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Combined Administration of Levetiracetam and Valproic Acid Attenuates Age-Related Hyperactivity of CA3 Place Cells, Reduces Place Field Area, and Increases Spatial Information Content in Aged Rat Hippocampus

Jonathan Robitsek,[1*] Marcia H. Ratner,[1*] Tara Stewart,[1] Howard Eichenbaum,[2] and David H. Farb[1*]

ABSTRACT: Learning and memory deficits associated with age-related mild cognitive impairment have long been attributed to impaired processing within the hippocampus. Hyperactivity within the hippocampal CA3 region that is associated with aging is mediated in part by a loss of functional inhibitory interneurons and thought to underlie impaired performance in spatial memory tasks, including the abnormal tendency in aged animals to pattern complete spatial representations. Here, we asked whether the spatial firing patterns of simultaneously recorded CA3 and CA1 neurons in young and aged rats could be manipulated pharmacologically to selectively reduce CA3 hyperactivity and thus, according to hypothesis, the associated abnormality in spatial representations. We used chronically implanted high-density tetrodes to record the spatial firing properties of CA3 and CA1 units during animal exploration for food in familiar and novel environments. Aged CA3 place cells have higher firing rates, larger place fields, less spatial information content, and respond less to a change from a familiar to a novel environment than young CA3 cells. We also find that the combination of levetiracetam (LEV) + valproic acid (VPA), previously shown to act as a cognitive enhancer in tests of spatial memory, attenuate CA3 place cell firing rates, reduce place field area, and increase spatial information content in aged but not young adult rats. This is consistent with drug enhancing the specificity of neuronal firing with respect to spatial location. Contrary to expectation, however, LEV + VPA reduces place cell discrimination between novel and familiar environments, i.e., spatial correlations increase, independent of age even though drug enhances performance in cognitive tasks. The results demonstrate that spatial information content, or the number of bits of information encoded per action potential, may be the key correlate for enhancement of spatial memory by LEV + VPA. © 2015 Wiley Periodicals, Inc.

KEY WORDS: place cells; memory; aging; levetiracetam; valproic acid

INTRODUCTION

Spatial disorientation is a common clinical manifestation of Alzheimer's disease (AD) (Henderson et al., 1989). Patients with mild cognitive impairment also have problems with spatial navigation and route learning (Benke et al., 2014). Understanding whether known cognitive enhancers may specifically change neural network activity of hippocampal pyramidal cells, or place cells, and thereby remedy deficits of learning and memory is a major experimental challenge. As an extension of this approach, a knowledge of how place cells may respond differentially to pharmacological interventions could contribute to the rational design of more effective therapeutics targeted to specific disorders of cognition. Non-demented older adults and patients with amnestic mild cognitive impairment exhibit elevated activity in the dentate gyrus (DG) and CA3 (Yassa et al., 2010a,b; Bakker et al., 2015). Animal models of age-related cognitive decline also implicate subregion-specific changes in hippocampal morphology and function (Wilson et al., 2006). For example, comparisons of CA3 pyramidal cell activity in aged and young animals indicate that aged cells have increased firing rates which appear to result from a loss of inhibitory control over neurotransmission in this subregion and have been hypothesized to contribute to age-related impairments in spatial information processing (Shetty and Turner, 1998; Cadacio et al., 2003; Stanley and Shetty, 2004; Wilson et al., 2005; Spiegel et al., 2013).

Hippocampal pyramidal cells establish specific firing patterns when an animal explores an environment (O'Keefe and Dostrovsky, 1971). The activity of place cells and the characteristics of their respective place fields have been associated with learning and memory function. While the basic properties of aged hippocampal place cells are similar to those in young adults (Barnes et al., 1983), functional differences become evident when animals are challenged with the need to process new information. For example, when young rats accustomed to exploring a

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[1] **Laboratory of Molecular Neurobiology, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, Massachusetts;** [2] **Department of Psychology, Center for Memory and Brain, Boston University, Boston, Massachusetts**

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*Correspondence to: David H. Farb, Laboratory of Molecular Neurobiology, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA. E-mail: dfarb@bu.edu

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familiar environment are subsequently moved to a novel environment, their CA3 pyramidal cells often remap and exhibit altered and unique place fields. In contrast, CA3 neurons in aged rats often fail to change their firing rates and place fields in response to environmental novelty (Wilson et al., 2005).

Reducing memory deficits associated with neurocognitive aging represents a major challenge of modern drug discovery. One promising lead comes from the observation that coadministration of LEV + VPA at sub-anticonvulsant doses improves learning and memory function in aged rats with spatial memory impairments (Koh et al., 2009). Here, we recorded simultaneously from both CA3 and CA1 neurons of aged and young adult rats to determine how acute coadministration of LEV and sodium VPA modulates place cell activity. Through this approach we plan to gain insight into how drugs might be acting to improve memory function and thus to probe memory mechanisms as well. We report that LEV + VPA attenuates CA3 place cell hyperactivity and improves aspects of place cell responding to environmental novelty by selectively increasing spatial information content, normalizing it to young adult levels. The results show that *in vivo* electrophysiological recordings of drug-induced changes in CA3 place cell activity may be a useful preclinical biomarker to parse out useful effects from adverse side effects during the discovery of therapeutic agents that ameliorate spatial memory deficits associated with aging or other neurocognitive disorders. Such a neural systems based approach to pharmacology may hold significant potential for the identification of therapeutic treatments to ameliorate memory deficits and other disorders in which memory function is implicated (Farb and Ratner, 2014).

MATERIALS AND METHODS

Subjects

Nine male Long-Evans rats (4 young; 6–8 months, and 5 aged; 24–28 months) were used in this study. Two of the young and five of the aged rats were prescreened for spatial learning ability in the Morris water maze at Johns Hopkins University before being shipped to Boston University School of Medicine (BUSM) for *in vivo* electrophysiological experiments. Animals from Johns Hopkins were kept in quarantine for 4 weeks upon arrival at BUSM. The remaining two young rats were obtained from Charles River Laboratories (Wilmington, MA) and were not tested on the water maze. Rats were individually housed in a climate-controlled vivarium maintained on a regular 12 hr/12 hr light/dark cycle in the Laboratory Animal Science Center at BUSM. Rats had *ad libitum* access to water but were mildly food deprived to 85% of their free-feeding weight. Rodent maintenance and research were conducted in strict accordance with the animal care guidelines stated in the NIH Guide for the Care and Use of Laboratory Animals. BUSM is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The Boston University Institutional Animal Care and Use Committee approved all procedures described in this study.

Water Maze Testing

Rats from Johns Hopkins were trained on a spatial navigation task in the Morris water maze using training trials to assess acquisition, and probe trials to assess search strategy in locating a submerged escape platform. Rats received three trials per day for eight consecutive days with a 60 s intertrial interval. The location of the platform remained constant in one quadrant of the maze, and the starting position for each trial was varied among four equally spaced positions around the perimeter of the maze. Every sixth trial was a probe trial during which the platform was retracted and unavailable for escape for the first 30 s of the trial; after this time it was raised and made available for escape. The probe trials assessed if, in searching for the escape platform, a rat developed a spatial bias. To test visual acuity and swimming ability, independent of the ability to process spatial information, each rat was given six cued training trials on the day after completion of the training trials. During these trials the submerged platform was replaced with a visible platform 2 cm above the surface of the water, and the location of the escape platform was varied randomly among the quadrants of the pool from trial to trial. Each rat was allowed 30 s to reach the platform and to remain there briefly before being returned to a holding cage for 5 s before the next trial. The primary measure obtained, referred to as the learning index, is derived from the probe trials that were interpolated after each set of five training trials. The learning index was computed as the average proximity of the rat (in cm) to the target platform location on probe trials two through four. Low index values represent more accurate search patterns acquired more rapidly during learning, while high index values indicate an inaccurate search strategy (Gallagher et al., 1993). Performance on the water maze separates the group of aged rats into two subsets: aged-unimpaired and aged-impaired. Aged-unimpaired rats learning index is within the range of young adult rats, whereas aged-impaired rats perform outside the range of both young adult rats and aged-unimpaired animals. However, due to the lengthy quarantine period when the rats arrived at BUSM, the cognitive status of the aged rats could have changed. For instance, aged-unimpaired rats may have become spatial memory impaired during the quarantine period. Therefore, we limited all subsequent analyses to comparisons of young adult versus aged rats without further stratifying the aged rats by cognitive status. This is consistent with the analysis methods used previously in studies within the same model of aging (Tanila et al., 1997; Wilson et al., 2004, 2005).

Surgery

Rats were anesthetized with isoflurane (3.5% for induction; 1.5–2% thereafter) in 100% oxygen delivered via a calibrated vaporizer (Vaporizer Sales and Services, Rockmart, GA). Glycopyrrolate (0.02 mg kg^{-1}, subcutaneous; s.c.) was administered to reduce salivary and bronchial secretions and prevent vagal bradycardia. Buprenorphine (0.05 mg kg^{-1}, s.c.) was also

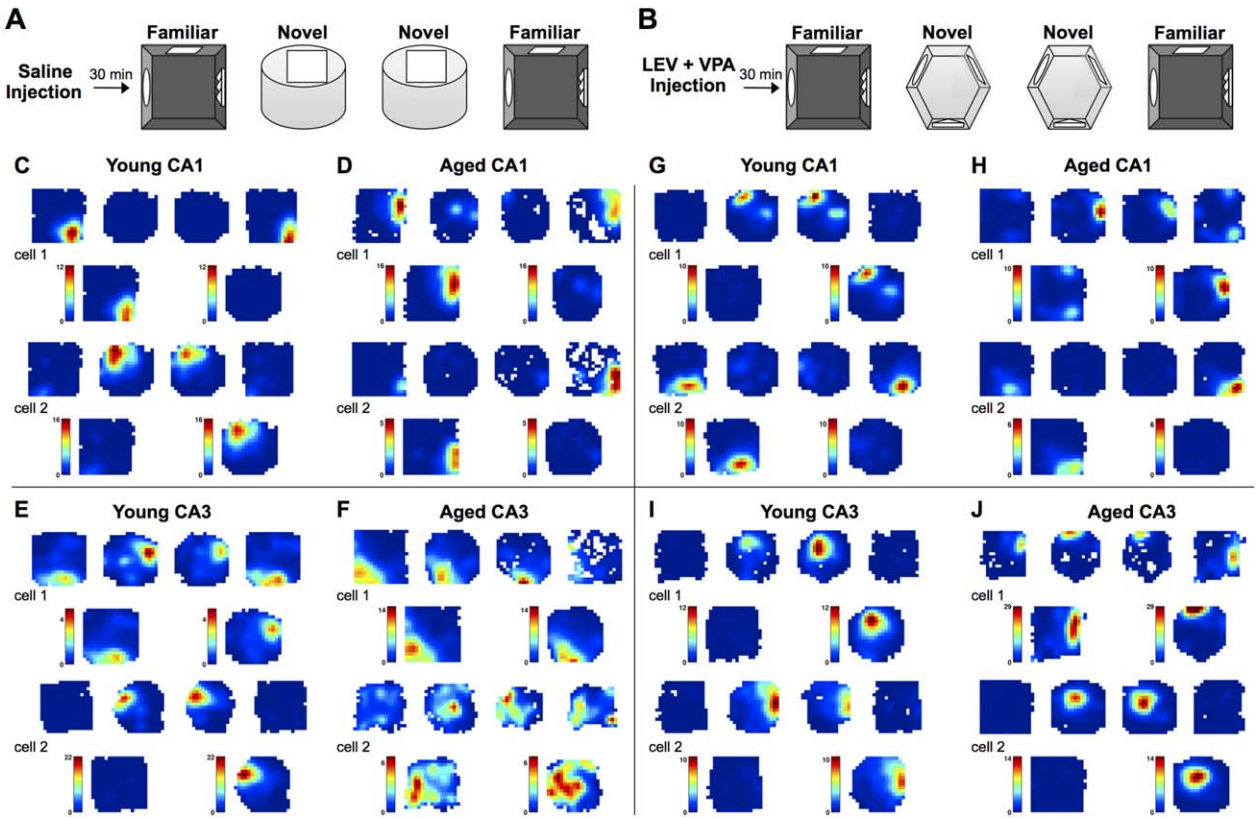

FIGURE 1. Representative place fields recorded from CA1 and CA3 hippocampal neurons in young and aged rats. Recording sequence of environments in Experiment 1, after saline vehicle (A) and in Experiment 2, after LEV + VPA (B) administration. Pseudocolor plots represent peak firing rate in spikes/sec, normalized to the cell's peak firing rate across the entire recording session (C–J). Top four place field maps in C–J correspond to each environmental exposure, whereas the two place field maps below are the merged familiar (square) and novel (circle or hexagon) maps, respectively.

administered for analgesia. Animals' heads were shaved, and the rats placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). A midline incision was made, the skull exposed, and bregma and lambda were made level. A small hole ($\sim$2 $\times$ 2 mm^2 diameter) was drilled into the cranium of the right hemisphere over dorsal hippocampus, 3.6 mm AP and 2.6 mm ML from bregma. The dura mater was removed and the electrode array was positioned onto the surface of the neocortex; the opening was sealed using Kwik-Sil silicone adhesive (World Precision Instruments, Shanghai, China). Six to eight additional holes were drilled for the placement of skull screws, two of which were intended for electrical grounds and the remaining four to six for securing the headstage to the skull, which was achieved in combination with dental acrylic.

Custom built microarrays containing 24 tetrodes were constructed. Each tetrode was comprised of four nichrome wires (12.5 μm diameter; California Fine Wire, Grover Beach, CA). Tetrode tips were cut and their ends were gold plated to lower impedance to 200 kΩ at 1 kHz. At the end of surgery, each tetrode was advanced $\sim$850 μm into the cortex. Post-operative analgesia was maintained with buprenorphine (0.05 mg kg^{-1}, s.c.) and carprofen (2.5 mg kg^{-1}, s.c.). Cephalexin (60 mg kg^{-1}, per oral) was administered for 7 days post-operatively to attenuate risk for infection.

Behavioral Training and Testing

To assess the effects of exposure to a novel environment, rats were familiarized to a black plywood box (60 cm $\times$ 60 cm) with three distinct two-dimensional white paper cues attached to the walls daily for 2 weeks prior to testing. Chocolate sprinkles or crushed Kellogg's Froot Loops™ were randomly distributed over the floor of the recording chamber to encourage continuous ambulation.

During testing, the animal explored each environment in the following order: Familiar–Novel–Novel–Familiar (Fig. 1). Each experimental trial consisted of four sessions, each of ten minutes duration, separated by $\sim$3 min pauses during which the rat was placed in a holding container (30 cm $\times$ 30 cm $\times$ 45 cm) while the base was wiped with 30% ethanol to remove olfactory cues and environments were alternated. Each environment was placed on the same black wooden base. The holding container was spun to mildly disorient the animals prior to re-entry (Tanila et al., 1997;Wilson et al., 2005). Rats had the opportunity to drink water between environmental exposures immediately prior to being returned to a given environment.

The novel environment in Experiment 1 (saline vehicle condition) was a grey aluminum cylinder (60 cm $\times$ 60 cm) with a single white cue card extending the height of the apparatus and

occupying 90° of arc. In Experiment 2 (LEV + VPA condition), the novel environment was a black plywood hexagon (60 cm × 60 cm), which had three white two-dimensional cues attached to the interior walls. Experiments were separated by 48 h.

Drug Preparation and Injections

For 5 days prior to the start of Experiment 1, each rat was given both an i.p. and s.c. injections of saline before daily screening to allow acclimation to the injection procedure. In Experiment 1, rats were given both i.p. and s.c. injections of sterile 0.9% saline (vehicle), respectively, at a volume equivalent to what they would be given when receiving LEV + VPA in Experiment 2. In Experiment 2, 2.5 mg kg^{-1} LEV (i.p.; Tecoland, Edison, NJ) and 50 mg kg^{-1} VPA (s.c.; Sigma–Aldrich, Natick, MA) were separately dissolved in vehicle and co-administered. Rats received injections 30 min prior to the start of a recording session. Fresh solutions of LEV + VPA were prepared daily. We used this low-dose combination of LEV+VPA, as the sub-anticonvulsant combination of these two antiepileptics has shown efficacy in reducing errors that aged-impaired rats make on the radial arm maze (Koh et al., 2009). Thus, we chose to examine the functional significance of this particular combination on place cell function to elucidate how the associated changes we measure in neuronal activity may mediate the behavioral improvement established in previous work.

Data Acquisition and Spike Sorting

All recordings used a Multi-Channel Acquisition Processor connected to a PC running RASPUTIN 2.6 (Plexon, Dallas, TX). Rats were connected to the pre-amplifiers with lightweight cables routed through a commutator (Plexon, Dallas, TX) to allow tangle-free movement throughout the environment.

Following a 5-day post-operative recovery period, tetrodes were gradually advanced toward the CA1 and CA3 pyramidal cell layers (~100–300 μm day^{-1}). To localize CA1 and CA3, accumulated distance of tetrode advancement, progressive increase in theta amplitude, and appearance of sharp-wave ripple events, theta-modulated spiking, and complex-cell spiking were used (Fox and Ranck, 1981; Buzsáki et al., 1983). If pyramidal cell activity was not identified on a tetrode during daily screening, it was advanced and allowed to settle for at least 1 h before further advancement. This process was repeated until tetrodes were positioned simultaneously in either the CA1 or CA3 pyramidal cell layers of the hippocampus.

Tetrode recordings were referenced to a common skull screw for local field potentials (LFPs), or an indifferent tetrode in the corpus callosum (for spikes), and differentially filtered for single unit activity (154 Hz–8.8 kHz) and LFPs (0.77–400 Hz). Spike signals were amplified 2,000–7,000× and digitized at 40 kHz, while LFP signals were amplified 1,000× and digitized at 1 kHz. Two LEDs on the headstage tracked the rat's position (VLSI 32; 20× gain; Plexon, Dallas, TX), which sampled x–y location in the environment at a rate of 30 frames/s (Cineplex, Plexon, Dallas, TX). Action potentials from single neurons were isolated using Offline Sorter 2.8.8 (Plexon, Dallas, TX). Conventional methods were used to identify putative pyramidal neurons and distinguish them from interneurons based on firing rates and waveform widths (Csicsvari et al., 1999). Waveform features such as valley amplitude, energy, peak-valley, principal components, and timestamps were extracted from the four wires comprising a single tetrode and then visualized to isolate activity from individual cells. In an effort to increase the probability that the selected units were isolated and not contaminated with spikes from other recorded units, the interspike interval histograms of each of these units was evaluated to ensure that there were no spikes within a 2 msec refractory period.

Spatial Firing Analysis

We focused our analyses on simultaneously recorded pyramidal neurons of the CA3 and CA1 subregions (tetrodes located in CA2 were included in CA1; see Martig and Mizumori, 2011). Only cells with clear amplitude differences between the four wires of a tetrode, complex spikes, and negative spike duration of more than 300 μs were included for further analyses using a series of custom MATLAB scripts (Mathworks, Natick, MA). Occupancy-normalized spatial firing rate maps were estimated using the total number of spikes that occurred at a given location divided by the total amount of time that was spent in the bin (3.5 cm × 3.5 cm), with distinct firing rate maps calculated for the familiar (F1 + F2) and novel (N1 + N2) environments. For a given bin to be included as valid, it had to be visited at least once during the session, and for at least 150 ms. The smoothed value for each bin was calculated as the mean for each bin, and all bins within 5 cm, where each bin is weighted by its distance from the central bin using a two-dimensional Gaussian kernel (Komorowski et al., 2009). Place fields were defined as six adjacent bins with firing rates >0.25 Hz, and place cells were defined as those pyramidal cells meeting the following criteria in one of the two environments: A place field with ≥100 spikes, a mean rate ≥0.1 Hz and peak rate ≥0.5 Hz. Data was only included for epochs where running speed was >2.0 cm s^{-1}.

To evaluate the effects of environment, age, and treatment on place cell characteristics we applied measures that assessed the discharge frequency and spatial selectivity of place cell firing. In addition to calculating mean and peak firing rates, the ratios of place cells that increased versus those that decreased their mean firing rates in response to novelty was also determined to further define how these cells responded to novelty under control (vehicle) and drug conditions.

To assess for the spatial selectivity and stability of place cell firing, across environments under the two treatment conditions we calculated the (1) place field area, (2) spatial information content, (3) and spatial correlations, for each place cell.

Place field area is defined as the sum of all bins within each environment in which the firing rate of the cell is ≥20% of the cell's peak firing rate (Hollup et al., 2001). In the case of multiple fields, field area is calculated as the sum of all bins meeting the inclusion criteria within each subregion. Place field area for the

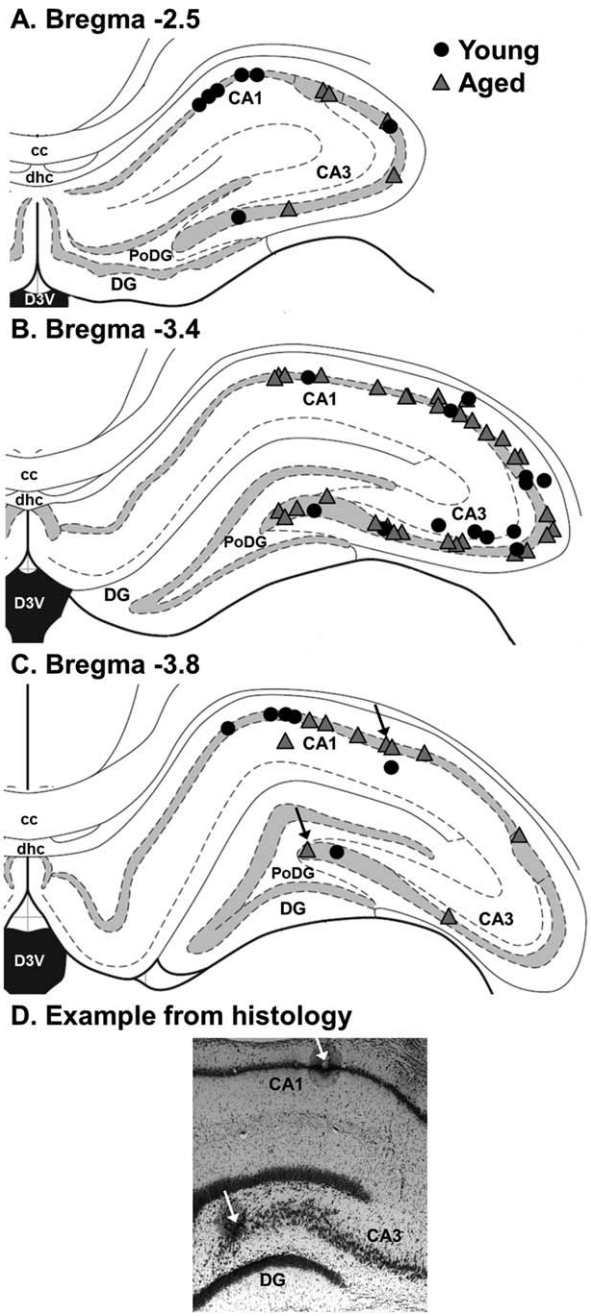

FIGURE 2. The anatomical location of tetrode recording terminals as identified by histological examination. Tetrode locations for young adult (circles) and aged (triangles) rats in hippocampal subregions CA3 and CA1 at 2.5 mm (A), 3.4 mm (B), and 3.8 mm (C) posterior to Bregma, using the atlas of Paxinos and Watson (2009). (D) Representative photomicrograph (×4) of a representative Nissl-stained section showing 2 tetrode locations in CA3 and CA1 (Arrows in C indicate sites in an aged rat from the section shown in D).

novel environments is calculated from transformed rate maps to facilitate comparison with the square enclosure that served as the familiar environment. Place field maps of the novel environments are transformed to match the shape of the familiar environment using the established method of Lever et al., (2002).

Spatial information content (Skaggs et al., 1993) provides a measure of how much information about the animal's position is conveyed by a single action potential measured in a place cell, expressed in bits/spike:

$$\textit{Information per spike} = \sum_{i=1}^{n} P_i \left(\frac{\lambda_i}{\lambda}\right) \log_2 \left(\frac{\lambda_i}{\lambda}\right)$$

where $i = 1,\ldots, n$ is the bin number, P_i is the probability of occupancy at bin i, λ_i is the mean firing rate for bin i, and λ is the overall mean firing rate of the cell. To reduce the degree that information content reflected behavioral inconsistencies in the number of visits to a specific location in the environment, P_i, is made into a uniform probability distribution over all the bins that are visited. That is, for every i, $P = 1/n$, where n is the total number of bins visited (Jung et al., 1994).

Spatial correlation was evaluated using a bin-to-bin Pearson correlation between the merged (F1 + F2; N1 + N2), smoothed, and transformed rate maps. Merged data were used in calculating the correlations to ensure that maximum area of environmental coverage would be represented in the firing rate maps. For statistical analyses, the correlation coefficients were subjected to Fisher's r-to-z transform prior to parametric tests:

$$z = \frac{1}{2} \ln \left(\frac{1-r}{1+r}\right)$$

where *ln* is the natural logarithm, and r is the sample correlation (Fisher, 1915). Means and standard errors were calculated from the z-transformed values, and then back-transformed for graphical presentation.

Histology

At the end of the study, rats were deeply anesthetized with 100 mg kg^{-1} (i.p.) pentobarbital and the recording electrode locations marked by passing anodal current (30 μA, 5 s) through the tetrodes. The animals were then transcardially perfused with 250 ml 0.9% saline, followed by 250 ml neutral buffered 10% formalin containing 10 ml glacial acetic acid and 10 g potassium ferrocyanide. Brains were extracted, cryoprotected in 20% then 30% sucrose solutions, and sectioned (50 μm) on a cryostat. Locations of the tetrode tips were confirmed by Prussian blue reaction as described (Tanila et al., 1997; Fig. 2). All brains were inspected for and found to be devoid of lesions and tumors.

Statistical Design

Full factorial repeated measures 2 × 2 × 2 ANOVAs were performed to assess for main effects and interactions between environment, age and treatment, with environment (familiar vs. novel) as the within-subjects factor, age (young vs. aged) and treatment (vehicle vs. LEV + VPA) as between-subjects factors, with separate comparisons performed for each subregion (CA1 vs. CA3). To further examine main effects and higher order interactions, standard ANOVA and two-tailed t tests were applied where applicable with the significance level set to

TABLE 1.

Number of CA3 and CA1 Place Cells From Young and Aged Rats Treated With Saline or LEV + VPA

Age	Number	Subregion	Drug treatment	
			Saline	LEV + VPA
Young	191	CA3	64	50
		CA1	50	27
Aged	336	CA3	121	132
		CA1	38	45
Total	527		273	254

Unit activity was recorded from the CA3 and CA1 hippocampal subregions following acute systemic administration of saline or LEV + VPA.

$P < 0.05$. *Post hoc* comparisons using repeated-measures or univariate ANOVA were performed to compare simultaneously recorded CA3 and CA1 activity separately within each age group and treatment for most measures described. For comparisons of proportions, a two-tailed Chi Squared test was used. Statistics were performed using JMP Pro 9.0.2 (SAS Institute, Cary, NC).

RESULTS

Morris Water Maze

As reported previously, spatial learning of aged rats was found to be impaired relative to young adult rats on the Morris water maze test (mean learning index ± SEM; young: 179.9 ± 13.6; aged 240.5 ± 13.6; $t_{(6)} = 3.15$; $P = 0.02$).

Identification of Place Cells

Neural data from 273 and 254 cells from Experiments 1 and 2, respectively, met the inclusion criteria for CA1 and CA3 place cells and were included for analyses (Table 1).

Place Cell Mean Firing Rates

Analysis comparing overall place cell mean firing rates across environments following vehicle administration in aged and young rats revealed that the mean firing rates of CA3 place cells increased with aging ($F_{(1,183)} = 12.38$; $P < 0.001$) (Fig. 3a). Analysis of aged CA3 place cells by environment following vehicle administration indicates that these cells discharge with a higher mean frequency in the novel than in the familiar ($t_{(120)} = 2.22$; $P = 0.028$) (Fig. 3a). Between subjects comparison of aged versus young CA3 cells in the novel environment with vehicle showed that the aged cells had higher mean firing rates ($t_{(183)} = 3.55$; $P < 0.001$; Fig. 3a). By contrast, the overall mean firing rates of CA1 cells in both aged and young rats were not influenced by age ($F_{(1,86)} = 3.29$; $P = 0.07$), nor by an interaction of age and environment following vehicle administration ($F_{(1,86)} = 0.57$; $P = 0.45$) (Fig. 3b). Comparison of the mean firing rates in CA3 versus CA1 place cells also did not reveal any subregional differences in overall firing rate values with vehicle ($F_{(1,112)} = 1.74$; $P = 0.19$).

Analysis of mean firing rates of CA3 place cells revealed an interaction between the factors for age, environment, and treatment ($F_{(1,363)} = 3.91$; $P = 0.048$). The elevated overall mean firing rates seen in aged CA3 cells were significantly reduced by treatment with LEV + VPA ($F_{(1,251)} = 4.16$; $P = 0.04$; Fig. 3a). Further comparison of the data stratified by environment and treatment revealed that this reduction was only significant when the aged rats were in the novel environment ($t_{(251)} = 2.93$; $P = 0.003$; Fig. 3a). Furthermore, LEV + VPA also effectively

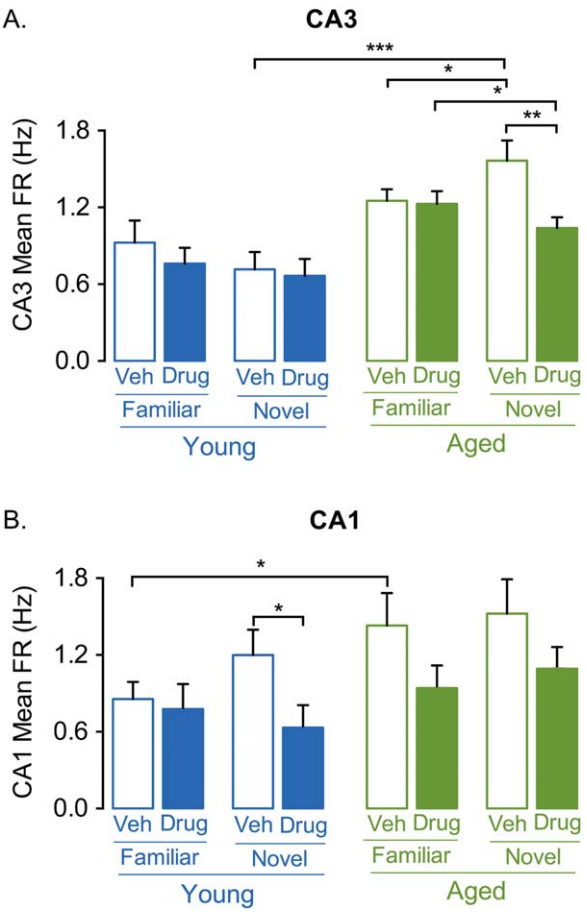

FIGURE 3. Mean firing rate of simultaneously recorded CA3 and CA1 place cells. Acutely administered LEV + VPA reduces the mean firing rate of aged CA3 place cells in novel but not familiar environments. A. Within subjects analysis shows that under vehicle control conditions the mean firing rate of aged CA3 cells was significantly higher in the novel environment. Between subjects comparisons revealed that the mean firing rate of aged CA3 cells in the novel environment was also significantly higher than that of young CA3 cells. LEV + VPA administration reduced the novelty-related increase in aged CA3 mean firing rates that occurred following vehicle injection by 34%, without affecting firing frequencies in the familiar environment. B. There is no significant difference in the overall mean firing rates of CA1 place cells across age or environments with vehicle. There was an interaction between the factors for age, environment, and treatment. Acute administration of LEV + VPA significantly reduced young CA1 mean firing rates in the novel environment. Significance is indicated by: * where $P < 0.05$; ** where $P < 0.005$; and * where $P < 0.001$.**

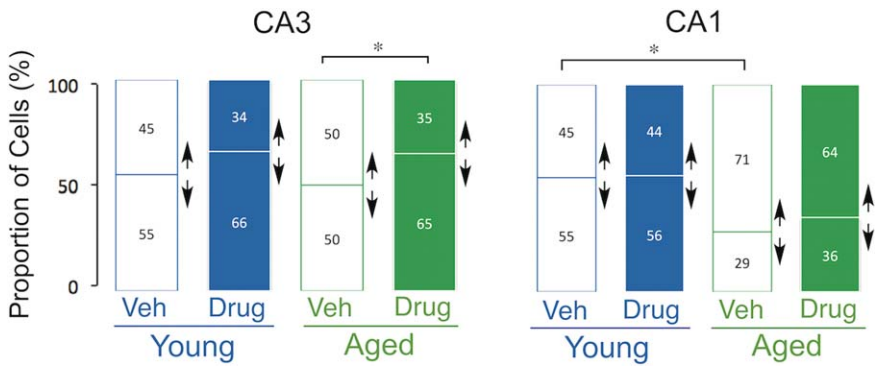

FIGURE 4. Proportion of simultaneously recorded CA3 and CA1 place cells increasing or decreasing firing rates in response to novelty. The proportion of young and aged CA3 cells with increased or decreased responding to novelty with vehicle is similar. Following LEV + VPA administration there was a significant increase in the proportion of aged CA3 cells that showed a decrease in responding to novelty. There was also a significant difference in the proportion of young and aged CA1 cells with increased or decreased responding to novelty with vehicle, with more aged cells showing an increase in responding to novelty, but this response was not modified by treatment with LEV + VPA. Significance is indicated by: * where $P < 0.05$.

changed the response to environmental novelty in aged CA3 cells such that upon introduction to the novel environment, aged CA3 cells decreased (rather than increased) their mean firing rates ($t_{(131)} = -2.36$; $P = 0.02$). Although LEV + VPA attenuated the response to novelty in aged CA3 cells, the mean discharge firing rate of these cells still remained elevated compared to that of young CA3 cells ($F_{(1,180)} = 8.09$; $P = 0.005$) in the familiar ($t_{(180)} = 2.62$; $P = 0.009$) and novel ($t_{(180)} = 2.32$; $P = 0.02$) environments respectively. In contrast to the aged CA3 cells, LEV + VPA had no significant effect on the mean firing rates of young CA3 cells.

Analysis of mean firing rates of CA1 place cells revealed an interaction between the factors for age, environment, and treatment ($F_{(1,156)} = 5.06$; $P = 0.03$) (Fig. 3b). Administration of LEV + VPA significantly reduced the mean firing rates young CA1 cells in the novel environment.

These results are consistent with our a priori hypothesis that acute systemic administration of a behaviorally efficacious dose of LEV + VPA would attenuate age-related increases in CA3 place cell firing rates (Koh et al., 2009). LEV + VPA also attenuated the novelty-induced increase in mean firing rate seen in aged CA3 cells [vehicle], without having any measurable effect on firing rates in the familiar environment suggesting that the drug may be affecting encoding of new information.

Ratios of Cells With Increased:Decreased Mean Firing Rates

To further characterize the response to novelty of the CA1 and CA3 populations we determined the ratios of cells that increased or decreased their firing rates upon exposure to the novel environment. With vehicle, the ratio (increased:decreased) was ~1:1 in CA3 of both age groups (young = 29 increased: 35 decreased; aged = 61 increased: 60 decreased; $\chi^2_{(1)} = 0.31$; $P = 0.58$; Fig. 4). In other words, a similar proportion of CA3 cells increased and decreased their firing rates upon introduction to a novel environment, regardless of age. In CA1, the ratio was ~1:1 in young cells and was 2.5:1 for aged CA1 cells, but this difference was not significant (young = 27 increased: 23 decreased; aged = 27 increased: 11 decreased; $\chi^2_{(1)} = 2.65$; $P = 0.10$).

Under LEV+VPA, the increased:decreased ratio for all cells in CA3 was 0.5:1, reflecting a larger number of cells decreasing their firing rate in response to a new environment as compared to vehicle (Saline = 89 increased: 96 decreased vs. LEV + VPA = 63 increased: 119 decreased; $\chi^2_{(1)} = 6.9$; $P = 0.009$) (Fig. 4). Although there was a similar shift present when comparing all CA1 cells, the ratio change was not significantly different from vehicle (Saline = 54 increase: 34 decrease vs. LEV + VPA = 41 decrease: 31 decrease; 1:1.3 ratio; $\chi^2_{(1)} = 0.32$; $P = 0.57$). No age-related differences in the ratios in either CA3 or CA1 were induced by acute administration of LEV + VPA. However, within-group comparisons revealed that LEV + VPA administration significantly altered the response of aged CA3 cells to environmental novelty as compared to vehicle (Saline = 60 increase: 61 decrease vs. LEV + VPA = 46 increase: 86 decrease; 0.5:1 ratio; $\chi^2_{(1)} = 5.63$; $p = 0.02$). Although a similar shift in the proportion of cells increasing:decreasing responding was seen in young CA3 cells this did not reach significance (Saline = 29 increase: 35 decrease vs. LEV + VPA = 17 increase: 33 decrease; 0.5:1 ratio; $\chi^2_{(1)} = 1.5$; $P = 0.25$). This indicates that within the aged rats, a greater number of cells now responded to the novel environment with a decrease, as opposed to an increase in firing rate following treatment. There was no effect of LEV + VPA treatment on the ratio for CA1 cells relative to vehicle for either young ($\chi^2_{(1)} = 0.64$; $P = 0.42$) or aged ($\chi^2_{(1)} = 0.41$; $P = 0.52$) rats.

Place Cell Peak Firing Rates

Analysis of CA3 peak rates by age and environment ($F_{(1,363)} = 5.92$; $P = 0.01$) indicated that aged CA3 cells responded to novelty with increased peak firing rates in both LEV + VPA and vehicle ($t_{(252)} = 3.76$; $P < 0.001$) (Fig. 5). In

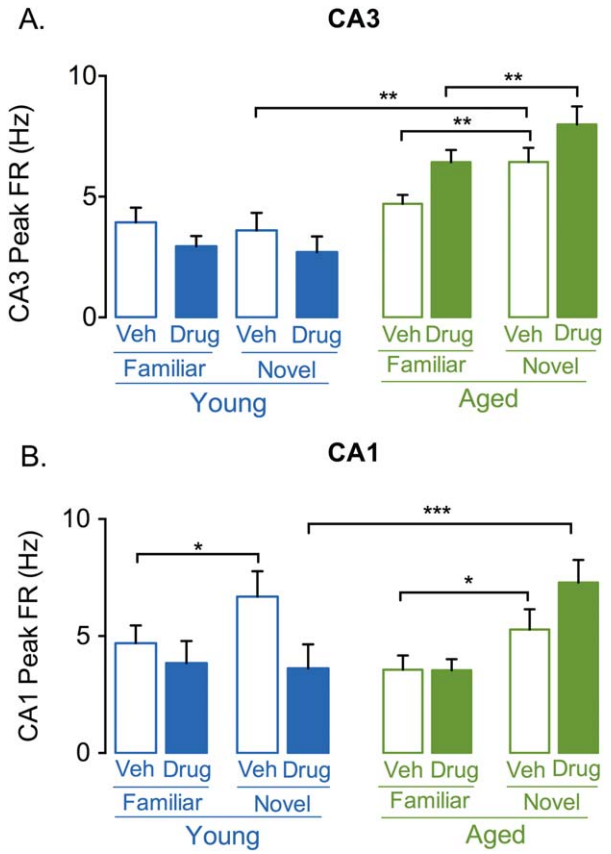

FIGURE 5. Peak firing rate of simultaneously recorded CA3 and CA1 place cells. Acute administration of LEV + VPA increases the peak firing rates of aged CA3 and CA1 place cells. A. CA3 peak firing rate was higher in aged than in young adult rats across environments. Peak firing rate of CA3 place cells increased with aging, and in response to environmental novelty following vehicle administration. B. Peak firing rate of CA1 place cells was enhanced by novelty across age groups after saline vehicle treatment. LEV + VPA increased the peak firing rates of aged CA1 place cells only in response to novelty. Significance is indicated by: * where $P < 0.05$; ** where $P < 0.01$; and * where $P < 0.001$.**

the novel environment aged CA3 peak rates were also higher than in young CA3 with LEV + VPA and vehicle, ($t_{(365)} = 5.29$; $P < 0.001$). Peak rates in CA3 also differed between age groups in response to treatment condition ($F_{(1,363)} = 5.41$; $P = 0.02$). Aged CA3 cells exhibited higher peak firing frequencies than young CA3 cells following vehicle administration ($t_{(183)} = 3.09$; $P = 0.002$) (Fig.5a). LEV + VPA increased the peak firing rates of aged CA3 cells relative to vehicle ($t_{(251)} = 2.75$; $P = 0.006$), but had no effect on young CA3 cells (Fig. 5a). Furthermore, CA3 peak rates were higher in aged rats than in young after LEV + VPA treatment ($t_{(180)} = -4.93$; $P < 0.001$). Thus, LEV + VPA administration increased the pre-existing elevation of CA3 peak rates in aged animals. There were no other significant effects of LEV + VPA on CA3 peak firing rates.

CA1 peak rates varied with the interaction between age, environment, and treatment ($F_{(1,156)} = 4.27$; $P = 0.0451$; Fig. 5b). The overall (F + N) peak firing rates of CA1 cells were similar in both aged and young rats with vehicle and were enhanced by novelty ($F_{(1,86)} = 6.76$; $P = 0.01$). LEV + VPA treatment abolished the novelty-induced increase in young CA1 peak firing rates. The peak firing rate of aged CA1 place cells was not significantly altered by treatment with LEV + VPA. However, CA1 peak rates in aged rats were significantly increased relative to young rats in the novel environment after administration of LEV + VPA ($t_{(70)} = 4.11$; $P < 0.01$).

Subregional comparisons of peak firing rates revealed that young CA3 cells were less responsive to novelty than young CA1 cells following vehicle administration ($F_{(1,112)} = 6.76$; $P = 0.01$). This difference in subregional response to novelty was not observed in the aged rats. There were no other significant subregional differences in either treatment group.

Spatial Information Content

Differences in the spatial selectivity of place cell firing were examined by calculating the spatial information content, a measure that reflects how well the action potentials of a cell predict the rat's location (Skaggs et al., 1993). Consistent with previous reports, there were no significant subregional differences in the spatial information content per spike in CA3 versus CA1 place cells as a function of age or environment, nor was there a main effect of treatment across subregions (Tanila et al., 1997; Wilson et al., 2004). However, there was a significant within subregion interaction between age and treatment in both CA3 ($F_{(1,363)} = 10.8$; $P = 0.001$) and CA1 ($F_{(1,156)} = 5.5$; $P = 0.02$) (Fig. 6). Acute treatment with LEV + VPA had no effect on the spatial information content per spike of young CA3 cells as compared with vehicle. However, consistent with our a priori hypothesis, LEV + VPA did enhance the spatial information content per spike in aged CA3 place cells as compared with vehicle ($t_{(251)} = 2.98$; $P = 0.003$). In addition, acute administration of LEV + VPA was associated with an increase

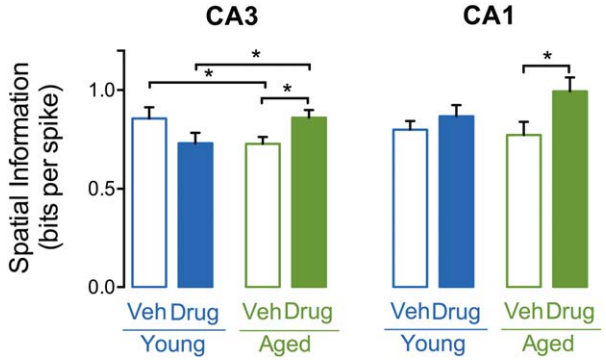

FIGURE 6. Spatial information content of spikes from simultaneously recorded CA3 and CA1 place cells. LEV + VPA increases the spatial information content of CA3 and CA1 place cell firing. Left: LEV + VPA increased spatial information content of CA3 cells in aged rats, so it is similar to that of young under vehicle. Right: LEV + VPA increased spatial information content of aged CA1 cells. [Data shown are averaged familiar and novel spatial selectivity values as there was no effect of environment on spatial selectivity.] Significance is indicated by: * where $P < 0.05$.

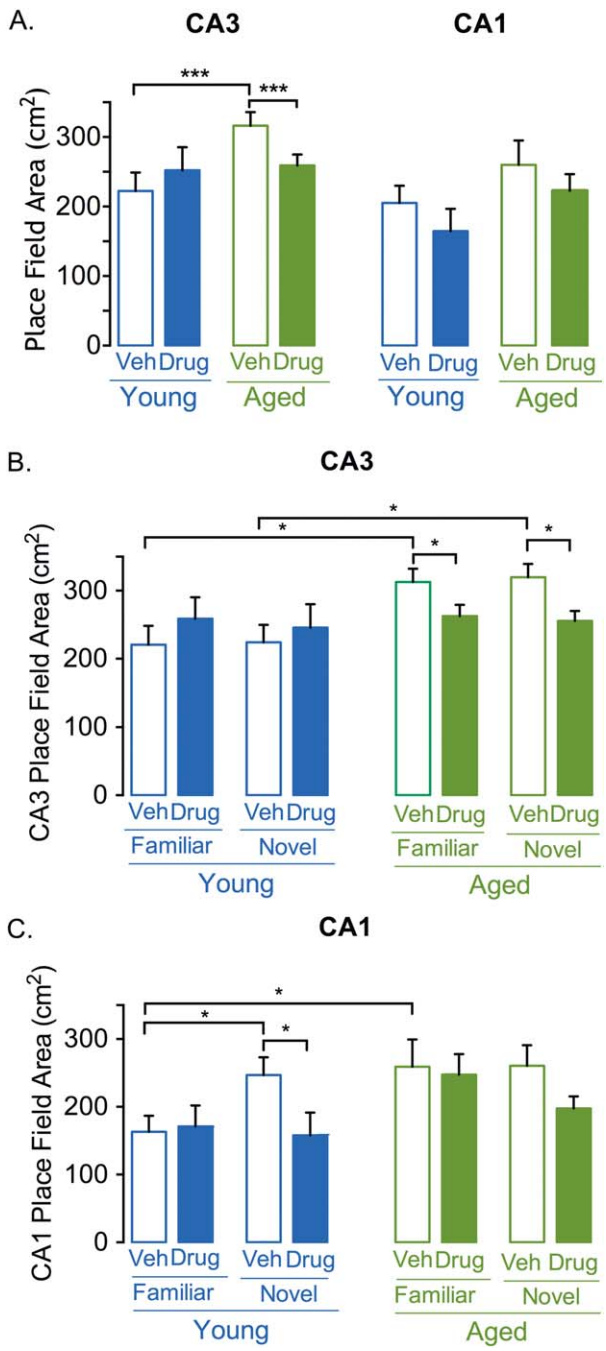

FIGURE 7. Place field area of simultaneously recorded CA3 and CA1 place cells. LEV + VPA reduces place field area. A. Effect of LEV + VPA on overall place field area by age and drug. B. Age effects on place field area by subregion showing that CA3 place field are larger in aged rats with vehicle and that this increase is abolished by LEV + VPA. C. Effects of environment and age on place field area showing that aged CA1 place fields are significantly larger than young place fields and that the area of young place fields increases in response to novelty whereas aged place fields do not. There were no significant age or drug-induced changes in the areas of CA3 place fields. Significance is indicated by: * where $P < 0.05$; and ** where $P < 0.005$.

in the spatial information content per spike in aged CA3 place cell as compared with young CA3 cells ($t_{(112)} = -2.19$; $P = 0.03$). Administration of LEV + VPA also increased spatial information content per spike in aged CA1 place cells as compared to vehicle ($t_{(81)} = 2.90$; $P = 0.005$). These data indicate that LEV + VPA significantly increases spatial information content of aged place cell firing and that this difference may be relevant to understanding the cognitive enhancing effects of LEV + VPA.

Place Field Area

Comparisons for subregional differences in overall place field area (F + N) with LEV + VPA or vehicle revealed no significant differences in CA1 place fields in either age group following vehicle administration. However, CA3 place fields of aged rats were significantly larger overall than young with vehicle ($F_{(1,75)} = 6.42$; $P = 0.01$) (see Fig. 7a). Following acute administration of LEV+VPA, place field area across environments was unchanged in CA1 in both age groups. In contrast, LEV + VPA significantly reduced the place field areas of aged CA3 cells relative to vehicle ($t_{(251)} = -2.62$; $P = 0.009$) to the point where these place fields were now equivalent in size in the young and aged CA3 place cells ($t_{(180)} = 0.25$; $P = 0.80$).

Comparisons for the effects of environment versus drug on place field area revealed that with vehicle aged CA1 place fields were significantly ($t_{(183)} = 3.29$; $P = 0.0012$) larger in the familiar environment than young (Fig. 7c). In addition, with vehicle, environmental novelty increased the place field area of young, but not aged CA1 cells ($t_{(49)} = 2.39$; $P = 0.021$); this response to novelty was not only abolished by LEV + VPA but the place field area was significantly smaller as compared to the vehicle control condition. There was no effect of LEV + VPA in CA3.

These data suggest that LEV + VPA administration alters the novelty-induced response of young CA1 place cells but has no significant effect on aged CA3 place cells under these same conditions.

Spatial Correlations of Place Fields in Familiar and Novel Environments

Spatial representations were compared by calculating the correlations between place field firing rate maps of the familiar and novel environments. The results of these analyses suggest that the effects of LEV + VPA on place field correlations are independent of spatial information content. With vehicle, spatial representations of the familiar and novel environments were more highly correlated in aged CA3 cells than in young ($t_{(183)} = 2.23$; $P = 0.027$; Fig. 8). There was no significant difference in the spatial correlations of aged versus young CA1 place fields which is consistent with this region receiving direct inputs from the entorhinal cortex and thus, not being entirely dependent on inputs from CA3 (Brun et al., 2002; Leutgeb et al., 2004). LEV + VPA abolished the age-related difference in CA3 place field similarity. In particular, the drug combination increased the similarity of CA3 place fields between

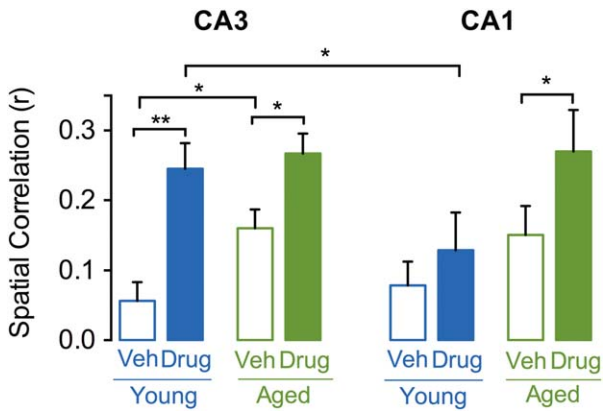

FIGURE 8. Place field correlations of simultaneously recorded CA3 and CA1 place cells. Aging increases the correlations between CA3 place fields in the familiar and novel environments. LEV + VPA administration enhances CA3 place field correlations in both young and aged rats, but appears to not significantly affect young CA1 place field correlations. Significance is indicated by: * where $P < 0.05$; ** where $P < 0.001$.

familiar and novel environments above the levels observed following vehicle administration in both young ($t_{(112)} = 4.38$; $P < 0.001$) and aged rats ($t_{(251)} = 2.06$; $P = 0.04$, Fig. 8).

In comparing the spatial correlation between subfields, after vehicle administration there was no difference between CA3 and CA1 in the spatial correlations of place fields across environments in young or aged rats. After LEV + VPA treatment, the spatial correlation in young CA3 was significantly higher than in young CA1 ($F_{(1,75)} = 4.72$; $P = 0.03$); Fig. 8), while there was no such difference in the aged rats, suggesting that LEV + VPA had a similar effect on spatial correlations in these animals. These findings suggest that the effects of LEV + VPA on spatial correlations are mediated primarily via actions on CA3 and, that this pharmacologic effect differentially influences aged and young CA1 place cells.

DISCUSSION

Hippocampal place cells in the CA3 subregion form distinct spatial maps for distinct environments (Alme et al., 2014) but with aging place cells become hyperactive and their corresponding place fields more rigid (Wilson et al., 2004; Wilson et al., 2005). However, it is unknown whether therapeutic agents that enhance performance in behavioral tests of spatial memory act via an acute modulation of place cell activity. To probe spatial memory mechanisms and to gain insight into how therapeutic agents might act as cognitive enhancers we asked whether LEV + VPA might selectively alter place cell mean and peak firing rates, place field area, spatial information and/or spatial correlations in young adult and aged subjects.

A major challenge of modern drug discovery in neuroscience is to parse out the therapeutic effects from side effects using objective measures, on a systems level, in an attempt to elucidate the mechanistic underpinnings of cognitive disorders subject to modulation and hopefully translate the findings from rodents to human. A major advantage of *in vivo* electrophysiology using high-density chronically implanted electrodes over measuring regional cerebral blood flow, for example, is found in the ability of this technology to quantitatively monitor the fundamental unitary element of rapid signaling, the action potential, of projection neurons as compared with interneurons from within and across brain subregions.

Mean and Peak Firing Rates of Aged vs Young CA3 and CA1 Place Cells in Familiar vs Novel Environments: Effects of LEV + VPA

Environmental novelty increases activity of CA3 and CA1 place cells

We find that environmental novelty is associated with an increase in mean and peak firing rates of aged CA3 but not CA1 place cells as recorded in parallel within each subject. This is consistent with a meta-analysis that examined published data from various laboratories suggesting that aged CA3 place cells exhibit hyperactivity in a novel environment (Wilson et al., 2005). When considered together, the data demonstrate that differences of subregions in responding to environmental novelty may underlie age-related deficits in the acquisition and encoding of novel spatial information in aged rats.

Aged rats encode differences between environments more slowly than young rats and the place fields of aged rats are not firmly tied to external cues. Changes in the effectiveness with which spatial representations are updated on the basis of external cues may contribute to age-related spatial learning deficits (Rosenzweig et al., 2003; Wilson et al., 2004). The vehicle control data reported here are consistent with these observations.

Effects of LEV + VPA on the firing rates of CA3 and CA1 place cells

This study was designed a priori to ascertain the real-time pharmacological effects of LEV + VPA on hippocampal pyramidal cell activity in aged versus young rats. The results demonstrate that acute systemic administration of LEV + VPA attenuates age-related CA3 place cell hyperactivity.

The dependent variable of interest (neuronal activity) was subject to potential modification by administration of the drug. Therefore, in an effort to avoid any drug-induced changes in basal activity that might persist following the initial administration of drug we did not counterbalance the timing of drug administration. Because we did not perform a dose response curve there was no need to counterbalance subsequent doses of drug (as would be required to control for a possible effect of one dose on another). We elected not to change the novel environments associated with drug versus vehicle administration so that drug was the only independent variable changed across trials. A repeated measures ANOVA was used for analysis to assess for effects of repeated exposures to the same environment. Another limitation of this study is that one

novel environment may have had a greater effect on neural activity than the other. However, since the order of drug administration and novel environment exposure was the same across ages and cell types (CA1 vs. CA3) the changes in neural activity reported herein are related entirely to administration of LEV + VPA.

Despite the aforementioned limitations of this study, the data are nevertheless consistent with previous reports comparing aged to young place cells in rats and with those in humans with mild cognitive impairment (Koh et al., 2009; Bakker et al., 2015). These findings suggest that LEV + VPA, which has previously been shown to enhance learning and memory performance in aged rats, may be acting in part by allowing novel information to predominate during spatial information encoding.

Spatial Selectivity of Aged vs Young CA3 and CA1 Place Cells in Familiar vs. Novel Environments: Effects of LEV + VPA

LEV + VPA increases the spatial selectivity of aged but not young CA3 and CA1 cells. An increase in spatial selectivity is thought to reflect a reduction in the occurrence of "spontaneous" spikes outside of the place field. A drug-induced increase in the signal to noise ratio in the old rats may account for the increase in spatial information content per spike. Environmental novelty normally increases firing rates of excitatory CA1 pyramidal cells while reducing rates for inhibitory interneurons (Nitz and McNaughton, 2004). Our results did not confirm earlier studies that found no significant difference in the spatial selectivity of place cells in old vs. young rats (Tanila et al., 1997; Wilson et al., 2004).

Spatial Correlations of Aged vs. Young CA3 and CA1 Place Cells in Familiar vs Novel Environments: Effects of LEV + VPA

In CA3, the spatial representations of familiar and novel environments are more similar in aged than young CA3 cells in vehicle as indicated by a higher spatial correlation. Surprisingly, however, LEV + VPA enhances CA3 place field similarity across age groups.

In young animals, LEV + VPA increases CA3 place field correlations. The results can't distinguish between a drug effect that causes novel place fields to more closely resemble the familiar ones or the reverse, in part because novel information can interfere with familiar information, complicating an interpretation of the directionality of drug action. However, it is tempting to speculate that increased inhibition in CA3 by LEV + VPA could allow novel sensory information to predominate and therefore to override the influence of previously stored information, resulting in place fields that are more highly correlated. This could occur if the influence of old information is effectively being ignored (Kremin and Hasselmo, 2007). Interestingly, in human subjects with amnestic mild cognitive impairment, functional imaging studies of regional cerebral blood flow show that levetiracetam normalizes the hyperactivity in the DG/CA3 and entorhinal cortices while increasing performance in pattern separation dependent tasks and reduces errors attributable to an over-riding effect on pattern completion (Bakker et al., 2015).

CA3 place cells receive inputs from the dentate gyrus via mossy fibers, the entorhinal cortex via the perforant path and auto-associative inputs via recurrent collaterals. This circuitry provides the underpinnings for the important role of CA3 units in pattern completion and pattern separation (Yassa and Stark, 2011). If LEV + VPA increases inhibition, novel information may override information stored previously in CA3 during working memory tasks. Such a pharmacological effect could account for the paradoxical increase in place field correlations while spatial selectivity also increases. Some evidence suggests that in principle there is both the time and opportunity for this to happen: (1) selective presynaptic inhibition in CA3 may regulate the amount of overlap between new and previously encoded information (Kremin and Hasselmo, 2007), and (2) CA3 cells take longer than CA1 cells to integrate and encode information about environmental novelty (Leutgeb et al., 2004), and (3) while these results with spatial correlations may at first seem incongruous with the improvement in spatial memory reported by Koh et al. (2009) the place fields of aged rats remap more slowly than those of young, and the effect of a particular drug on place field correlations may be in parallel or tangential to its effects on memory function (Robbe et al., 2009). This spatial selectivity paradox for CA3 will await future studies using LEV-+ VPA and similar pharmacological probes to differentially modulate acquisition versus delayed recall tasks.

In CA1, the network is predominantly feed-forward and does not exhibit this paradoxical behavior in response to LEV-+ VPA. LEV + VPA could be inhibiting or altering sensory input or acquisition, allowing previously stored information to dominate in aged and young rats. An interference with sensory inputs and encoding of novel information via enhanced inhibition could leave stored information intact (Hirshman et al., 2003; Fisher et al., 2006). While CA3 drives CA1 the entorhinal cortex contributes direct projections to CA1 as well (Brun et al., 2002; Leutgeb et al., 2004), leaving enhanced inhibition as a plausible alternative explanation.

Place Field Area of Aged vs. Young CA3 and CA1 Place Cells in Familiar vs. Novel Environments: Effects of LEV + VPA

In aged CA3 with vehicle, aged CA3 cells exhibit enlarged place fields as compared with young CA3 cells, consistent with a previous report based on a metaanalysis of published data (Wilson et al., 2005). Importantly, we find that LEV + VPA administration reduces place field area of aged CA3 cells relative to vehicle, thereby eliminating the age-related difference in place field size regardless of environment. This suggests that LEV + VPA modifies aspects of place field properties that reflect the influence of novel spatial cues.

In aged CA1 cells with vehicle, aged CA1 cells do not remap in response to novelty but young CA1 cells enlarge their place fields in response to novelty. CA1 cell place field area is enlarged for aged cells as compared with young in the familiar

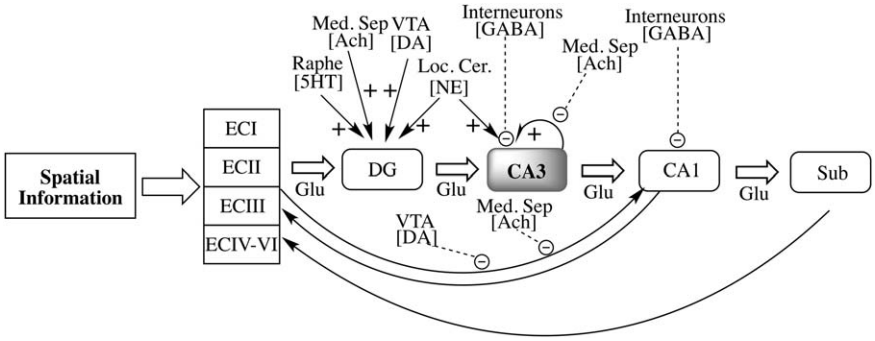

FIGURE 9. Pharmacological modulation of hippocampal circuitry implicated in learning and memory. Cartoon shows interactions between inhibitory and excitatory inputs. Putative targets for treating age related learning and memory impairments include enhancement of GABA inhibitory tone and attenuation of glutamate mediated excitation. A loss of functional inhibitory interneurons has been implicated in CA3 pyramidal cell hyperactivity. Acute administration of LEV + VPA may attenuate the hyperactivity in this region of the hippocampus in part by enhancing GABAergic neurotransmission (Speigel et al., 2013; Wakita et al., 2014). Muscarinic and nicotinic acetylcholine receptors provide for suppression of feedback excitation and enhancement of afferent input respectively (Sava and Markus, 2008). Ach = acetylcholine; DA = dopamine; DG = dentate gyrus; ECI = entorhinal cortex layer I; ECII = entorhinal cortex layer II; ECIII = entorhinal cortex layer III; GABA = Gamma Amino Butyric Acid; Glu = -glutamate; Loc. Cer. = Locus Coeruleus; Med. Sep. = Medial Septum; NE = norepinephrine; Raphe = raphe nucleus; Sub = subiculum; VTA = Ventral Tegmental Area.

environment, suggesting that plasticity of CA1 place cells is reduced in aged rats (Shen et al., 1997).

The response of young CA1 cells to LEV + VPA reveals that drug abolishes the novelty-induced change in place field area. This suggests that acute administration of LEV + VPA may interfere with the normal response to novelty seen in young rats.

The size of place fields in CA3 versus CA1 depends on the location of recording electrodes along the hippocampal transverse axis (Lee et al., 2004; Mizumori et al., 1995; Igarashi et al., 2014). To control for this variable microarrays were positioned above the hippocampus based on the same stereotaxic coordinates in both young and aged rats. Tetrode location along the transverse axis would be unlikely to account for the drug-induced within subject differences in place field area as tetrode location along the axis did not change from day to day.

Mechanisms via Which LEV + VPA May Be Modulating Neuronal Activity

Behavioral studies comparing LEV + VPA to LEV or VPA alone suggest that at sub-anticonvulsant doses these two drugs have a synergistic effect on memory function (Koh et al., 2009). While the exact mechanism of action exerted by LEV and VPA at these concentrations has not been fully elucidated, it is possible that co-administration of these two drugs enhances inhibitory neurotransmission and reduces excitatory neurotransmission. Enhanced inhibitory neurotransmission mediated by VPA was demonstrated by the observation that VPA inhibits LTP induction, an effect that is abolished by the $GABA_A$ receptor channel blocker picrotoxin (Zhang et al., 2003). VPA has also been shown to increase brain levels of GABA within 15 minutes after administration, possibly by blocking reuptake of this important inhibitory neurotransmitter (Nau and Löscher, 1982; Fraser et al., 1999). Acute administration of VPA has been shown to increase synaptic GABA concentrations in the ventral hippocampus dialysate by 200% when administered at 400 mg/kg (IP) but reduces synaptic GABA levels by 50% when administered at a 100 mg kg^{-1} dose (Biggs et al., 1992). No study to date has shown whether acute administration of VPA plus LEV at the doses used in our work exerts a similar enhancement of extracellular GABA concentrations in the brain. VPA also attenuates neuronal excitation mediated by NMDA-type glutamate receptors, further exemplifying its role in reduced excitatory neurotransmission (Löscher, 1999). Similarly, another report showed that acutely administered VPA (50 mg kg^{-1}) attenuated methamphetamine-induced glutamate release (Ito et al., 2006).

The mechanism of action of LEV has not been fully elucidated (Löscher and Hönack, 1993). Binding to the synaptic vesicle protein 2A allows modulation of synaptic neurotransmitter release, which has been proposed as the primary mechanism of action for LEV (Lynch et al., 2004; Gillard et al., 2006). LEV has also been shown to remove the Zn^{2+}-induced suppression of presynaptic $GABA_A$ receptor-mediated inhibition, resulting in a decrease in glutamate-mediated excitatory transmission, which has been implicated in the modulation of the excitatory synapse between mossy fibers and CA3 neurons (Wakita et al., 2014).

A reduction in parvalbumin expressing interneurons in the stratum pyramidale CA3 region has been reported (Shetty and Turner, 1998). A loss of functional inhibitory interneurons in the hilus has also been reported and thus the aforementioned mechanism of action may counteract the inhibitory/excitatory imbalance thought to underlie the spatial memory deficits associated with aging (Speigel et al., 2013). Other possible modes of action include effects on calcium currents (Niespodziany et al., 2001; Costa et al. 2006) and potassium currents (Madeja

et al., 2003; Huang et al., 2009). Although LEV may be acting to modulate calcium currents it does not appear to act by blocking sodium currents (Zona et al., 2001; Madeja et al., 2003; Costa et al., 2006). More work is required to assess how acute co-administration of these drugs may change synaptic neurotransmitter levels or affect synaptic plasticity in the aged hippocampus and improve aspects of spatial memory.

In conclusion, the results are consistent with the hypothesis that aberrant hippocampal pyramidal cell firing rates underlie the deficits in memory function seen in aged rats which may be mediated in part by a loss of functional inhibitory interneurons in the hilus (Spiegel et al., 2013). As previously mentioned the loss of inhibitory control over CA3 neuronal activity may impair the ability to encode new memories by allowing previously encoded information to pass directly to CA1 with little or no influence from novel stimuli. Memory function depends on a delicate balance between inhibitory and excitatory neurotransmission, all of which is subject to modulation by therapeutic agents (Fig. 9). This hypothesis is supported in part by the observation that hyperactivity as well as too much inhibition both are associated with learning and memory deficits and moreover, that restoration of normal neuronal firing can improve performance.

For example, inhibition of NMDA receptor-mediated excitatory neurotransmission via acute administration of the noncompetitive NMDA receptor antagonist memantine augments experience-dependent place field expansion in aged rats (Burke et al., 2008). This approach will also be of use to evaluate the proposed effects of endogenous neuroactive molecules such as the neurosteroid pregnenolone sulfate. Pregnenolone sulfate is present in human and rodent brain at physiologically relevant concentrations and meets most of the criteria for an endogenous neurotransmitter/neuromodulator. PregS likely plays a significant role in modulation of glutamatergic excitatory synaptic transmission underlying learning and memory, yet the molecular target(s) and a linage to in vivo systems level modulation of synaptic activity underlying the behavioral pharmacological effects for its action awaits identification (Smith, Gibbs, Farb Psychopharmacology 2014; Smith et al. Mol Pharm. 2014). By contrast, infusion of the cholinergic agonist carbachol into the medial septum is associated with an impairment of learning and memory function in young rats while aged rats show improvements in acquisition of new spatial information (Sava and Markus, 2008). Administration of a $GABA_A$ alpha 5-selective positive allosteric modulator improves memory function in aged rats (Koh et al., 2013). Although the mechanism via which LEV + VPA at sub-anticonvulsant doses attenuates pyramidal cell hyperactivity in aged rats has not been fully elucidated, the current findings coupled with observations from other studies reveal several possible receptor targets known to be involved in regulating neuronal activity within the hippocampus. Whether selectively enhancing inhibitory or attenuating excitatory neurotransmission ultimately proves to be the optimal pharmacologic intervention, these results demonstrate that *in vivo* electrophysiological analysis is a powerful tool that can be effectively used in the preclinical development of novel cognitive enhancers. Results from human functional imaging studies suggest that data from preclinical *in vivo* electrophysiological studies such as this one are translational and may even facilitate the discovery of lead compounds for treating cognitive disorders (Bakker et al., 2012, 2015).

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