Age-Related Decrease in Theta and Gamma Coherence Across Dorsal Ca1 Pyramidale and Radiatum Layers

Tara K. Jacobson,[1] Brandy Schmidt,[1] James R. Hinman,[1] Monty A. Escabí,[1,2,3] and Etan J. Markus[1]*

ABSTRACT: In both humans and rodents, aging is linked to impairments in hippocampus dependent learning. Given such deficits, one would expect corresponding changes in hippocampal local field potentials, which represent the integration of multiple inputs onto a given dendritic field within the hippocampus. The current experiment examined coherence of theta and gamma in young and aged rats at submillimeter and millimeter distant locations both within and across layers in CA1 of the dorsal hippocampus. The degree to which different dendritic layers show coherent oscillations indicates the uniformity of the inputs and local circuitry, and may form an important element for processing information. Aged rats had lower coherence in all frequency ranges; this was most marked within a layer as the distance between electrodes increased. Notably, unlike younger rats, in the aged rats coherence was not affected by running on the maze. Furthermore, despite the previously reported effects of cholinergic activation on theta frequency and power, there was no effect of the cholinomimetic physostigmine on coherence. These data indicate an age related fragmentation in hippocampal processing that may underlie some of the observed learning and memory deficits. © 2015 Wiley Periodicals, Inc.

KEY WORDS: local field potentials; oscillations; hippocampus; encoding; novelty; physostigmine; EEG; cholinergic

INTRODUCTION

The functional role of large amplitude rhythmical activity in the hippocampus has long been of interest (Jung and Kornmuller, 1938). With the accumulation of evidence linking the hippocampus to learning and memory, determining the possible influence of these oscillations on the learning process has become increasingly important. After more than 6 decades of research, theta (~7–8 Hz) oscillations have been associated with arousal, orientation, exploration, attention, voluntary movement, motivation, emotion, as well as learning, and memory (Smith and Mizumori, 2006; Manns et al., 2007a; Montgomery and Buzsaki, 2007; Gupta et al., 2012; Hirshhorn et al., 2012; Lega et al., 2012). Similar, yet more recent attention has been devoted to the impact that that faster frequency gamma oscillations (>30 Hz) may have on memory and attention (Bragin et al., 1995; Chrobak and Buzsaki, 1998a; Montgomery and Buzsaki, 2007; Sederberg et al., 2007; Colgin et al., 2009; Jutras et al., 2009; Colgin and Moser, 2010; Nyhus and Curran, 2010). These rhythmic field potentials reflect the given input to an area that arise through interactions between synaptic inputs, excitatory pyramidal cells and interneurons (Buzsaki, 2002; Csicsvari et al., 2003; Logothetis, 2003; Einevoll et al., 2013). Hippocampal area CA1, the principle cortical output structure of the hippocampus, is the focus of this study. Inputs generating oscillations in CA1 include the medial septum (MS), entorhinal cortex (EC), and CA3 subfield (Monmaur and Thomson, 1983; Bragin et al., 1995; Buzsaki and Chrobak, 1995; Ylinen et al., 1995; Kocsis et al., 1999; Colgin et al., 2009; Pignatelli et al., 2012). Inputs to CA1 from both CA3 and the MS concentrate most consistently in the stratum radiatum layer of the pyramidal cells dendritic field close to the cell body along the apical dendrites (Frotscher and Leranth, 1985; Nyakas et al., 1987; Ishizuka et al., 1990) while EC projections align farther from the cell body in the stratum lacunosum-moleculare (Amaral and Witter, 1989; Amaral, 1993). Coherent oscillations within CA1 reflect the extent to which these inputs from multiple afferents are similar across layers (Gray, 1994; Chrobak and Buzsaki, 1998b; Kocsis et al., 1999; Ang et al., 2005; Fries, 2005). Alternatively, coherence measured between distant regions can reflect coordination between brain regions (Igarashi et al., 2014). Thus, in the current experiment, greater coherence within and across layers in CA1 may reflect more synchrony of inputs and circuits during information processing.

Theta and gamma coherence likely reflect this anatomical connectivity and not just physical distance between electrode sites (Sabolek et al., 2009). Levels

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of coherence are similar during theta associated behaviors (running, rapid eye movement sleep, and cholinergic activation) despite differences in overt behavior (Penley et al., 2012). However, coherence varies across the hippocampal septo-temporal axis indicating that the signals reflecting coherence are largely independently generated (Hinman et al., 2011). Notably, coherence between different locations can increase in response to task demands (Fell et al., 2003; Montgomery et al., 2008, 2009; Penley et al., 2013; Schmidt et al., 2013).

One hallmark of aging is impairment in hippocampal dependent memory (Barnes et al., 1980; Rapp et al., 1987; Light, 1991; Zyzak et al., 1995). Aging is also associated with declining functions within the cholinergic system; reduced acetylcholine (ACh) receptor binding (Gill and Gallagher, 1998), reduced choline uptake (Decker et al., 1988), and other degenerative changes to cholinergic neurons (for a review see Schliebs and Arendt, 2011). Cholinergic enhancement manipulations (cholinergic agonists or acetylcholinesterase inhibitors) can improve memory impaired aged animals (Brandeis et al., 1990; Quirion et al., 1995; Carnicella et al., 2005; Hernandez et al., 2006; Deiana et al., 2011). ACh is linked to theta activity within the hippocampus (Bland, 1986; Stewart and Vanderwolf, 1987; Olpe et al., 1987; Lee et al., 1994; Podol'skii et al., 2001; Hasselmo, 2006; Barry et al., 2012) and the cholinergic system may be particularly important in encoding novelty (Hasselmo et al., 2002; Hasselmo, 2006; Manns et al., 2007b; Jeewajee et al., 2008; Cutsuridis et al., 2010; Hasselmo and Sarter, 2011; Barry et al., 2012; Douchamps et al., 2013). Thus, hippocampal theta oscillations are reliant upon cholinergic input, which shows degradation with age. However, there are very few investigations into explicit differences in rhythmic oscillatory activity between adult and aged rats and their relationship to spatial novelty processing. Given the potential role of coherence in learning, one would expect changes in coherent oscillations in aged animals.

We recently showed (Jacobson et al., 2013) an age related decline in theta and gamma power in conjunction with maze exploration and response to novelty. It is unknown if there are age-related changes to theta or gamma coherence in response to environmental novelty. In this study, we reanalyzed the local field potentials to assess theta and gamma coherence as adult and aged animals explored a familiar (a retrieval situation) or novel (an encoding situation) maze configuration. Furthermore, we examined whether these aging changes were sensitive to cholinergic manipulations.

MATERIALS AND METHODS

Detailed methods of surgical and recording procedures in these animals have been previously described (Jacobson et al., 2013). Data were collected from 7 adult (12.2 ± 0.15 months) and 8 aged (23.3 ± 0.32 months) Fisher 344 male rats (Harlan, IN and Taconic, NY) and all procedures performed in accordance with the University of Connecticut's Institutional Animal Care and Use Committee.

Behavioral Task

Prior to and post surgery, animals were trained to alternate between the ends of two radial maze arms in a fixed (familiar) position. After surgery to implant electrode arrays, animals were re-trained to alternate on the familiar trajectory for at least 1 week prior to recordings. Given previous findings of changes during aging in both power and theta-gamma coupling, the effects of a novel trajectory and a cholinergic agonist were examined on measures of coherence. Local field potentials were collected first while rats rested quietly in their home cage (H1), then during two maze running epochs (M1 and M2) with an injection of either saline or physostigmine (0.1 mg/kg S.C.) separating the maze epochs, and a final epoch again in their home cage (H2; Fig. 1A). During each session rats first ran the familiar trajectory while the second maze epoch was either re-exposure to the same configuration following saline (Familiar Saline) or physostigmine (Familiar Physostigmine) injections, or exposed to one of three novel maze configuration following administration of saline (Novel Saline) or physostigmine (Novel Physostigmine).

Surgery

Two electrode arrays (four 50 μm tungsten wires, CA Fine Wire, Grover Beach, CA) were arranged and spaced using fused silica tubing (Polymicro Tubing, Phoenix, AZ), were implanted into dorsal hippocampus (array 1 at AP −3.5 mm, ML 2.5 mm from bregma, and DV 2.5 mm from the skull and array 2 at AP −4.5 mm, ML 3 mm from bregma, and DV 3.3 mm from the skull). Each array was cut on a small diagonal and targeted to sample local field potentials in different layers (Fig. 1B).

Recording and Data Analysis

Wide-band electrical activity was recorded (1-1893 Hz, 3787 samples/sec) using Neuralynx Data Acquisition System (Bozeman, Montana) then viewed and analyzed offline to exclude bad segments (signal loss or clipping) and data during food consumption and turning at the end of each arm then downsampled to 473.4 samples/s (Neuralynx, Bozeman, MT), thus all maze data is based on only when the animal is running on the maze. Each running segment was concatenated into a single string of data (Sabolek et al., 2009; Hinman et al., 2011; Penley et al., 2013; Long et al., 2014) and then truncated to 60 s to ensure each segment of data was the same length for coherence analysis. All signal analysis was conducted using custom-written programs in MatLab (Mathworks, Natick, MA). Briefly, coherence measures the linear association between two signals as a function of the frequency; the coherence (Bullock et al., 1990) between electrode pairs was computed using the Welch average periodogram estimation procedure with a spectral resolution of 2 Hz; only regions of

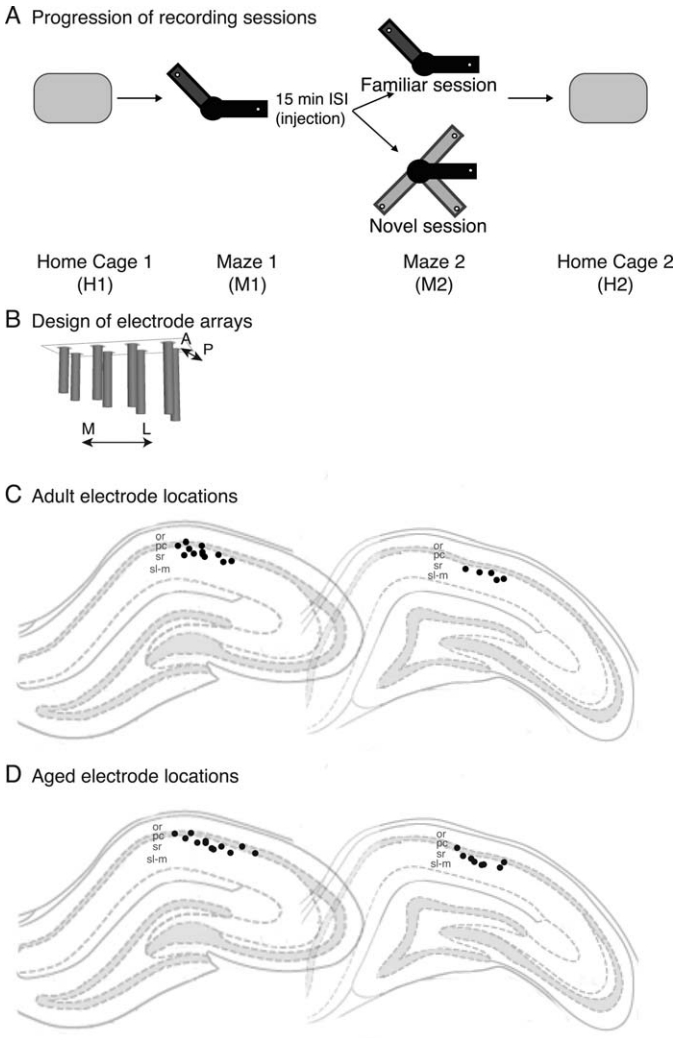

FIGURE 1. A: Diagram of maze trajectories. Each session consisted of recording each animal in four different epochs (H1, M1, M2, and H2) under different conditions (Familiar/Novel trajectory and Saline/Physostigmine). The first epoch was in the home cage outside the maze room (H1). The configuration of the first maze epoch (M1) was always the same trajectory. Following the M1 epoch rats were given either Saline or Physostigmine, then 15 min later explored either the familiar trajectory again, or one of three novel trajectories (M2). Finally, rats were recorded once again in the home cage (H2). B: Depiction of electrode configuration that was implanted into dorsal hippocampus. Adjacent electrodes are electrodes in the same medial (M) -lateral (L) plane that are next to each other in the array. Distant electrodes are in different anterior (A) -posterior (P) planes of the dorsal hippocampus. C: Electrode locations included in analyses for adult rats. D: Electrode locations included in analyses for aged rats.

the coherence falling above the 95% threshold were considered significant (Sabolek et al., 2009; Hinman et al., 2011, 2013; Penley et al., 2012; Schmidt et al., 2013). For each electrode pair, the statistically significant area in the theta (4.5–12 Hz), low gamma (25–55 Hz) and high gamma (65–140 Hz) bands were calculated and normalized by the frequency range (expressed as average coherence value per Hz) to facilitate comparison across frequency ranges and animals.

Since there are well documented differences in coherence across layers (Bullock et al., 1990; Kocsis et al., 1999; Montgomery et al., 2009) and with distance along the septo-temporal axis (Sabolek et al., 2004; Hinman et al., 2011; Penley et al., 2012, 2013; Long et al., 2014) effort was made to ensure that pairs of electrodes analyzed in this experiment were in similar positions along the septo-temporal axis and along the proximal-distal axis. Since our main interest was determining if there were any differences in coherence between adult and aged rats, only electrodes clearly identified to fall within these parameters were included in analyses (Figs. 1C,D). Therefore, this data set is limited to electrodes within the dorsal CA1 *stratum radiatum* (*sr*) and the *pyramidal cell* (*pc*) layers at similar positions in the aerial plane. Coherence was measured between submillimeter distant (adjacent electrodes within the same array) electrode pairs within the same layer (*stratum radiatum*) or between *stratum radiatum* and *pyramidal cell* layers; and across 1 millimeter distant (different electrode arrays) within the same layer (*stratum radiatum*).

Histology and Electrode Placement

Oscillatory activity was monitored on-line while animals were resting in their home cage and actively exploring the maze as an initial indicator of electrode position (see Buzsaki, 2002). However, for data analysis electrode locations were determined by the histological assessment. Following testing, rats were euthanized with CO_2 and perfused intracardially with saline followed by 10% phosphate buffered formalin solution. Brains were extracted and further fixed in formalin, cryoprotected in 30% sucrose, then sliced into 40 μm sections and stained with 0.25% Thionin. Consecutive sections were examined to follow electrode tracts and determine the layer in which each electrode tip resided.

RESULTS

Coherence Across Epochs

A main goal of the study was to determine differences in coherence attributed to age. Coherence between electrode pairs

TABLE 1.

Number of Animals/Recording Session for Each Condition

			Adjacent		Distant
			sr-sr	*sr-pc*	*sr-sr*
Familiar	Saline	Adult	6/20	3/11	2/6
		Aged	5/23	6/25	2/8
	Physostigmine	Adult	6/24	3/12	2/11
		Aged	5/23	6/27	2/6
Novel	Saline	Adult	6/12	3/7	2/5
		Aged	5/9	6/10	2/3
	Physostigmine	Adult	6/8	3/4	2/4
		Aged	5/9	6/10	2/3

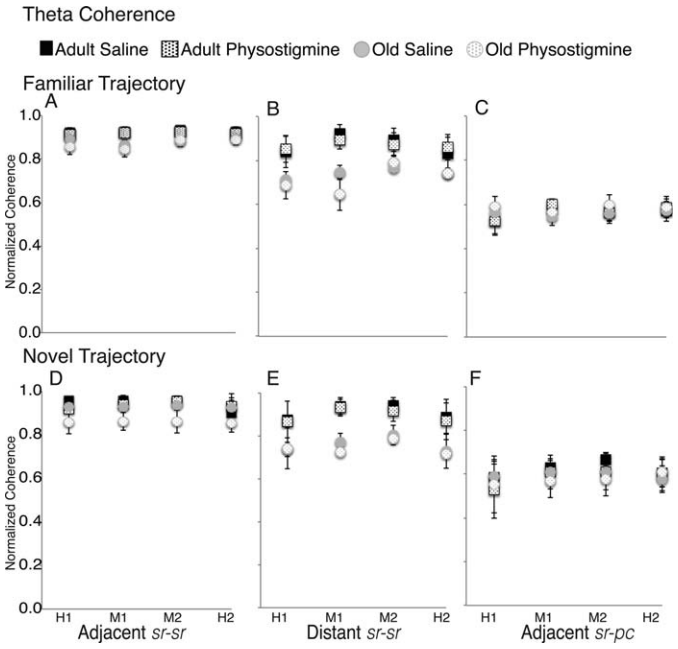

FIGURE 2. Theta coherence across epochs and environments. Each column represents one electrode pairing, rows illustrate the maze trajectory. A: Recordings from sessions that follow the familiar M1-familiar M2 trajectory configuration for adjacent electrodes with placements in *stratum radiatum*. B: Sessions following the familiar M1 -familiar M2 trajectory with 1mm distant electrodes with both placements in *stratum radiatum*. Sessions following the familiar M1-familiar M2 trajectory with adjacent electrodes in different layers, *stratum radiatum* and *pyramidal cell*. D: Sessions following the familiar M1-novel M2 trajectory with adjacent same layer placements. E: Sessions following the familiar M1 -novel M2 trajectory with distant same layer placements. F: Sessions following the familiar M1 -novel M2 trajectory with adjacent different layer placements. For both adjacent and distant pairs within *sr* aged rats have lower coherence than the adult rats. Electrodes at distant sites increased on the maze compared to in the home cage.

within the same lamina (*sr – sr*) and between lamina (*sr – pc*) was calculated and further separated into adjacent (submillimeter) and distant (1 mm) pairs (see Table 1). A Repeated Measures ANOVA was used since coherence between electrode pairs was measured within the same rat during different behavioral epochs (H1, M1, M2, and H2), with age, drug, and environment as between subject factors. Baseline age differences were determined during the first epoch as the animal sat in its home cage before any behavioral or drug manipulation. Measures of effect size (partial eta^2) are also reported as an indication of the percentage of variance from each factor and interaction that is explained by that factor.

Previously (Jacobson et al., 2013) showed how theta and gamma power were affected by the age of these animals, familiarity of the maze trajectory, and by treatment with the cholinergic agonist physostigmine. This study determines the degree to which these changes in power also manifest as changes in coherence. Main effects of age, drug, and environment are presented first, followed by results for each epoch.

Theta Coherence

Theta coherence at adjacent electrodes within *stratum radiatum* (*sr-sr*) was significantly lower in aged rats compared to adult rats (main effect of age: rANOVA $F_{1,120} = 5.54$, $P < 0.05$) with no other significant main effects nor interactions of either drug or environment (all $P > 0.1$), thus aged rats had consistently lower coherence, however coherence was not different for either age group across different environments nor was affected by cholinomimetic administration. Repeated measures across epoch showed no main effects or interactions with age, drug, nor environment (all $P > 0.1$). Measure of effect size corroborate the age effect indicating that age accounts for 4.4% (partial $\eta^2 = 0.044$) of the variance, while all other factors account for 1% or less (all partial $\eta^2 \leq 0.01$). Thus despite novelty and cholinomimetics increases in theta power in aged and young rats (Jacobson et al., 2013; Penley et al., 2013) this change in power did not affect coherence within the theta band between adjacent electrodes (Figs. 2A,D).

For electrode pairs within the same layer (*sr-sr*) but at a 1 mm distance aged rats again had lower coherence, main effect of age (rANOVA $F_{1,45}$=13.20, $P < 0.001$; partial $\eta^2 = 0.258$). There were no significant effects or interactions with environment or drug (all $P > 0.1$; all partial $\eta^2 \leq 0.01$). There was a main effect of epoch (rANOVA $F_{3,114} = 7.59$, $P < 0.001$; partial $\eta^2 = 0.166$) and an epoch by age interaction (rANOVA $F_{3,114} = 3.56$, $P < 0.05$; partial $\eta^2 = 0.086$) thus regardless of the novelty of the environment, aged rats had a lower coherence and were affected differently by exploration, or the move from home cage to maze, than the younger rats. The age effect had the largest effect size accounting for 25.8% of the variance. There were no other significant interactions with epoch (all $P > 0.1$; Figs. 2B,E).

For adjacent electrodes in different layers (*sr-pc*) coherence was lower than pairs in the same layer, and there was no main effect of age, environment, nor drug (all $P > 0.1$; all partial $\eta^2 < 0.01$). There was a main effect of epoch (rANOVA $F_{3,147} = 3.27$, $P < 0.05$; partial $\eta^2 = 0.032$). There were no other significant interactions of epochs (all $P > 0.1$; all partial $\eta^2 < 0.01$). Thus, coherence at adjacent electrodes in different layers shows no effect of age, environment, nor drug, but had different patterns of coherence across holder and maze exploration epochs (Figs. 2C,F).

Taken together the data indicate decreased theta coherence in the aged animals within the *sr* layer where the levels of coherence in young animals was high. Notably novelty and cholinergic activation did not affect coherence in either age group.

Low Gamma Coherence

Low gamma coherence at adjacent electrodes within *stratum radiatum* (*sr-sr*) was significantly lower in aged rats compared to adult rats (main effect of age: rANOVA $F_{1,120} = 35.04$, $P < 0.001$; partial $\eta^2 = 0.226$), with no main effects of

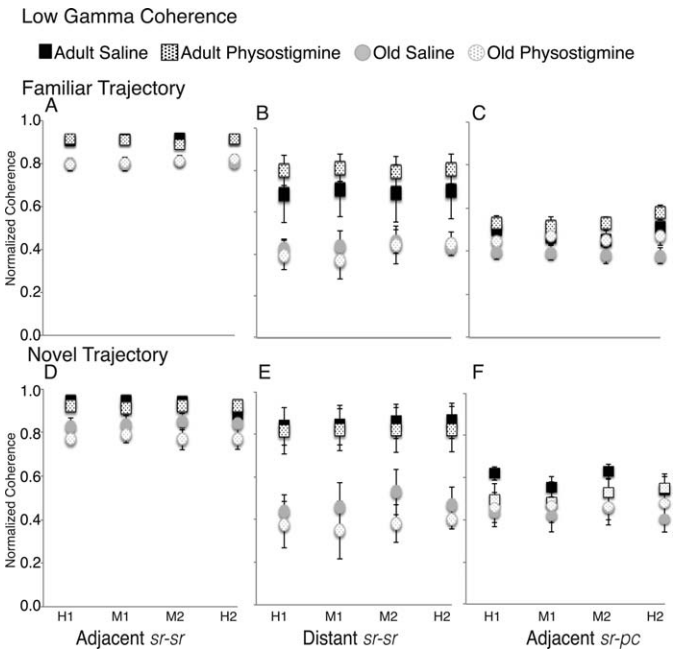

FIGURE 3. Low gamma coherence across epochs and environments. Columns represent different electrode pairings. Tope row represents all familiar M1 - familiar M2 sessions, bottom row shows all familiar M1 - novel M2 sessions. A: Familiar sessions for adjacent *sr* pairs. B: Familiar sessions for distant *sr* pairs. C: Familiar sessions for adjacent *sr-pc* pairs. D: Novel sessions for adjacent *sr* pairs. E: Novel sessions for distant *sr* pairs. F: Novel sessions for adjacent *sr-pc* pairs. Aged rats have reduced low gamma coherence for all electrode pairings. At distant *sr* pairs and adjacent *sr-pc* pairs coherence increased during maze exploration.

environment, drug, epoch, nor any significant interactions (all $P > 0.1$; all partial $\eta^2 < 0.01$). Therefore there was no effect of the behavioral manipulations or drug for either age group, though in the aged rats low gamma coherence was reduced (Figs. 3A,D).

Electrodes pairs within the same layer (*sr-sr*) 1 mm apart showed lower coherence in the aged than adult rats (rANOVA $F_{1,38} = 27.23$, $P < 0.001$; partial $\eta^2 = 0.412$). There was no effect of either environment, drug, nor any interactions (all $P > 0.1$; all partial $\eta^2 < 0.01$). There was a main effect of epoch (rANOVA $F_{3,114} = 4.17$, $P < 0.01$; partial $\eta^2 = 0.099$) and an epoch by age interaction (rANOVA $F_{3,114} = 3.58$, $P < 0.05$; partial $\eta^2 = 0.086$). Thus similar to theta coherence in adjacent electrodes, regardless of environment or drug condition aged rats again had lower coherence than adult rats, and had a different pattern of coherence across epochs than the younger rats (Figs. 3B,E).

Between electrodes in different layers (*sr-pc*) aged rats had decreased low gamma coherence (rANOVA $F_{1,49} = 31.81$, $P < 0.001$; partial $\eta^2 = 0.053$). There were no other main of effects or interactions (all $P > 0.1$; all partial $\eta^2 \leq 0.01$). Thus aside from an age-related decrease in low gamma coherence there was no effect of behavioral manipulation or drug (Figs. 3C,F).

In all, these data indicate an-age related reduction in low gamma coherence both between location within and across laminar layers. The aging effect is especially striking as the distance between the electrodes increases (note Figs. 3B,E). Notably, the overall effects of behavioral manipulations and cholinergic activation was minimal.

High Gamma Coherence

Coherence between adjacent electrodes within the same layer (*sr-sr*) in the high gamma range was lower in aged rats (main effect of age: rANOVA $F_{1,120} = 18.63$, $P < 0.001$; partial $\eta^2 = 0.134$). There was no main effect of environment or drug (both $P > 0.1$, partial $\eta^2 < 0.01$). There was a main effect of epoch (rANOVA $F_{3,360} = 5.15$, $P < 0.01$; partial $\eta^2 = 0.041$) and an epoch by environment interaction (rANOVA $F_{3,360} = 2.61$, $P = 0.05$; partial $\eta^2 = 0.021$), thus overall aged rats had lower coherence that was not affected by environment nor drug, and the pattern of coherence for both groups was different across epochs and influenced by the novelty of the environment (Figs. 4A,C).

High gamma coherence within the same layer (*sr-sr*) 1mm apart showed a main effect of age (rANOVA $F_{1,38} = 13.47$, $P < 0.01$; partial $\eta^2 = 0.262$), also with no main effects of environment nor drug (both $P > 0.1$, partial $\eta^2 < 0.01$). There

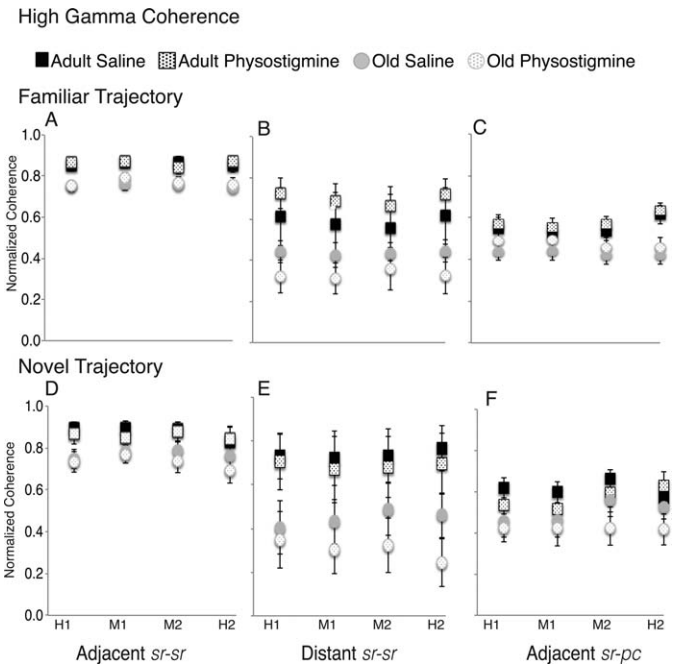

FIGURE 4. High gamma coherence across epochs and environments. Columns represent different electrode pairings. Tope row represents all familiar M1 - familiar M2 sessions, bottom row shows all familiar M1 - novel M2 sessions. A: Familiar sessions for adjacent *sr* pairs. B: Familiar sessions for distant *sr* pairs. C: Familiar sessions for adjacent *sr-pc* pairs. D: Novel sessions for adjacent *sr* pairs. E: Novel sessions for distant *sr* pairs. F: Novel sessions for adjacent *sr-pc* pairs. Aged rats have lower high gamma coherence for all electrode pairings. At distant *sr* pairs and adjacent *sr-pc* pairs coherence increased during maze exploration.

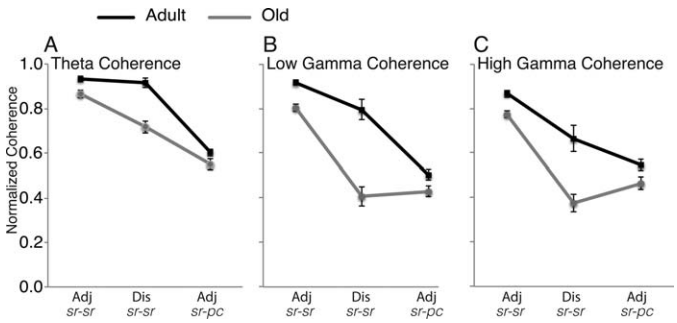

FIGURE 5. Coherence across distance and layers during exploration of the highly familiar environment. A: Theta coherence in both adult and aged rats decreased across distance and layer. B: Low gamma coherence decreased across distance and layer in both age groups. C: High gamma coherence also decreased across distance and layer for both age groups.

was no main effect of epoch ($P > 0.1$), although there was a significant epoch by age interaction (rANOVA $F_{3,114} = 4.92$, $P < 0.01$; partial $\eta^2 = 0.115$). Overall these results indicate that aged rats had lower high gamma coherence, and the pattern of coherence across epochs was different for the age groups (Figs. 4B,D).

High gamma coherence at adjacent electrodes in different layers (sr-pc) was decreased in age rats (main effect of age: rANOVA $F_{1,49} = 6.92$, $P < 0.01$; partial $\eta^2 = 0.065$). There was no main effect of environment or drug (both $P > 0.1$; partial $\eta^2 < 0.01$). There was a main effect of epoch (rANOVA $F_{3,147} = 2.54$, $P = 0.05$; partial $\eta^2 = 0.025$), a significant epoch by age by environment interaction (rANOVA $F_{3,147} = 2.86$, $P < 0.05$; partial $\eta^2 = 0.028$). This suggests that there is still consistently lower high gamma coherence across different layers, and that the pattern of coherence for both age groups across epochs may be influenced by environment (Figs. 5C,E).

Coherence Across Layers

To better characterize age-related coherence within and across lamina, we focused on the data from the M1 epoch (first maze run before any trajectory or drug manipulations).

There was a main effect of layer, such that theta coherence decreased across both layer and distance (rANOVA $F_{1,54} = 347.01$, $P < 0.001$), and was lower in aged rats (rANOVA $F_{1,27} = 96.61$, $P < 0.001$). There was also a layer by age interaction (rANOVA $F_{1,54} = 109.63$, $P < 0.001$) indicating the decrease across distance was more pronounced in the aged rats (Fig. 5A).

Likewise, both low and high gamma coherence decreased across layer and distance (rANOVA $F_{1,54} = 216.43$, $P < 0.001$, and $F_{1,54} = 544.20$, $P < 0.001$, respectively), was lower in aged rats (rANOVA $F_{1,27} = 854.25$, $P < 0.001$, and rANOVA $F_{1,27} = 2162.78$, $P < 0.001$), and also a layer by age interaction (rANOVA $F_{1,54} = 190.71$, $P < 0.001$, and rANOVA $F_{1,54} = 246.23$, $P < 0.001$; Figs. 5B,C).

To rule out the possibility that differences in coherence were due to decreased running speed in the aged animals, data was selected from epochs that were similar in running speed in both age groups (mean ± sem for adult = 11.97 ± 0.87; mean ± sem for aged = 11.06 ± 0.75; $t_{58} = 0.79$, $P > 0.1$, Fig. 6).

A 1-tailed *t*-test was conducted on theta, low gamma, and high gamma coherence for each electrode pair. Theta coherence was significantly lower in aged rats for all electrode pairs (adjacent sr-sr: $t_{43} = 3.00$, $P < 0.01$; distant sr-sr: $t_{22} = 3.19$, $P < 0.01$; adjacent sr-pc: $t_{20} = 3.91$, $P < 0.01$). Low gamma coherence was significantly lower in aged rats for all electrode pairs (adjacent sr-sr: $t_{43} = 3.70$, $P < 0.01$; distant sr-sr: $t_{22} = 3.00$, $P < 0.01$; adjacent sr-pc: $t_{20} = 5.47$, $P < 0.01$). The age effects were less pronounced for high gamma coherence

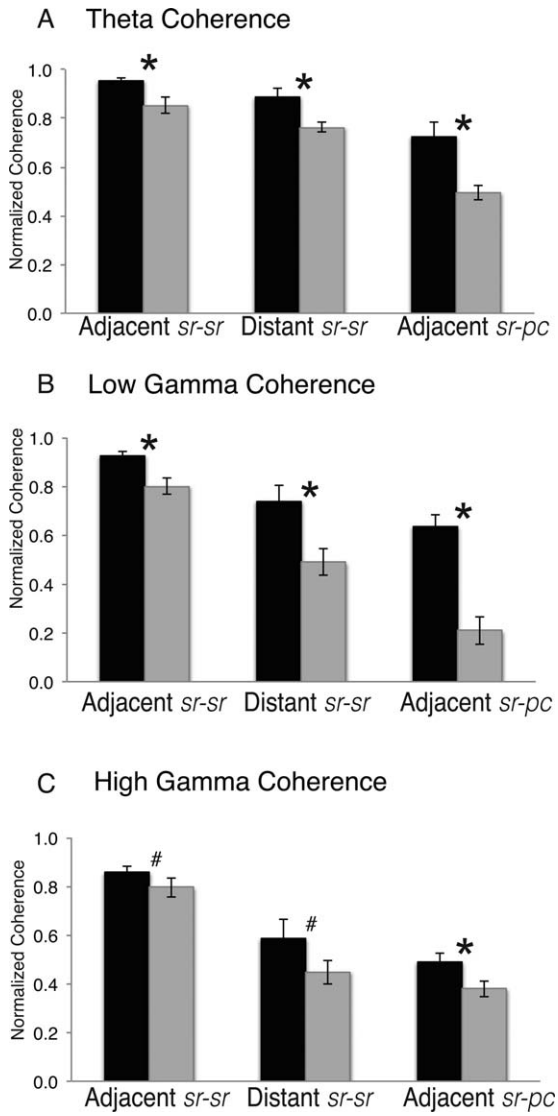

FIGURE 6. Coherence during exploration of the highly familiar environment matched for running speed. A: Theta coherence at each electrode pair while aged and adult rats ran at similar speeds. Theta coherence was decreased in aged rats at each electrode pair. B: Low gamma coherence was decreased in aged rats at each electrode pair while running at similar speeds. C: High gamma coherence was decreased in aged rats at adjacent *sr-pc* electrode pairs, while a similar trend existed at both adjacent and distant *sr-sr* electrode pairs. * $P < 0.05$; # $0.05 < P < 0.1$

with a trend for significance at both adjacent and distant sr-sr electrode pairs ($t_{43} = 1.35$, $P = 0.09$ and $t_{22} = 1.4$, $P = 0.09$, respectively), while coherence at adjacent sites across layers was significantly lower in aged rats ($t_{20} = 2.12$, $P < 0.05$).

Taken together the data indicate an age related decrease in coherence spanning theta and gamma bandwidths. These differences remain even when running speed is matched. The age effects were most pronounced for low gamma especially across the lamina and as distance increased.

DISCUSSION

This experiment examined coherence of theta and gamma between different layers in the dorsal hippocampus CA1 region in adult and aged rats. Coherence was measured under different overt behavioral state (in home cage and exploring the maze), environmental modifications (familiar and novel maze trajectories) and under conditions of cholinergic activation.

Overall, the aged rats consistently showed lower coherence in both theta and gamma ranges, with only theta coherence across a different layer unaffected by age. The age-related decreased coherence was observed both while in their home cage and exploring the maze, and could not be attributed simply to aging differences in running speed.

In adult rats, behavioral state (exploring maze) increased coherence across distance and layers. This was not seen for adjacent electrodes within a layer, presumably because of a ceiling effect given the high coherence between adjacent recording electrodes. Importantly, behavioral state had no effect on coherence in the aged animals despite lower baseline coherence levels. Finally, novelty and cholinergic activation had no effect on theta or gamma coherence in the adult or aged animals.

One possible reason for lower coherence observed in the aged animals could stem from lower theta and gamma power in these animals. This however was not the case; power in both the theta and gamma bands was comparable in both groups when they ran the familiar trajectory (Jacobson et al., 2013). Analysis of coherence for the same epochs however, reveal that aged rats have reduced coherence compared to adult rats in all epochs, and manipulations that differentially increased theta in the adult (a novel trajectory) and aged (physostigmine) animals had no impact on the lower coherence found in the same aged animals. It has been previously demonstrated that novelty can increase theta power and decrease the peak theta frequency (Jeewajee et al., 2008). We also show (Jacobson et al., 2013) that exploring a novel maze configuration increased theta and gamma power in both adult and aged rats (although to a lesser degree than in adults), however coherence between and within layers was unaffected by this increase in power and was not changed with cholinergic agonism. In addition, the difference in coherence could be due to the slower running speeds observed in aged animals (Hinman et al., 2011), however, as noted above, coherence was lower in aged rats even during periods of no exploration or locomotion in their home cage. Furthermore, when controlling for speed, aged rats still have lower coherence than adult rats. This finding also parallel evidence that coherence is decreased in aged humans (Vysata et al., 2014).

In agreement with previous studies (Sabolek et al., 2009; Penley et al., 2012), we further demonstrate the importance of similarity of input rather than absolute distance on coherence by showing laminar dissimilarities within CA1. Both theta and gamma coherence were lower across different layers at close electrode pairs than for coherence at a father physical distance in the same layer (Fig. 5). This supports the role of different external inputs in generating these oscillations (Sullivan et al., 2011; Penley et al., 2012; Colgin, 2014).

Further, gamma coherence was more affected than theta coherence by both distance and layer, which highlights the role of local interneuron circuits in generating gamma oscillations (Sabolek et al., 2009; Buzsaki and Wang, 2012). In fact the most extensive (~50%) age-related decrease in coherence found in this study was for gamma coherence across distance. While little is known regarding changes in local inhibitory circuits during aging, Stanley et al. found a selective loss of GABA interneurons across layers in CA1 reducing the amount of depolarization-induced GABA release in slice recordings of aged rats (Stanley et al., 2012). There are two considerations in determining the coherence between two sites, a consistent phase relationship, and a similar amplitude modulation. Changes in either or both of these factors would decrease the cooperative information processing between sites. Whether the decrease in coherence results from independent changes in frequency at different sites or differential modulation of the amplitude of oscillations, the end result in terms of cooperative processing is likely the same. This ultimately raises further questions regarding the mechanisms of both theta and gamma oscillations and how these oscillations may or may not be coherent along different septo-temporal aspects of the hippocampus in both aged and adult rats.

Previous studies have shown that in aged rats, low doses of cholinomimetics improved performance on a spatial task (Stemmelin et al., 1999), facilitated the establishment of stable place fields in a novel environment (Sava and Markus, 2008), increased power and lowered theta frequency in response to novelty (Jeewajee et al., 2008; Jacobson et al., 2013). Despite these effects on theta power, the age-related decline in theta coherence observed in the current results was unaffected by cholinomimetics. Similarly we have shown an age-related reduction in theta modulation of gamma, which is also unaffected by behavior or physostigmine (Jacobson et al., 2013). Thus, it would seem that whatever facilitory effects cholinomimetics have on behavior they are not based on changes to theta modulation of gamma or overall coherence of similar CA1 inputs.

The data indicate fundamental changes in the function of hippocampal circuitry during aging. Theta and gamma coherence was reduced in old rats during both in the home cage and running on the maze, and unlike younger animals this was unaffected by behavioral state. This could indicate a less homogenous pattern of external inputs and local interneuron networks across regions. We have shown that in younger

animals the degree of coherence across hippocampal regions increases in relation to task demands (Schmidt et al., 2013). The coherence data from the present study indicates that during aging hippocampal circuitry becomes more modular or fragmented. While overall increases may be seen in theta and gamma power under different conditions, a lack of changes to coherence precludes the advantages attained with coordinated processing. The current findings are the first to report any age-related differences in coherence, and suggest that fragmentation in information processing may underlie some of the learning and memory deficits found during aging.

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