the radius of dendrite; \( Sl \) is the average length of spines \([\text{mean} \pm \text{SD}; \text{rat}: 0.69 \pm 0.29 \mu m; \text{C57BL/6}: 0.71 \pm 0.27 \mu m; \text{129/SvEv}: 0.62 \pm 0.25 \mu m]\); \( Sd \) is the average diameter of spine head \([\text{mean} \pm \text{SD}; \text{rat}: 0.35 \pm 0.16 \mu m; \text{C57BL/6}: 0.35 \pm 0.15 \mu m; \text{129/SvEv}: 0.35 \pm 0.14 \mu m]\)

\[ \cos \theta = \frac{Dr + Sd}{Dr + Sl} \]

\( N, n, \) and \( Dr \) were determined individually for each segment, whereas \( Sl \) and \( Sd \) were determined as the average values for each animal group.

**Simulations**  
Effect of \( I_{h} \) on resonance and “sag.” Simulations were performed on a single-compartment model (cylinder of 100-μm diameter and 100-μm length) using the NEURON simulation environment (Hines and Carnevale 1997). Membrane properties were set to: \( C_m = 1 \mu F/cm^2 \) and \( R_m = \tau_{slow}/C_m \) (from \( \tau_{slow} = R_m/C_m \) and using mean \( \tau_{slow} \) from experiments). Kinetics of the h conductance were: \( \tau_{h} = 47 \) ms and \( V_h = -82 \) mV (Magee 1998). The maximal conductance \( (g_h) \) was set to 22 mS/cm², to yield a resonance frequency \( (f_R) \) similar to that observed in the rat. To simulate the lower \( f_R \) observed in mice, we either reduced \( g_h \) or jointly increased \( \tau_{nax} \) and reduced \( V_{nax} \). To match experimental observations, we simulated membrane potential sag at −65 mV and resonance at −75 mV using current injection waveforms identical to those used experimentally.

Passive models of reconstructed neurons. Whole cell reconstructions were imported into the NEURON simulation environment (Hines and Carnevale 1997), and models were created by pairing each cell’s digitized anatomy with its measured \( \tau_{slow} \) (recorded in synaptic blockers plus either ZD7288 or CsCl). Default membrane parameters for each cell were set to \( C_m = 1 \mu F/cm^2, R_i = 150 \) Ω-cm, and \( R_m = \tau_{slow}/C_m \). No active conductances were included. The whole cell reconstructions were based solely on dendritic diameter and length and did not include spines. To account for the extra surface area in spines, \( R_m \) was divided and \( C_m \) was multiplied by a “spinescale” parameter (Golding et al. 2005; Stuart and Spruston 1998), which was defined as the ratio of the total surface area, including spines, to the surface area without spines, according to the following equation

\[ \text{spinescale} = \frac{SA_{shaft} + SA_{spines}}{SA_{shaft}} = \frac{\pi d + NA}{\pi d} \]

where \( SA_{shaft} \) is the surface area of dendritic shaft for a 1-μm-long segment; \( SA_{spines} \) is the surface area contributed by spines in a 1-μm-long segment; \( d \) is the average diameter of dendritic shaft; \( N \) is the estimated spine density from the Feldman and Peters (1979) equation; and \( A \) is the average surface area of an individual spine.

The average surface area of an individual spine in rats and C57BL/6 was set to 0.85 μm², which is the sum of the head and neck surface areas reported for rats in Harris and Stevens (1989). The individual spine surface area in 129/SvEv was set to 0.83 μm², which was obtained by scaling the Harris and Stevens (1989) neck surface area by 90%. Spines in 129/SvEv were shorter than, but had head diameters similar to, those of the other groups, so we reasoned that differences in spine length and surface area were due to a shorter spine neck.

**Statistical analyses**

A two-factor ANOVA test, Bonferroni-corrected (InStat 3.0; GraphPad Software), was used to assess the differences among the three animal groups and between pharmacological treatments. Where ANOVA revealed significant differences, a Student’s \( t \)-test was performed to assess the significance between groups. When SDs across groups were significantly different according to Bartlett’s test, a Dunn-corrected, nonparametric ANOVA was used. Data are reported as means ± SE.

**RESULTS**

**Quantitative morphology**

Because rats have larger brains with larger hippocampi than those of mice (Kalisch et al. 2006; Kovacevic et al. 2005; Ma et al. 2005; Sahin et al. 2001), we first compared gross hippocampal anatomy, as measured from our slices. As expected, slices of rat hippocampi were larger than those from mice, with larger average lengths and widths (Fig. 1; ANOVA, Bonferroni-corrected; length: \( P < 0.0001 \); width: \( P < 0.0001 \)). Surprisingly, the mean distance from individually labeled soma along the apical dendritic tree to the hippocampal fissure was similar across groups (Fig. 1, ANOVA, \( P = 0.248 \)). The observed similarities were not due to a dorsoventral sampling bias because the mean slice positions along the dorsoventral axis were not different among groups (see METHODS for quantification detail). The findings suggest that the apical dendritic lengths of CA1 pyramidal neurons may be conserved across these two species, despite differences in total hippocampal volume. Although the extent of area CA1 was similar in rats.
and mice, the width of the dentate gyrus was smaller in mice (Fig. 1C, nonparametric ANOVA, Dunn-corrected; \( P < 0.001 \)), which contributes to a difference in total hippocampal volume across species.

We further quantified dendritic morphology using 3D reconstructions of DAB-processed neurons (Fig. 2A). Total dendritic length was similar across all animal groups, but neurons of 129/SvEv mice appeared slightly shorter due to a decreased dendritic length within stratum radiatum compared with that of rats (Fig. 2, A and B; nonparametric ANOVA, Dunn-corrected; total length: \( P = 0.012 \); length in s. oriens: \( P = 0.186 \); length in s. radiatum: \( P = 0.019 \); length in s. lacunosum-moleculare: \( P = 0.098 \)). Total membrane surface area and volume were also significantly reduced in the 129/SvEv mouse strain compared with those of rats, due to differences within stratum radiatum (Fig. 2B; nonparametric ANOVA, Dunn-corrected; total surface area: \( P = 0.004 \); surface area in s. oriens: \( P = 0.029 \); surface area in s. radiatum: \( P = 0.002 \); surface area in s. lacunosum-moleculare: \( P = 0.091 \); total volume: \( P = 0.002 \); volume in s. oriens: \( P = 0.025 \); volume in s. radiatum: \( P = 0.001 \);

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**FIG. 1.** Geometry of hippocampal slices taken from rats and C57BL/6 and 129/SvEv mice. A: photos of slices showing hippocampal geometry across the dorsoventral axis in the 3 groups. A neurobiotin-filled cell is visible in CA1 of each slice. B: diagram of a typical measurement of hippocampal width, length, soma-to-hippocampal fissure distance, and dentate gyrus width. C: average measure of hippocampal width and length in slices with stained neurons; average distance from each visible soma to the hippocampal fissure, as measured along the apical dendritic tree; and average width of the dentate gyrus, ***\( P < 0.001 \). (\( n = 22 \) rats, 21 C57BL/6 mice, and 14 129/SvEv mice).
FIG. 2. Morphology of CA1 pyramidal neurons of rats and C57BL/6 and 129/SvEv mice. A: representative reconstructed neurons from each animal group. Dotted lines represent the borders between stratum radiatum and stratum lacunosum-moleculare for each cell. B: average dendritic length, membrane surface area, and volume within different strata of CA1. *P < 0.05. C: Sholl analyses for intersections and dendritic length. Radius of concentric circles was different for each cell and was set to give a total of 20 apical circles. Scaled distance from soma refers to the number of Sholl spheres, increasing from 1 to 20 with distance from the soma. Negative distances refer to the basal dendrites. Stars represent regions of significant difference (*P < 0.05). Rat vs. 129/SvEv mice: difference in intersections at Sholl distance 11 and in dendritic length at Sholl distances 11–13. Rat vs. C57BL/6 mice: difference in dendritic length at Sholl distances 4 and 13. C57BL/6 vs. 129/SvEv: differences in dendritic length at Sholl distances 10–11. n = 5 rats, 6 C57BL/6 mice, and 8 129/SvEv mice.

Neurons of C57BL/6 mice were intermediate to both rats and 129/SvEv and were not significantly different from either. Consistent with these data, Sholl analyses performed on the neuron reconstructions revealed that the number of intersections and dendritic length were decreased in distal stratum radiatum of 129/SvEv mice compared with those in rats (Fig. 2C). Additionally, C57BL/6 had less dendritic length in prox-
inal stratum radiatum and at the stratum radiatum/stratum lacunosum-moleculare border.

**Analysis of spines**

Whole cell reconstructions provide a measure of the membrane surface area along the dendritic shaft. Because dendritic spines also significantly contribute to the membrane surface area and cell volume, we quantified spine density across the dendritic arbor in each of the three groups. Spiny segments were reconstructed from six different regions across the dendritic tree in the three animal groups (Fig. 3, A and B; \( n = 3 \) segments per region per animal, 54 total). Average distances from the soma differed slightly but not significantly between rats and mice for the same cell regions (Fig. 3C; standard ANOVA; LMt: \( P = 0.153 \); LMM: \( P = 0.635 \); RTd: \( P = 0.244 \); RTm: \( P = 0.550 \); Rt: \( P = 0.175 \); OD: \( P = 0.346 \)).

**FIG. 3.** Spine densities in CA1 pyramidal neurons of rats and C57BL/6 and 129/SvEv mice. A: sample cell and regions where spiny reconstructions were made. 1: lacunosum-moleculare thin (LMt); 2: lacunosum-moleculare medium (LMM); 3: radiatum/thick distal (RTd); 4: radiatum/thick medial (RTm); 5: radiatum thin (Rt); 6: oriens distal (OD). \( n = 3 \) rats, 3 C57BL/6 mice, and 3 129/SvEv mice reconstructions for each of the 6 regions (total of 54 reconstructed segments). B: sample photos of spines from LMM from the 3 groups. The rat spiny segment is accompanied by the 2D image of its corresponding 3D reconstruction. C: average distance from the soma for the reconstructed spiny segments of the different cell regions. D: average dendritic diameter of the reconstructed spiny segments. Significance bars above the graph show significance across animal groups within a single-cell region. Bars below show significance across cell regions for all animal groups. E: average spine length and spine head diameter of all spines reconstructed within each animal group. F: average estimated spine densities derived by correcting raw spine counts to account for hidden spines using the Feldman and Peters (1979) geometric equation. Significance bars above the graph show significance across animal groups within a single-cell region. Bars below show significance across cell regions for all animal groups. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).
RTm: $P = 0.111$; Rt: $P = 0.608$; OD: $P = 0.957$). Dendritic diameter was larger in rats than that in 129/SvEv mice in LMm and larger in rats than that in both mice in RTm (Fig. 3D) (Across animal species: ANOVA, Bonferroni-corrected; LMt: $P = 0.174$; LMm: $P = 0.011$; RTd: $P = 0.080$; RTm: $P = 0.016$; Rt: $P = 0.035$; OD: $P = 0.482$. Across region: nonparametric ANOVA, Dunn-corrected; $P < 0.0001$). The spine head diameter was similar across all three groups, but total spine length was reduced in 129/SvEv compared with that in both rats and in C57BL/6 mice (Fig. 3E). ANOVA, Bonferroni-corrected; head diameter: $P = 0.729$; spine length: $P < 0.0001$). Spine densities of the different regions were determined by counting the number of visible spines per $\mu$m length. However, this method of counting will be affected by dendrite diameter because more spines will be obscured from view by larger diameter dendrites than smaller ones. We used the geometric equation of Feldman and Peters (1979) to correct for spines that were hidden by the dendritic shaft. This approach allowed us to compare across groups of animals, regardless of average diameter, and to give us more accurate spine density approximations for modeling. Corrected values revealed larger spine densities in rats than 129/SvEv mice in radiatum/thick/medial and larger densities in rats than both mice in lacunosum-molecular/medium (Fig. 3F) (Across animal species: standard ANOVA, Bonferroni-corrected; average: $P = 0.039$; LMt: $P = 0.227$; LMm: $P = 0.001$; RTd: $P = 0.569$; RTm: $P = 0.031$; Rt: $P = 0.130$; OD: $P = 0.407$. Across cell region: nonparametric ANOVA, Dunn-corrected; $P < 0.0001$).

**Resting membrane properties**

Dendritic geometry will determine cellular physiology, given that membrane surface area is inversely proportional to $R_s$ (Rall 1977). Because CA1 pyramidal neurons of 129/SvEv mice had less surface area than those of rats, we hypothesized that they might also be physiologically distinct from rat neurons. We measured passive membrane properties in the three animal groups in the presence of CNQX, APV, bicuculline, and picROTOX to block fast glutamatergic and GABAergic transmission. Rat neurons had passive membrane properties similar to those previously reported by our lab and by others (Table 1; Fan et al. 2005; McDermott et al. 2003; Staff et al. 2000). C57BL/6 neurons had a more hyperpolarized resting membrane potential ($V_m$) than that of the other two groups (Table 1; ANOVA, Bonferroni-corrected; $P = 0.0001$). 129/SvEv neurons had significantly larger input resistances ($R_i$) than those of the other groups, as might be predicted from their smaller membrane surface area (ANOVA, Bonferroni-corrected; $P = 0.0001$). Both fast and slow time constants ($\tau_{fast}$, $\tau_{slow}$) were similar across all animal groups (ANOVA, Bonferroni-corrected; $\tau_{slow}$: $P = 0.103$, $\tau_{fast}$: $P = 0.810$; 50 neurons total; rat: 20; C57BL/6: 16; 129/SvEv: 14).

<table>
<thead>
<tr>
<th>Property</th>
<th>Rat (n = 20)</th>
<th>C57BL/6 (n = 16)</th>
<th>129/SvEv (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane potential, mV</td>
<td>$-64.6 \pm 0.8$</td>
<td>$-67.5 \pm 0.6^*$</td>
<td>$-62.8 \pm 0.6$</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>$65.6 \pm 4.4$</td>
<td>$65.4 \pm 1.7$</td>
<td>$93.1 \pm 6.7^*$</td>
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<tr>
<td>Slow time constant, ms</td>
<td>$22.4 \pm 1.5$</td>
<td>$22.2 \pm 0.9$</td>
<td>$26.1 \pm 1.6$</td>
</tr>
<tr>
<td>Fast time constant, ms</td>
<td>$0.8 \pm 0.1$</td>
<td>$0.8 \pm 0.0$</td>
<td>$0.8 \pm 0.0$</td>
</tr>
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Values are means ± SE. *Significantly different from the other two groups.

Because $I_h$ is a prominent contributor to $V_m$, $R_i$, and $\tau$, we also measured these parameters in the presence of the $I_h$ blockers ZD7288 or CsCl. The $I_h$ blocker ZD7288 had similar effects on the passive properties for neurons of all groups. ZD7288 increased $R_i$, increased $\tau_{slow}$ and hyperpolarized $V_m$ (Fig. 4, A–C), as would be expected after blocking $I_h$. CsCl likewise increased $R_i$. However, in CsCl-treated cells, $\tau_{slow}$ increased only in rats, whereas in C57BL/6 it did not significantly change, and in 129/SvEv it decreased. Wash-in of CsCl also resulted in depolarization of $V_m$ in all three groups of animals (Fig. 4D). For consistency, after drug wash-in, we held the cells at the $V_m$ measured before drug application.

**Action potential properties**

In addition to passive properties, we also measured certain active properties. Active properties of CA1 pyramidal neurons in control aCSF varied widely among the three groups of animals. The action potential amplitude measured in rats was consistent with previously reported values (Staff et al. 2000; Fig. 5B). Compared with rats, C57BL/6 had a hyperpolarized peak and decreased maximum dV/dt, resulting in an action potential of smaller amplitude (Fig. 5, A and B). In contrast, 129/SvEv neurons had a hyperpolarized threshold and an action potential of larger amplitude compared with that of rats. 129/SvEv neurons also had a larger half-width than that of neurons of C57BL/6 (Fig. 5B; ANOVA, Bonferroni-corrected; threshold: $P = 0.003$; max dV/dt: $P = 0.014$; amplitude: $P < 0.0001$; half-width: $P = 0.035$).

The inclusion of compounds intended to block $I_h$ affected active properties uniquely, depending on the compound used and animal group. CsCl increased action potential half-width in rats and C57BL/6, but not in 129/SvEv (Fig. 5C). ZD7288 decreased amplitude, decreased maximum dV/dt and increased half-width in rats and C57BL/6. It also increased threshold in rats and decreased peak amplitude in the C57BL/6 mice. The only measured effect of ZD7288 in 129/SvEv mice was an increase in the action potential half-width (Fig. 5D).

**Membrane resonance**

$I_h$ blockers affected both the passive and active membrane properties of rat and mouse CA1 pyramidal neurons. Additional information on $I_h$ in the different species was obtained by analyzing the voltage “sag,” which results from the membrane charging more rapidly than the activation or deactivation of $I_h$. The sag ratio and timing of the peak hyperpolarization can reveal differences in the amount of active $I_h$ at rest. Sag ratios were not significantly different in the three groups of animals, although there appeared to be a trend with rats having more sag (Fig. 6A; ANOVA; $P = 0.293$). The time-to-peak of the sag, however, was significantly longer for both mouse groups than that for the rats (Fig. 6B; standard ANOVA, Bonferroni-corrected; $P = 0.001$), suggesting there may be some difference in $I_h$. In addition to voltage sag, $I_h$ causes the membrane potential to resonate at a frequency that is directly related to the amount of active $I_h$ (Hu et al. 2002; Hutcheon and Yarom 2000; Narayanan and Johnston 2007). To further explore possible differences in $I_h$ between rats and mice, we measured the intrinsic resonance properties of the neurons in control aCSF using a “chirp” stimulus (Narayanan and...
Because I_h activation and membrane resonance are voltage-dependent, we measured resonance at two different potentials: −65 and −75 mV. At both of these membrane potentials, rats had significantly higher resonance frequencies and strengths than those of both strains of mice (Fig. 6, C and D; ANOVA, Bonferroni-corrected; frequency at −65 mV: P < 0.0001; frequency at −75 mV: P < 0.0001; strength at −65 mV: P < 0.0001; strength at −75 mV: P < 0.0001).

The observed lower resonance frequency in mice suggested that mice may have less active I_h near rest, relative to that of rats. This could be the result of either fewer total h channels or a difference in h channel kinetics, effectively reducing any h conductance (g_h) available to influence resonance (Narayanan and Johnston 2007). To distinguish between these two possibilities, we simulated the effects of either reducing maximal h conductance (g_h), or altering the biophysical properties of h channels (half activation voltage, V_{1/2}, and activation time constant, τ_{act}) as might be expected if mice were to have a reduced HCN1:HCN2 ratio relative to rats (Chen et al. 2001; Ulens and Tytgat 2001). Using a single compartment model with R_m = 22 kΩ·cm^2 (our measured value in rats), and experimentally determined properties of g_h (V_{1/2} = −82 mV, and τ_{act} = 47 ms; Magee 1997), we were able to reproduce the sag ratio, time-to-peak, and resonance values observed in rats (Fig. 7, model 1) by setting g_h to 22 nS/cm^2. Lowering g_h (Fig. 7, models 2), or changing V_{1/2} and τ_{act} (Fig. 7, model 3) were both able to reproduce reduced sag ratio, delayed time-to-peak, (Fig. 7, B and C), and lower resonance frequencies (Fig. 7D), similar to our observations in mice. Similar shifts in resonance frequency could also be obtained by altering V_{1/2} and τ_{act} in isolation (data not shown). Changing R_m to 26 kΩ·cm^2 (our
measured value in 129/SvEv) did not affect resonance or membrane voltage sag (data not shown).

Simulations using passive membrane models

Because of the many differences reported here among rats and the two mouse strains, we used our morphological and physiological data to create models of mouse CA1 neurons for use in future studies done on C57 and 129 strains of mice. We used \( \tau_{\text{slow}} \) measured in CsCl or ZD7288 to calculate \( R_m \) for each cell, and individual cell morphology was combined with its calculated \( R_m \) to create a single-cell model in NEURON. When compared with the experimental data, models that did not account for spine surface area gave \( R_N \) values approximately twofold larger than the actual experimental values (Fig. 8, A and B). To account for the surface area contributed by spines, an additional model was created for each neuron by dividing \( R_m \) and multiplying \( C_m \) in a region-dependent manner by the spinescale values shown in Table 2. These spinescales were calculated using our corrected spine densities, dendritic diameters, and previously reported spine surface area measurements (Harris and Stevens 1989). After applying the spinescale, \( R_N \) closely fit the experimental values for all groups, despite significantly different passive membrane properties, cell morphologies, and spine densities across animal groups (Fig. 8, B and C; no difference across groups: standard ANOVA, \( P = 0.490 \)).

DISCUSSION

In this study we compared the cellular morphologies and passive and active membrane properties of CA1 pyramidal neurons of rats and C57BL/6 and 129/SvEv mice. Neurons from rats and C57BL/6 mice had similar structure, with few differences in dendritic morphology and membrane physiology. In contrast, we discovered several distinguishing anatomical and physiological characteristics of the 129/SvEv mouse strain. Moreover, based on measurements of membrane reso-
nance and voltage sag, we report the amount of active \( I_h \) at rest to be a significant difference between rats and both mouse strains.

Similarities across species and strains

We observed surprising similarities in area CA1 between the two species. On the gross hippocampal level, the CA1 region appears to be very similar in rats and mice. Despite mice having smaller hippocampi, the distance from CA1 \textit{stratum pyramidale} to the hippocampal fissure is conserved and total dendritic length of CA1 pyramidal neurons is comparable between the two species. This might imply the difference in hippocampal size may be the result of fewer neurons as opposed to similar numbers of smaller neurons. Alternatively, our observations suggest the CA1 pyramidal neurons appear to be more densely packed, so the number of neurons might be similar because of an increased density in mice relative to that of rats. At the single-neuron level, the membrane time constant was also conserved across animal groups. The membrane time constant is an important factor affecting the spread of voltage signals in these neurons (Rall 1977) and it provides an underlying similarity in the way that rats and mice may temporally process inputs.

Also surprising was how well our single-cell models—which incorporated a variety of morphological and physiological measurements—re-created experimental steady-state \( R_N \) measurements across species. This attests to the accuracy of our physiological measures of \( \tau_{slow} \), our whole cell morphological measurements, and our spine density approximations. It also suggests that there was no factor affecting passive membrane properties uniquely in mice compared with rats that we did not account for in the models. Our mouse neuron models provide the first mouse CA1 pyramidal cell models to date.

Neurons from 129/SvEv mice are morphologically and electrophysiologically distinct from neurons from rats

Although the mouse strains exhibited both similarities and differences to the rat in various parameters, differences at the cellular level were more pronounced in the 129/SvEv strain than those in the C57BL/6 strain, which generally had characteristics intermediate to the rat and 129/SvEv. 129/SvEv mice had smaller neurons than those of rats, with less dendritic length, membrane surface area, and cell volume in \textit{stratum radiatum}. In addition, they had thinner diameter dendrites and lower spine densities in regions \textit{lacunosum-moleculare}/\textit{medium} and \textit{radiatum}/\textit{medial} than rats, as well as a shorter average spine length than that of both rats and their C57BL/6 counterparts. The combination of these factors suggests that they had fewer spines and less surface area in these regions compared with those of rats. Consistent with these data and combined with the observation of a longer membrane time constant compared with that of both rats and C57BL/6, 129/...
SvEv had larger input resistances. In contrast to the 129/SvEv strain, the C57BL/6 strain exhibited fewer and more limited morphological distinctions. These included decreased dendritic length at the stratum radiatum/stratum lacunosum-moleculare border, decreased dendritic diameter in RTm, and decreased spine density in LMm. Physiological differences between rats and C57BL/6 included a hyperpolarized \( V_m \) and differences in active properties. These differences are suggestive of potentially important species- and strain-specific attributes of hippocampal connectivity.

The 129/SvEv mouse strain also exhibited a unique pharmacological response relative to that of the other two groups—neurons from the 129/SvEv animals displayed a decrease in \( \tau_{\text{slow}} \) in CsCl. This is inconsistent with the expected increase that would occur if a resting conductance (i.e., \( I_h \)) is blocked. One possible explanation is a species/strain-specific difference in the affinity of the Na\(^+\)/K\(^+\)-ATPase for extracellular Cs\(^+\). In addition to blocking \( h \) channels, Cs\(^+\) is also known to compete with K\(^+\) to be transported across the membrane via the Na\(^+\)/K\(^+\)-ATPase (Sachs 1977), block inwardly rectifying K\(^+\) channels (Fukushima 1982), reduce uptake of intracellular Ca\(^{2+}\) by SERCA pumps (Kargacin et al. 2005), and interfere with K\(^+\) buffering in glia (Janigro et al. 1997). Cs\(^+\) can also cause a depolarization of \( V_m \) (Fernandez et al. 2001; Ghamari-Langroudi and Bourque 2001). The 129/SvEv mice may be more sensitive to some of these effects relative to the two other groups. Additionally, whereas ZD7288 affected the action potential amplitude of rats and C57BL/6, the action potential amplitude of 129/SvEv mice remained unaffected.

It is interesting that we found significant differences between these two strains of mice. In contrast to these findings, one previous study reported that passive membrane properties were similar between C57 and 129 strains (Nguyen et al. 2000b). However, methodological differences, most notably blind versus visualized patching and recording at room temperature versus physiological temperature, may explain these discrepancies.

Mice of the 129 strain have been reported to have deficiencies in long-term potentiation (LTP) (Nguyen et al. 2000a) and have a mixed history with the Morris water maze. Some variants perform well on the maze (Brooks et al. 2005; Contet et al. 2001; Montkowski et al. 1997), whereas other variants perform poorly (Owen et al. 1997). In contrast, C57 strains have generally been reported to perform well in the Morris water maze (Montkowski et al. 1997; Stavnezer et al. 2002). The combined morphological and physiological differences observed in 129/SvEv CA1 pyramidal neurons could negatively affect neuronal information processing and the animal’s performance in hippocampal-dependent learning tasks.
Both mouse strains have less Ih active at rest than rats

The differences in anatomical and physiological properties observed among the groups do not appear to be species specific with one exception—membrane resonance. C57BL/6 and 129/SvEv had lower resonance frequencies and strengths than those of rats at both membrane potentials tested. The membrane potential resonance observed in CA1 pyramidal neurons occurs in the theta-frequency range (Leung and Yu 1998; Pike et al. 2000) and is critically dependent on the distribution and characteristics of ion channels expressed throughout the membrane, most notably, channels of the HCN family (Hu et al. 2002; Hutcheon and Yarom 2000). At membrane potentials near rest, the resonance frequency is dependent primarily on the interaction of $R_m$, $C_m$, and $I_h$ (Narayanan and Johnston 2008). Because the three animal groups had a similar $\tau_{slow}$ and $C_m$ is similar for several neuron types (Chitwood et al. 1999; Gentet et al. 2000), and given the relationship $\tau_{slow} = R_mC_m$, we propose that the differences in resonance frequency between rats and mice are due to differences in $I_h$. Because resonance frequency is directly related to the magnitude of $I_h$, the lower resonance frequencies observed in mice suggest they have less $I_h$ than that of rats.

The magnitude of $I_h$ is determined by its maximal conductance ($g_h$), its voltage dependence of activation ($V_{1/2}$), and its activation time constant ($\tau_{act}$; Narayanan and Johnston 2008). The level of expression of h channels will determine $g_h$, whereas the molecular identity of the channels will largely determine $V_{1/2}$ and $\tau_{act}$. We modeled both scenarios in our single-compartment simulations and found that either decreasing $g_h$ or shifting $\tau_{act}$ and $V_{1/2}$ resulted in membrane voltage transients displaying sag and resonance similar to experimental observations from mice, consistent with the hypothesis that less $I_h$ is active at the resting membrane potential in mice than that in rats.

Our results suggest that mice have either a lower $g_h$ or a different h-channel subunit composition. Although membrane resonance is useful for detecting local differences in $I_h$, steady-state $R_N$ provides the best indicator of $g_h$ because it more efficiently engages $I_h$ and $V_{1/2}$. Resulted in membrane voltage transients displaying sag and resonance similar to experimental observations from mice, consistent with the hypothesis that less $I_h$ is active at the resting membrane potential in mice than that in rats.

Table 2. Spinescale values for each segment

<table>
<thead>
<tr>
<th>Segment</th>
<th>Rat</th>
<th>C57BL/6</th>
<th>129/SvEv</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. oriens distal</td>
<td>2.51</td>
<td>2.74</td>
<td>2.45</td>
</tr>
<tr>
<td>S. radiatum thick/medial</td>
<td>1.69</td>
<td>1.78</td>
<td>1.56</td>
</tr>
<tr>
<td>S. radiatum thick/distal</td>
<td>1.60</td>
<td>1.81</td>
<td>1.74</td>
</tr>
<tr>
<td>S. radiatum thin</td>
<td>1.86</td>
<td>2.69</td>
<td>2.04</td>
</tr>
<tr>
<td>S. lacunosum-moleculare medium</td>
<td>2.10</td>
<td>1.78</td>
<td>1.78</td>
</tr>
<tr>
<td>S. lacunosum-moleculare thin</td>
<td>1.71</td>
<td>1.66</td>
<td>1.53</td>
</tr>
</tbody>
</table>
likely due to h-channel subunit composition. In both rats and mice, pyramidal neurons in CA1 have h channels composed of HCN1 and HCN2 isoforms (Mooßmann et al. 1999; Shin and Chetkovich 2007). Channels comprised of HCN2 activate at a more hyperpolarized \( V_m \) and are slower to activate relative to channels made up of HCN1 alone (Chen et al. 2001; Santoro et al. 2000; Ulens and Tytgat 2001). Heteromerization of the two subunits in CA1 pyramidal neurons is also possible (Much et al. 2003), resulting in channels having properties intermediate to the two (Ulens and Tytgat 2001; but see Chen et al. 2001). Mice would have less \( I_h \) active at rest than that of rats if they had a decreased HCN1:HCN2 ratio.

Finally, because neurons from the 129/SvEv group had larger \( R_N \) and a slightly higher \( R_m \), it was not clear whether the decrease in \( f_g \) was attributable to \( R_m \) as opposed to altered \( I_h \). Simulations revealed that there was little effect on \( f_g \) caused by increasing \( R_m \) from 22 to 26 k\( \Omega \)-\( \text{cm}^2 \) (data not shown), suggesting that the lower \( f_g \) in the 129/SvEv group, like the C57BL/6 group, was due to less active \( I_h \).

Several studies show that mice do not perform as well as rats on the Morris water maze. Rats use complex spatial strategies to find the hidden platform in the maze (Frick et al. 2000; Lipp and Wolfer 1998), whereas mice use simpler, route-dominated techniques (Whishaw et al. 2001). On both the single-cell and hippocampal network levels, a decrease in \( I_h \) could hinder the spatial processing abilities of the mouse CA1. The density of \( I_h \) is distributed in a gradient across the apical tree of CA1 pyramidal neurons. Along the apical trunk in \textit{stratum radiatum}, there is a sevenfold linear increase in current density relative to the soma (Magee 1998), increasing to 60-fold in distal \textit{stratum lacunosum-moleculare} (Lorincz et al. 2002). \( I_h \) affects signal processing of CA1 pyramidal neurons in several ways. By attenuating distal signals (Golding et al. 2005) and normalizing temporal summation (Desjardins et al. 2003; Magee 1999), it removes the location dependence of inputs integrated at the soma. Additionally, it inhibits dendritic (Poolos et al. 2002) and cellular (Fan et al. 2005) excitability, decreases the amplitude and duration of distal Ca\textsuperscript{2+} spikes (Tsay et al. 2007), and constrains LTP at perforant path synapses (Nolan et al. 2004). The inhibitory effects of \( I_h \) on signal propagation may provide a way for the neuron to respond dynamically to a wide range of physiological inputs (Nolan et al. 2004). \( I_h \) also preferentially filters low-frequency inputs (Nolan et al. 2004; Poolos et al. 2002), conferring on CA1 pyramidal neurons high-pass filtering capabilities. In combination with the low-pass filtering caused by passive properties of the cell membrane, \( I_h \) helps tune individual CA1 neurons to frequencies in the theta range (Hu et al. 2002; Hutcheon and Yarom 2000). The somatodendritic gradient in \( I_h \) also mediates gradients in optimal tuning frequency (Hutcheon and Yarom 2000) and intrinsic phase response along the somatodendritic trunk (Narayan and Johnston 2008). However, it should be noted that, because a majority of the studies identifying the properties and distribution of \( I_h \) have used rats (Desjardins et al. 2003; Fan et al. 2005; Golding et al. 2005; Hu et al. 2002; Hutcheon and Yarom 2000; Lorincz et al. 2002; Magee 1998, 1999; Narayan and Johnston 2007; Poolos et al. 2002), whereas some of the functional aspects of dendritic \( I_h \) have relied on mice (Nolan et al. 2004; Tsay et al. 2007), extrapolation of these results across species and strains should be performed with caution.

There is also evidence that \( I_h \) may affect the overall expression of the hippocampal theta oscillation as well as theta-related phenomena in CA1 pyramidal neurons. Forebrain-specific HCN1 knockouts show enhanced theta power (Nolan et al. 2004). Interestingly, when compared with rats, mice also show enhanced theta power (Buzsáki et al. 2003). \( I_h \) may also play a role in the generation of phase precession in CA1, a phenomenon in which a neuron will fire progressively earlier in the theta cycle as an animal traverses space (O’Keefe and Recce 1993; Skaggs et al. 1996). Two rhythm generators oscillating at slightly different frequencies could cause such a phase shift (O’Keefe and Recce 1993), with the mechanism likely involving either a network or intrinsic oscillation or both (Maurer and McNaughton 2007). Because mouse pyramidal neurons resonate at lower frequencies than those of neurons in rats, phase precession could be altered in mice, possibly affecting their spatial memory processing.

Conclusions

The differences we found in CA1 pyramidal neurons across rodent species and strains may significantly influence hippocampal function, highlighting the necessity of considering animal type in the design and interpretation of experiments on CA1 pyramidal neurons. This study presents several anatomical and physiological distinctions that exist between a single rat strain and two strains of mice. There are likely to be many more differences that were not directly measured here, as well as differences in other strains of rats and mice. In light of our results, researchers should be cautious about overgeneralizing phenomena observed in a single species and strain.

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