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RESEARCH ARTICLE

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Dendrites of the dorsal and ventral hippocampal CA1 pyramidal neurons of singly housed female rats exhibit lamina-specific growths and retractions during adolescence that are responsive to pair housing

Yi-Wen Chen[1] | Ada Akad[1] | Ruka Aderogba[1] | Tara G. Chowdhury[1] | Chiye Aoki[1,2]

[1]Center for Neural Science, New York University, New York, New York 10003

[2]Neuroscience Institute, Langone Medical Center, New York University, New York, New York 10016

Correspondence
Chiye Aoki, PhD, 4 Washington Place, Room 809, New York, NY 10003.
Email: ca3@nyu.edu

Present address
Tara G. Chowdhury, Department of Behavioral Neuroscience, Oregon Health and Science University School of Medicine, Portland, Oregon

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Abstract

Adolescence is accompanied by increased vulnerability to psychiatric illnesses, including anxiety, depression, schizophrenia, and eating disorders. The hippocampus is important for regulating emotional state through its ventral compartment and spatial cognition through its dorsal compartment. Previous animal studies have examined hippocampal development at stages before, after or at single time points during adolescence. However, only one study has investigated morphological changes at multiple time points during adolescence, and no study has yet compared developmental changes of dorsal versus ventral hippocampi. We analyzed the dorsal and ventral hippocampi of rats to determine the developmental trajectory of Golgi-stained hippocampal CA1 neurons by sampling at five time points, ranging from postnatal day (P) 35 (puberty) to 55 (end of adolescence). We show that the dorsal hippocampus undergoes transient dendritic retractions in stratum radiatum (SR), while the ventral hippocampus undergoes transient dendritic growths in SR. During adulthood, stress and hormonal fluctuations have been shown to alter the physiology and morphology of hippocampal neurons, but studies of the impact of these factors upon adolescent hippocampi are scarce. In addition, we show that female–female pair housing from P 36–44 significantly increases branching in the dorsal SR and reduces branching in the ventral SR. Taken together with data on spine density, these results indicate that pyramidal cells in the dorsal and ventral CA1 of female adolescents are remodeled differently following single housing. Social housing during adolescence elicits pathway-specific changes in the hippocampus that may underlie behavioral benefits, including stability of emotion regulation and superior cognition.

KEYWORDS

apical dendrites, dendritic spines, Golgi stain, pair housing, Sholl analysis

1 | INTRODUCTION

Adolescence is a critical period of life, characterized by maturation of cognitive, reproductive and social skills and capacities in all mammals (Hazen, Schlozman, & Beresin, 2008; Sisk & Foster, 2004). Adolescence is also the most common stage for the emergence of neuropsychiatric illnesses, such as schizophrenia, depression, substance abuse, anxiety disorders, and eating disorders (Kessler et al., 2007; Paus, Keshavan, & Giedd, 2008). Previous studies suggest that abnormal trajectories in brain development during adolescence may contribute to the pathophysiology of these disorders (Merikangas, Nakamura, & Kessler, 2009; Paus et al., 2008).

There is mounting evidence indicating that increases in circulating steroids during adolescence, which accompany reproductive maturation, influence the development of neurons in the hippocampus and other brain regions (Cooke & Woolley, 2005; Giedd et al., 2006). Imaging studies of

healthy brains indicate that the human brain undergoes dynamic changes throughout adolescence and that the change in gray matter volume over time has an inverted U-shape pattern (Casey, Jones, & Hare, 2008; Giedd, 2004; Gogtay et al., 2004). The loss of gray matter in the prefrontal cortex, temporal lobe, and basal ganglia is surmised to be a result of massive pruning of synapses and development of the white matter, reflecting the growth of myelin that is delayed, relative to axonal growth (Casey, Tottenham, Liston, & Durston, 2005; Gogtay et al., 2004). Rodent models also shed light upon our understanding of healthy brain development during adolescence. Some specific examples include overproduction and rapid pruning of dendritic branches, dendritic spines, synapses, and/or neurotransmitter expression during adolescence in the amygdala (Zehr, Todd, Schulz, McCarthy, & Sisk, 2006), nucleus accumbens (Teicher, Andersen, & Hostetter, 1995) and prefrontal cortex (Andersen & Teicher, 2004; Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Drzewiecki, Willing, & Juraska, 2016; Koss, Belden, Hristov, & Juraska, 2014; Willing & Juraska, 2015).

As for the hippocampus, adolescents perform differently from adults on hippocampal-dependent learning and memory tasks (McCormick & Mathews, 2010). The rate of cell proliferation in the dentate gyrus of the hippocampal formation, which is the highest at 2 weeks postnatal for rodents, declines progressively but is still higher during adolescence than in adulthood (Bayer & Altman, 1974; He & Crews, 2007; Schlessinger, Cowan, & Gottlieb, 1975). Spines are pruned during adolescence (Afroz, Parato, Shen, & Smith, 2016) and this pruning ensures optimal hippocampus-dependent spatial memory (Afroz et al., 2016). However, it remains unexplored whether remodeling of hippocampal circuit during adolescence involves dendritic branch remodeling as well as spine pruning.

The cortical and limbic regions that continue to mature during adolescence, such as prefrontal cortex, amygdala, and hippocampus, are also some of the most stress-reactive areas in the brain (McEwen, 2005). The hippocampus, in particular, shows plasticity in its response to stress (Conrad, Ortiz, & Judd, 2017; McEwen, 1999) and gonadal hormones (Woolley & McEwen, 1992) independently and jointly (McLaughlin et al., 2010). The hippocampus is not a functionally uniform structure. Instead, function differs along the septo-temporal axis of the hippocampus, with the dorsal hippocampus being more important for spatial learning and memory performance and with the ventral hippocampus being more preferentially involved in regulation of stress, emotion, and affect (Bannerman et al., 2002; Fanselow & Dong, 2010; Moser, Moser, & Andersen, 1993). Accumulated evidence suggests that the dorsal versus ventral hippocampus also differ in cell morphology, topographical arrangement of neuronal connectivity, and patterns of gene expression (Fanselow & Dong, 2010; Swanson & Cowan, 1977). Previous findings from our laboratory add to this list of differences: there is a dorsal-ventral distinction in the changes in dendritic branching that are elicited by food-restriction stress during adolescence (Chowdhury et al., 2014a). Adolescence is a period of psychiatric vulnerability which, in turn, is influenced by anxiety that is proposed to be regulated more by the ventral than the dorsal hippocampus (Bannerman et al., 2002; McHugh, Deacon, Rawlins, & Bannerman, 2004). However, we have data indicating otherwise (Aoki, Chen, Chowdhury, & Piper, 2017). It has also been shown that dorsal and ventral hippocampal lesions interact with puberty to result in different behavioral deficits in adulthood (Lipska, Jaskiw, & Weinberger, 1993; Pacteau, Einon, & Sinden, 1989). However, no study has examined or directly compared changes in the structure of pyramidal neurons in the dorsal and ventral hippocampus during the developmental period specifically spanning adolescence.

Environmental enrichment and social experience have been shown to alter both hippocampal-dependent behaviors and neuronal structure in the hippocampal CA1 (Faherty, Kerley, & Smeyne, 2003; Huttenrauch, Salinas, & Wirths, 2016; Lauterborn, Jafari, Babayan, & Gall, 2015). In both animals and humans, social interaction is important for parental care, pair bonding and cooperation (Clark & Dumas, 2015; Rilling & Young, 2014; Trezza, Campolongo, & Vanderschuren, 2011). Absence of exposure to age-appropriate social interactions can be detrimental to the organism and the gene pool. For humans, social isolation during adolescence (both voluntary and involuntary) is associated with several neuropsychiatric illnesses, including schizophrenia, anxiety, and depression (Rubin, Coplan, & Bowker, 2009). Adolescent rodents also live in groups and exhibit higher levels of social behaviors than younger or adult animals (Panksepp et al., 2007). Therefore, rodent models of adolescent social stress can be used for understanding the impact of social stress in adolescent humans.

Most studies involving laboratory rodents have focused on changes elicited within brains of males, leaving female brains to be relatively understudied. Few studies have found that exposure to enriched rearing environment from weaning leads to sex differences in dendritic morphological changes in dentate gyrus (Juraska, Fitch, Henderson, & Rivers, 1985) and hippocampal CA3 region (Juraska, Fitch, & Washburne, 1989). Previous studies also suggest that females have an increased sensitivity to chronic social stress, and that social instability stress reduces hippocampal neurogenesis in female adolescent rats (McCormick, Nixon, Thomas, Lowie, & Dyck, 2010). Sex differences in behaviors (Galef & Sorge, 2000) and corticosterone (Hurst, Barnard, Hare, Wheeldon, & West, 1996) responses to housing conditions have been reported for rats as well. Although adolescents exhibit heightened levels of social behavior and social isolation alters neurotrophin levels in the hippocampus (Meng, Li, Han, Shao, & Wang, 2011; Scaccianoce et al., 2006), it remains unexplored whether deprivation of social interaction due to single housing or the experience of pair housing during adolescence impact dendritic morphology of neurons in the female hippocampus.

In this context, our present set of experiments had three objectives. First, we measured dendritic complexity and spinous complexity in female rats throughout adolescence, to determine the developmental trajectory of the hippocampal CA1 neurons. Secondly, we investigated whether the dorsal and the ventral hippocampi show similar or different patterns of development. Finally, we evaluated the contribution of social housing during early to mid-adolescence. The analysis reveals that dendritic arbor and spines of pyramidal cells in the hippocampal CA1 are remodeled during mid-adolescence in ways that differ for the dorsal versus ventral hippocampus and are influenced by social housing. Together, our findings contribute to our basic understanding of how wiring of the hippocampus changes across adolescence and shed light on the mechanisms of the behavioral benefits derived from social housing during adolescence.

2 | EXPERIMENTAL PROCEDURES

2.1 | Animals

All procedures relating to the use of animals were in accordance with the Institutional Animal Care and Use Committee of New York University (Animal Welfare Assurance #11–1374).

Two cohorts, totaling 36 female Sprague-Dawley rats, were purchased from Hilltop Lab Animals INC and delivered to the New York University animal facility on postnatal Day 28 (P28). All animals were handled and weighed daily, kept on a 12-h light:12-h dark cycle (lights off at 19:00 h), and given food and water *ad libitum* until they were sacrificed as described below under 'Brain collection and tissue processing'.

Twenty of these animals (cohort 1) were used to analyze dendritic development of hippocampal CA1 neurons. Upon arrival, these animals were pair-housed with another female. Starting on P32, animals were singly housed and assigned to one of five age groups, to be euthanized either at P35, P40, P44, P50, or P55. Each animal was weighed on P32, prior to grouping, so as to equalize the mean body weights across the age groups. Each animal was weighed daily.

The second cohort of 16 rats was used to determine the effects of pair housing during adolescence upon dendritic remodeling. Upon arrival, these animals were weighed, then pair-housed with another female. On P36, animals were assigned into two groups of eight animals each: singly-housed or pair-housed with the same female. The only criterion used to house the animals singly or as a pair was their body weights: the mean weight of the two groups was equalized. All animals of the second cohort were weighed daily and euthanized on P44.

While it is known that ovarian hormones affect hippocampal structure and function, we did not consider the estrous cycle phase as a biological variable, because it has been shown that pubescent female Sprague-Dawley rats (P35–41) exhibit only partial cycling (Hodes & Shors 2005). Vaginal smears of animals at P50 indicated that two were in diestrus, and the other two were in transition from estrus to metestrus. At P55, two were in diestrus, one was in transition from proestrus to estrus and the fourth was in metestrus.

2.2 | Brain collection and tissue processing

Animals in the first cohort were euthanized by being deeply anesthetizing with urethane (34%; 0.65–0.85 mL/g body weight, i.p.) between the hours of 12–4 pm, during the light cycle. The animals were decapitated, and the brains were quickly removed from the skull. The brain was divided along the coronal plane into three blocks of 2–3 mm thickness and processed immediately for Golgi–Cox impregnation using the FD Rapid GolgiStain kit, according to the instructions of the manufacturer (FD NeuroTechnologies, Ellicott City, MD).

Animals in the second cohort were euthanized on P44 between the hours of 4–6 pm, at the end of the light cycle. Animals were deeply anesthetized using urethane (34%; 0.65–0.85 mL/g body weight, i.p.) prior to transcardial perfusion with phosphate-buffered saline containing heparin (10,000 U per 500 mL, Henry Schein) followed by 500 mL of 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) at a flow rate of 50 mL/min. The right hemisphere was divided along the coronal plane into three blocks of 2–3 mm thickness and processed immediately for Golgi–Cox impregnation using the FD Rapid Golgi Stain kit according to the instructions of the manufacturer. The left hemisphere was reserved for another study involving immunocytochemistry.

For all brains collected from cohort 1 and 2, coronal sections, 250 μm thick, were prepared, using the Leica VT1000M Vibratome (Leica Microsystems GmbH, Wetzlar, Germany).

2.3 | Morphological analysis

CA1 pyramidal neurons were chosen from two compartments along the septotemporal axis of the hippocampus, namely rostral–dorsal, and caudal–ventral hippocampal regions, heretofore referred to as 'dorsal' and 'ventral', respectively. Dorsal cells were taken from coronal sections corresponding to −2.80 to −4.30 mm in the anterior–posterior axis from Bregma, and 2.50 to 3.50 mm deep from the skull surface. Selected neurons from ventral hippocampus were chosen from coronal sections corresponding to −4.80 to −6.04 mm in the anterior–posterior axis from Bregma, and 7.00–9.40 mm deep from the skull surface, using the rhinal fissure as a local landmark (Figure 1a,b) (Chowdhury et al., 2014a). Golgi-impregnated neurons were filled completely, such that pyramidal cell body, apical, and basal dendrites, and dendritic spines were clearly visible. Experimenters traced and analyzed the neurons while kept blind to the identity of the experimental groups of the animals. Cells to be traced were chosen such that their dendritic processes were not artificially broken within 300 μm of radius from the soma and were minimally overlapping with neighboring pyramidal cells. The cell body and apical dendrites of cells were traced in three dimensions using the Neurolucida system 11.07 (MicroBrightField Bioscience, Williston, VT) attached to an Olympus BX51 microscope (Olympus, Tokyo, Japan), using a 20× objective. Neurolucida Explorer software package was used to analyze the manual tracing of the neuron using the 'Sholl Analysis' option to quantify the number of intersections that the apical dendrite and its branches made with imaginary spheres centered at the center of the soma, beginning at a radius of 20 μm and increasing in distance by 20 μm (Chowdhury et al., 2014a,b). In cohort 1, Sholl analysis was performed on 12–20 cells per group from four animals (4–5 cells per animal). In Experiment 2, Sholl analysis was performed on 30–37 cells for both groups, with each group consisting of eight animals (3–5 cells per animal).

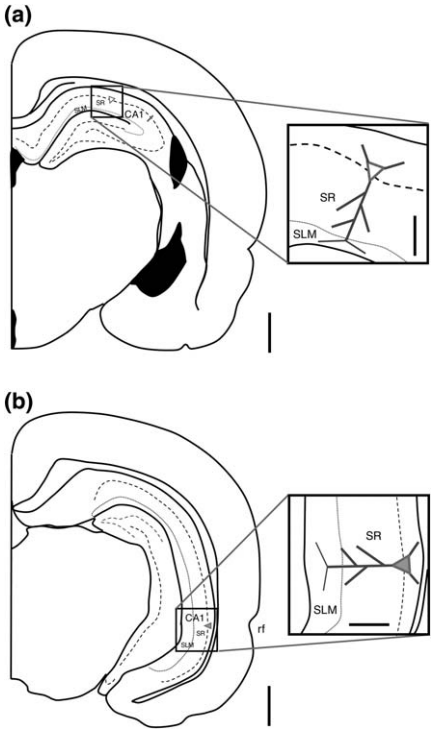

FIGURE 1 Schematics showing the outline of typical coronal sections selected for sampling CA1 pyramidal cells of the rostral–dorsal ['dorsal', (a)] and caudal–ventral ['ventral' (b)] hippocampus; see "Methods" for further details of the range of coordinates used to sample from the two compartments of the hippocampus. The inserts show orientations of pyramidal neurons and two distinct laminas of CA1, stratum radiatum (SR) and stratum lacunosum-moleculare (SLM). Calibration bar represents 2 mm for the hemispheres and 200 μm for the neurons

Spine density and percentage of mature spines were also analyzed. At least five segments of dendrites were sampled, consistently within stratum radiatum (SR). Because pyramidal neurons are undergoing overall growth during adolescence, the distance of SR from soma varied with age. The chosen dendritic branches occurred between 80 and 160 μm from the soma for brains collected at P35; 100 and 200 μm from the soma for brains collected at P40 and P44; 120 and 220 μm from the soma for brains collected at P50 and P55. Within SR, the segments were chosen to be near where the number of intersections made by the dendritic branches with imaginary spheres in the Sholl analysis were maximum for each age group.

We also analyzed spine density in stratum lacunosum-moleculare (SLM). Here, too, the distance of SLM from soma varied with age. Thus, the chosen dendritic branches occurred 260 and 360 μm from the soma for brains collected at P35; between 300 and 400 μm from the soma for brains collected at P40 and P44; between 340 and 440 μm from the soma for brains collected at P50 and P55). Five dendritic segments from SLM were analyzed per brain. In SLM, the segments were chosen to reside within 100 μm from the most distal imaginary sphere of the Sholl analysis.

For both SR and SLM, dendritic segments were traced using the Neurolucida software along with dendritic spines, using a 100× objective. Dendritic spines were categorized based on their morphological characteristics into four groups: thin (immature), filopodia (immature), stubby, and mushroom spines. The relative proportion of mature spines was calculated for each dendritic segment as the ratio of the density of mushroom and stubby spines to the total spine density (Chowdhury et al., 2014a,b).

2.4 | Statistical analysis

All statistical analyses were conducted, while keeping the experimenter blinded to the environmental condition and the animal's age at the time of euthanasia. Data from the Sholl analysis were analyzed using a repeated measure two-way ANOVA to reveal the overall group effect. Two-tailed Student's *t* test was used to assess the difference when two groups were being compared. Two-way ANOVA was used to evaluate the significance of differences among the five age groups and between the two hippocampal regions, using postnatal days and dorsal or ventral hippocampal CA1 as the two factors. One-way ANOVA was used when three or more groups were being compared within each hippocampal CA1 region. Significant pairs were determined using Tukey's HSD *post hoc* test. For all analyses, neuron was regarded as the independent unit of analysis (*N* value), and we pooled neurons from multiple animals of the same age and rearing condition. Detection of outliers was performed for each group, using the ROUT method (Motulsky & Brown, 2006). This method led to the detection of two values, no animal contained cluster of outliers, and the group mean

values was not altered by excluding the outliers. Thus, the data are presented without removing the outliers. Statistical analysis was done by SPSS statistical software 24.0 (IBM Corp., Armonk, NY) and GraphPad Prism 7.0. p values of $<.05$ were considered statistically significant.

3 | RESULTS

3.1 | Dendritic remodeling of CA1 pyramidal cells differs between dorsal and ventral hippocampus during adolescence

Figure 2a and f show examples of Neurolucida-traced Golgi-stained cells from the dorsal and ventral hippocampus of each age group. Sholl analysis was used to quantify apical dendritic branching of dorsal CA1 pyramidal neurons at incremental distances from the cell body. SR is a functionally distinct compartment of CA1, receiving excitatory afferents primarily but not exclusively from CA3 via Shaffer collaterals (Amaral, 2007). In contrast, SLM receives excitatory afferents selectively from lateral amygdala (Pikkarainen, Ronkko, Savander, Insausti, & Pitkanen, 1999), perforant path and thalamus (Cavdar et al., 2008). Since these contrasting afferent patterns contrasting functions, developmental changes in SR and SLM will be described separately.

3.2 | Remodeling in SR

Two-way ANOVA, showed a significant interaction between age (P35–55) and hippocampal region (dorsal vs. ventral) ($F(4,146) = 3.985$, $p = .004$), and a main effect of hippocampal regions ($F(1,146) = 31.24$, $p < .0001$) on the number of dendritic intersections in the SR. Two-way ANOVA also revealed a significant interaction between age and hippocampal region ($F(4,146) = 3.602$, $p = .008$), and main effect of hippocampal regions ($F(1,146) = 22.46$, $p < .0001$) on the total dendritic length in the SR.

In the dorsal SR, one-way ANOVA revealed a significant effect of age on the number of dendritic intersections (Figure 2b, $F(4,79) = 3.149$, $p = .019$]. Tukey's HSD *post hoc* analysis showed that the number of intersections was significantly decreased from P35 to P50 by 23% (Figure 2b, mean ± SEM at P35: 6.930 ± 0.477; at P50: 4.373 ± 0.544; $p < .05$). Cells from the P55 group had significantly more branches (55%) than cells from the P50 group (Figure 2b, P50: 4.373 ± 0.544; P55: 6.787 ± 0.503; $p < .05$). One-way ANOVA also revealed a significant effect of age on total dendritic lengths in the dorsal SR [Figure 2c, $F(4,79) = 3.021$, $p = .023$]. Tukey's HSD *post hoc* analysis showed that total dendritic lengths were significantly decreased by 39%, from P35 to P50 (Figure 2c, P35: 952.95 ± 65.98 μm; P50: 579.13 ± 83.95 μm; $p < .05$). Cells from the P55 group had significantly longer branches (49%) than cells from P50 the group (Figure 2c, P50: 579.13 ± 83.95 μm; P55: 864.7 ± 61.665 μm; $p < .05$).

In the ventral CA1, the effect of age was strikingly different from that of dorsal CA1. In the ventral SR, the number of intersections was significantly increased from P35 to P50 by 32% (Figure 2g, P35: 3.114 ± 0.37; P50: 4.82 ± 0.49; $p = .03$). Total dendritic length in the ventral SR also increased by 56% (Figure 2h, P35: 406.27 ± 54 μm; P50: 632.03 ± 59.21 μm; $p = .05$).

3.3 | Remodeling in SLM

Two-way ANOVA, using age (P35–55) and hippocampal regions (dorsal vs. ventral) as factors, showed a main effect of hippocampal regions ($F(1,146) = 6.097$, $p = .015$) on the number of dendritic intersections in the SLM.

In the dorsal SLM, the age effect on dendritic branching was similar to that in the SR: the number of intersections was significantly decreased from P35 to P50 by 46% (Figure 2d; P35: 2.11 ± 0.41; P50: 1.13 ± 0.26; $p = .036$). On the other hand, cells from the P55 group had significantly more branches than cells from the P50 group (Figure 2d; P50: 1.13 ± 0.26; P55: 2.33 ± 0.34; $p = .016$). The number of branches in the SLM of dorsal CA1 more than doubled during these 5 days of adolescence. Total dendritic lengths in the dorsal SLM also increased by 75% during these 5 days (Figure 2e; P50: 256.13 ± 60.88 μm; P55: 448.23 ± 62.46 μm; $p = .047$).

In the ventral SLM, total dendritic length increased marginally—by 36% from P50 to P55 (Figure 2j, P35: 278.79 ± 23.55 μm; P50: 378.23 ± 48.95 μm; $p = .078$). The number of intersections also increased by 33% (Figure 2i, P35: 1.98 ± 0.15; P50: 2.63 ± 0.39), but this difference did not reach statistical significance ($p = .12$).

3.4 | Dendritic spines of CA1 pyramidal cells in the dorsal hippocampus mature with age without changes in spine density

Dendritic spines are sites specialized for excitatory synaptic inputs onto pyramidal cells (Harris & Kater, 1994) and as such, provide indications of the extent of excitatory synaptic integration capable by each neuron. Spine density analysis was conducted to determine the effects of age on overall spine density as well as the relative densities of immature spines (thin spines and filopodia) and mature spines (mushroom and stubby spines) on CA1 pyramidal cells of the CA1 (High mag images as Figure 3a,b). Examples of dendritic segments from SR of the dorsal hippocampus at P35 and P55 groups are shown in Figure 3c and d, respectively.

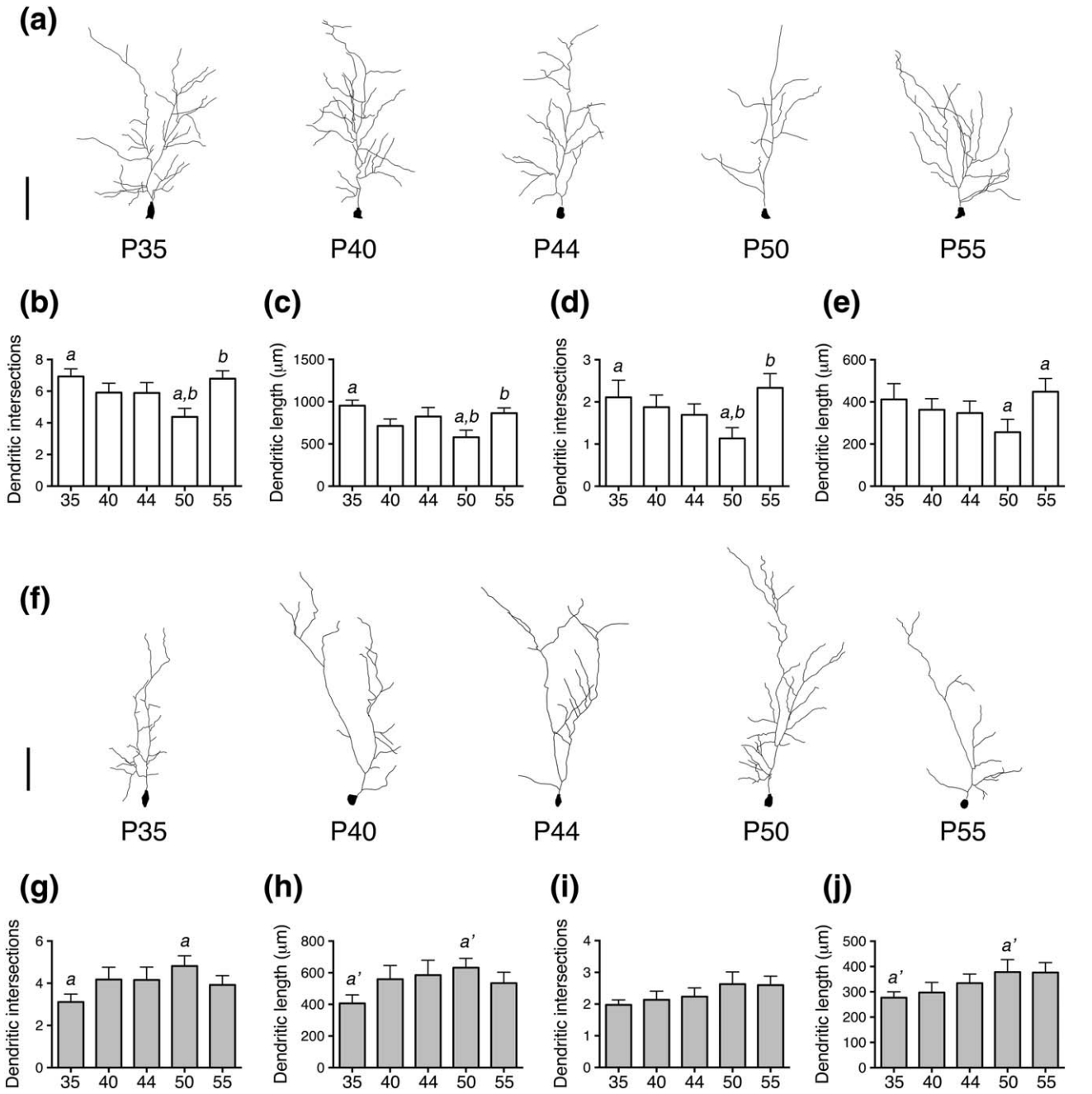

FIGURE 2 Apical dendrites of the dorsal and ventral hippocampal CA1 neurons exhibit growth and retraction during adolescence. (a) CA1 pyramidal cells of the dorsal hippocampus transiently decrease in complexity during adolescence. Representative Neurolucida tracings of dorsal hippocampal CA1 pyramidal cells from each age point. Calibration bar represents 100 μm. The average number of dendritic intersections and the total dendritic length in the SR and SLM were calculated for each cell within each age group. In dorsal hippocampal CA1, comparisons of dendritic branching across age groups revealed that the average number of dendritic intersections in the SR (b), and in the SLM (d) transiently decrease during adolescence. Comparisons of dendritic branching across age groups also revealed that the total dendritic length in the SR (c), and in the SLM (e) transiently decrease during adolescence. (f) CA1 pyramidal cells of the ventral hippocampus transiently increase in complexity during adolescence. Representative Neurolucida tracings of ventral hippocampal CA1 pyramidal cell from each age point. Calibration bar represents 100 μm. In ventral hippocampal CA1, comparisons of dendritic branching across age groups revealed that the average number of dendritic intersections in the SR (g), and in the SLM (i) transiently increase during adolescence. Comparisons of dendritic branching across age groups also revealed that the total dendritic length in the SR (h), and in the SLM (j) transiently increase during adolescence. Data from each group were compared across ages from P35 to P55. Bar graphs represent means + SEM ($N = 15–20$ cells per group). Same letters indicate significant differences between age groups as assessed by one-way ANOVA, followed by Tukey's HSD test. Same letters with the prime symbol indicate marginally significant differences ($p < .08$) between groups as assessed by Student's *t* tests

In the dorsal SR, one-way ANOVA showed no significant effect of age on spine density [Figure 3e, $F(4,90) = 0.811$, $p = .52$]. On the other hand, one-way ANOVA revealed a significant effect of age on the proportion of spines in dorsal SR that were mature [Figure 3f, $F(4,90) = 5.959$, $p = .0003$]. Tukey's HSD *post hoc* analysis showed that the proportion of spines that were mature was significantly higher at P44 (78.54 ± 3.45%), P50 (85.6 ± 5.04%), and P55 (81.1 ± 3.46%), compared with the P35 Group (61.547 ± 2.34%; Figure 3f, all $p < .05$).

This pattern for the dorsal SR was recapitulated in the dorsal SLM: one-way ANOVA showed no significant effect of age on the spine density [Figure 3g, $F(4,90) = 0.671$, $p = .61$], while one-way ANOVA revealed a significant effect of age on the proportion of spines that were mature [Figure 3h, $F(4,90) = 6.831$, $p < .0001$]. Tukey's HSD *post hoc* analysis showed that the proportion of mature spines was significantly higher at P50 (84.73 ± 2.2%) and P55 (87.41 ± 2.62%), compared with P35 (63.841 ± 4.82%; Figure 3h, $p < .05$) and P40 groups (69.47 ± 3.55%; Figure 3h, $p < .05$).

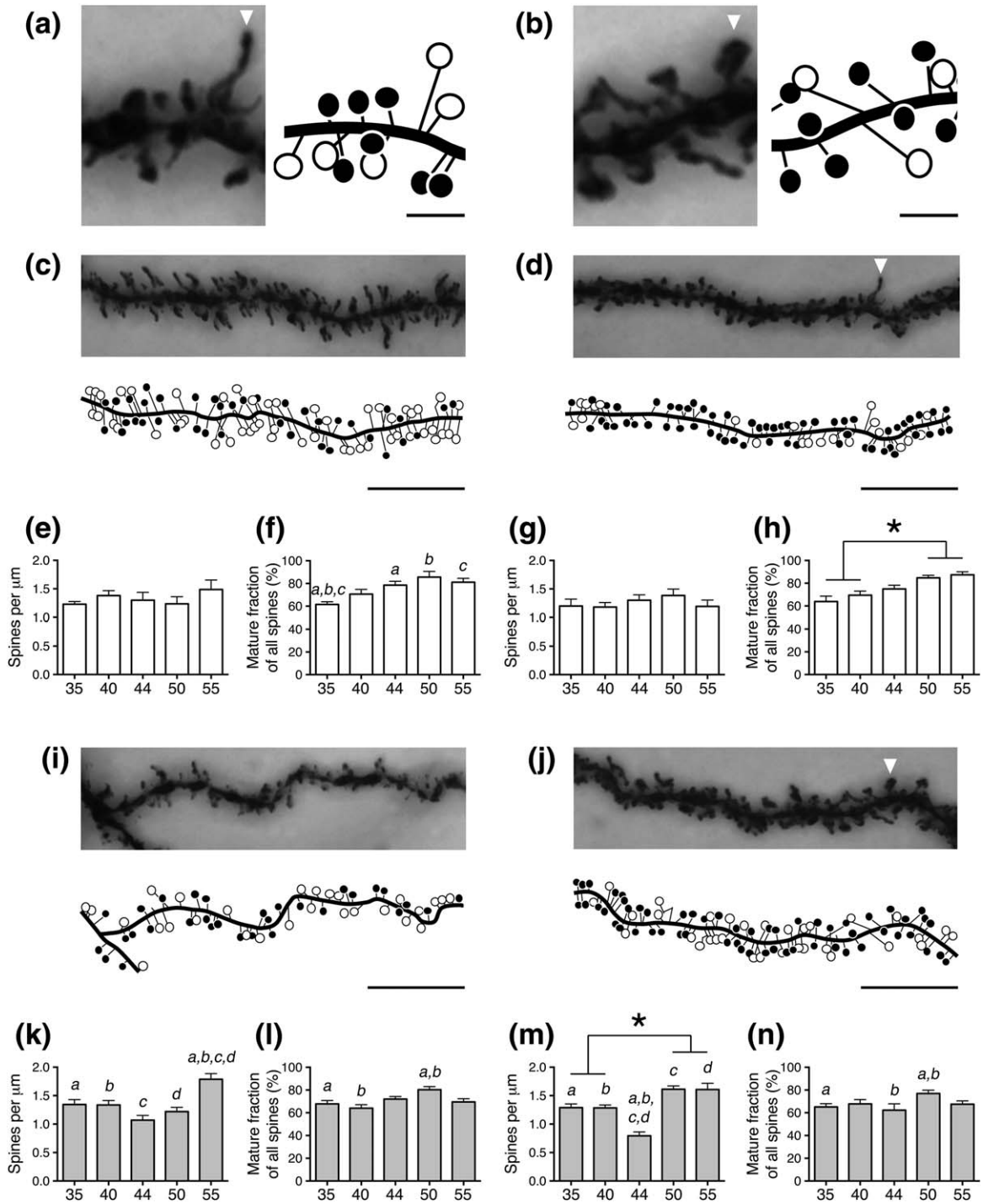

FIGURE 3.

3.5 | Dendritic spines of CA1 pyramidal cells in the ventral hippocampus mature with age and increase in spine density

In ventral SR, the effect of age on dendritic spine density was different from that of dorsal CA1. Examples of dendritic segments from the SLM of the ventral hippocampus at P35 and P55 groups are shown in Figure 3i and j, respectively. One-way ANOVA revealed a significant effect of age on the dendritic spine density in the SR of ventral CA1 [Figure 3k, $F(4,96) = 7.313$, $p < .0001$]. Tukey's HSD *post hoc* analysis showed that the spine density was significantly higher at P55 (1.79 ± 0.1 spines per μm), compared with all other groups (P35: 1.34 ± 0.09 spines per μm; P40: 1.34 ± 0.08 spines per μm; P44: 1.07 ± 0.08 spines per μm; P50: 1.22 ± 0.07 spines per μm; Figure 3k, all $p < .05$).

One-way ANOVA also showed a significant effect of age on the proportion of spines that were mature in the ventral SR [Figure 3l, $F(4,96) = 3.489$, $p = .01$]. Tukey's HSD *post hoc* analysis showed that the proportion of spines that were mature was significantly higher at P50 (80.28 ± 2.79%), compared with the P35 (67.81 ± 3.01%; Figure 3l, $p < .05$) and P40 (64.05 ± 3.11%; Figure 3l, $p < .05$) groups.

In the ventral SLM, one-way ANOVA showed a significant effect of age on dendritic spine density [Figure 3m, $F(4,100) = 19.01$, $p < .0001$]. Tukey's HSD *post hoc* analysis showed that the spine density was significantly higher at P50 (1.612 ± 0.059 spines per μm) and P55 (1.608 ± 0.11 spines per μm), compared with P35 (1.289 ± 0.066 spines per μm; Figure 3m, $p < .05$) and P40 (1.283 ± 0.052 spines per μm; Figure 3m, $p < .05$). Surprisingly, Tukey's HSD *post hoc* analysis also revealed that the spine density was significantly lower at P44 (0.793 ± 0.07 spines per μm), compared with all other groups (Figure 3m, all $p < .05$). The proportion of mature spines was also significantly higher at P50 (76.97 ± 2.9%), compared with the P35 (65.11 ± 2.96%; Figure 3n, $p < .05$) and P44 (62.31 ± 5.53%; Figure 3n, $p < .05$) groups.

3.6 | The effects of pair housing during adolescence are the opposite for dorsal versus ventral hippocampal CA1

Previously, we showed that environmental enrichment (wheel access) and stress (food restriction) elicit pathway-specific changes upon CA1 pyramidal neurons in the dorsal and ventral hippocampus of adolescent female rats (Chowdhury et al., 2014a). In that study, all animals had been singly-housed. Thus, it remained to be determined whether pair housing during adolescence also causes dendritic remodeling in the hippocampal CA1. Tissue from another cohort of 16 adolescent rats was used to address this question.

Dorsal CA1 pyramidal neurons' apical dendrites in pair-housed animals had overall marginally significantly greater number of dendritic intersections with Sholl spheres, relative to those from singly-housed controls [Figure 4a, $F(1,60) = 1.724$, $p = .19$]. The average number of intersections in the SR was significantly greater in the pair-housed group (4.673 ± 0.359) than in the singly-housed group [3.725 ± 0.281; Figure 4b; $t(60) = 2.095$, $p = .04$]. There was a trend for increased average dendritic lengths in the SR of pair-housed animals (615.753 ± 51.74 μm), compared with singly-housed controls [516.45 ± 40.93 μm; Figure 4c; $t(60) = 1.515$, $p = .13$].

In contrast to dorsal SR, dorsal SLM showed no difference in the number of intersections or dendritic lengths between singly-housed (intersections: 2.056 ± 0.25; length: 441.2 ± 46.37 μm) and pair-housed animals [intersections: 1.913 ± 0.27; Figure 4d; $t(60) = 0.389$, $p = 0.7$; length: 444.8 ± 46.36 μm; Figure 4e; $t(32) = 0.056$, $p = .96$].

FIGURE 3 Dendritic spine analysis reveals that the proportion of spines that are mature increases with age in the dorsal hippocampus, while spine density increases with age in the ventral hippocampus. Left panels (a) and (b) show the higher magnification of the representative projection of Golgi-stained apical dendritic segments in the SR of the dorsal hippocampus from the P55 group [panel (d)] and apical dendritic segments in the SLM of the ventral hippocampus from P55 group [panel (j)], created by joining portions in different planes of focus. White triangles indicate the specific spine showed in panel (a) and (d) (immature) and in panels (b) and (j) (mature). In the right panels (a) and (b), Neurolucida tracings of the same dendritic fragments are showed. Filled circles represent spines categorized as mature (mushroom or stubby spines). Empty circles represent spines categorized as immature (thin or filopodia spines). Calibration bar represents 2 μm. Representative projection of Golgi-stained apical dendritic segments in SR of the dorsal hippocampus from the P35 group [(c), top panel] and from the P55 group [(d), top panel], created by joining portions in different planes of focus. In the bottom of panels (c) and (d), Neurolucida tracings of the same dendritic fragments are shown. The calibration bar represents 10 μm. Density and proportion of mature spines in the SR and the SLM were calculated for each dendritic segment within each age group. In the dorsal hippocampal CA1, total spine density measured in the SR (e) and in the SLM (g) showed no significant difference across the ages. The proportion of mature spines in the SR (f) and the SLM (h) increased with age. Representative projection of Golgi-stained apical dendritic segments in SLM of the ventral hippocampus from P35 group [(i), top panel], and from P55 group [(j), top panel], created by joining portions in different planes of focus. In bottom panels (i) and (j), Neurolucida tracings of the same dendritic segments are showed. Calibration bar represents 10 μm. In the ventral hippocampal CA1, total spine density measured in the SR (k) and in the SLM (m) increased with age. The proportion of mature spines in the SR (l) and the SLM (n) increase transiently, with the peak at P50. Data from each group were compared across ages from P35 to P55. Bar graphs represent means + SEM ($N = 15–31$ fragments per group). Same letters indicate significant differences between age groups as assessed by one-way ANOVA, followed by Tukey's HSD test. The asterisk indicates significant difference across combined age groups, as assessed by the same statistical tests

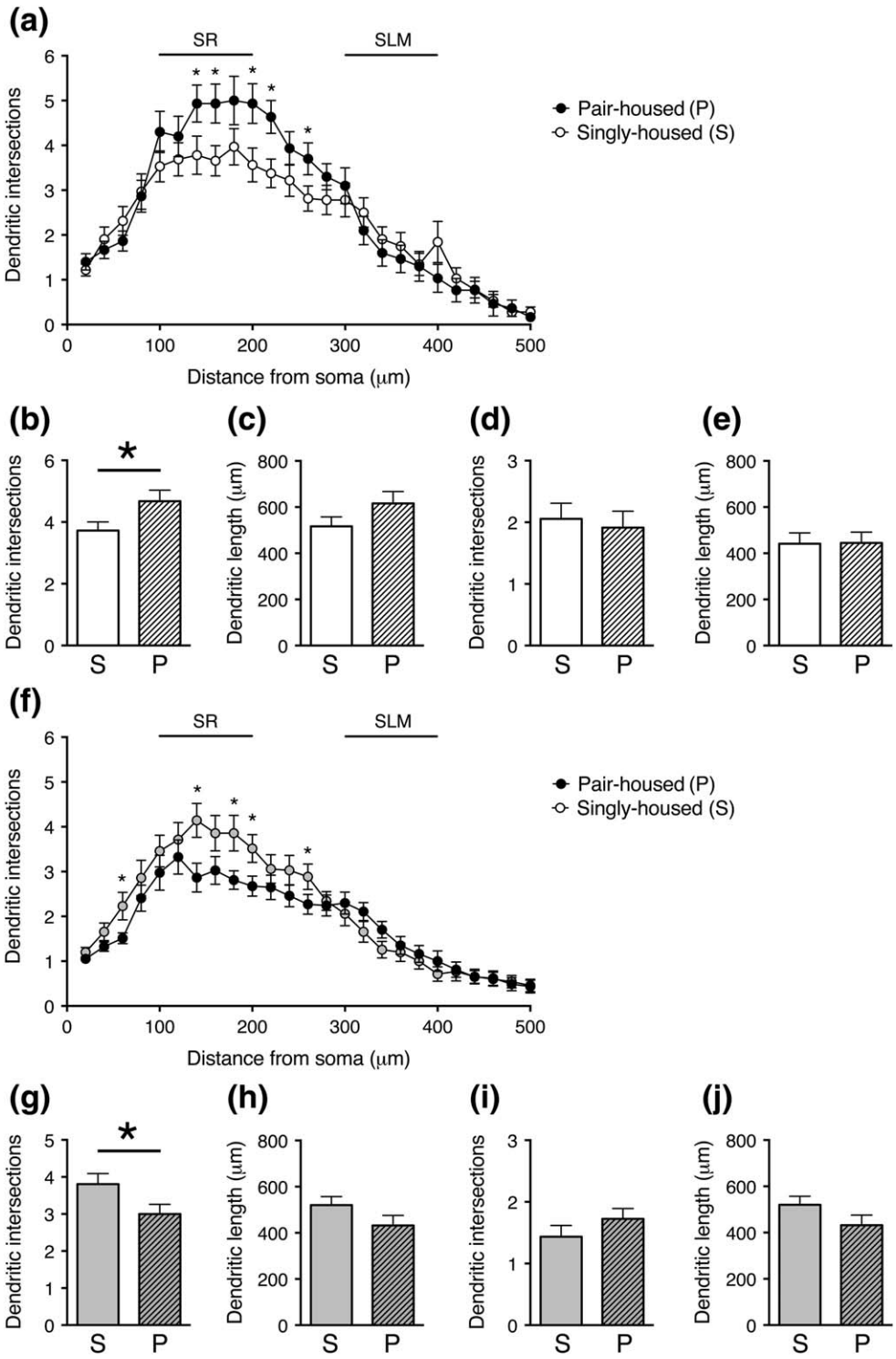

FIGURE 4 Pair housing during early to mid-adolescence has opposite effects on apical dendritic branching in dorsal versus ventral hippocampal CA1. The number of intersections with Sholl spheres centered at the soma of pair-housed animals' and singly-housed controls' dorsal (a) and ventral (f) CA1 at P44 are plotted against radii of the sphere as the means + SEM (dorsal: $N = 30$ cells for pair-housed, 32 cells for singly-housed group; ventral: $N = 37$ cells for pair-housed, 35 cells for singly-housed group). In the dorsal hippocampal CA1, pair housing during adolescence increases the average number of dendritic intersections in the SR (b), but not in the SLM (d). There is also a trend toward an increase in total dendritic lengths in the SR (c), but not in the SLM (e). In ventral hippocampal CA1, pair housing during adolescence decreases the average number of dendritic intersections in the SR (g), but not in the SLM (i). No significant difference between groups was found for total dendritic lengths in the SR (h) or the SLM (j). S, singly-housed controls. P, pair-housed animals. Asterisks indicate statistical significance with $p < .05$ by Student's t tests

Sholl analysis of neurons from the ventral hippocampus showed effects of pair housing that were the opposite of those observed in the dorsal hippocampus: pyramidal neurons of the pair-housed group exhibited marginally significantly fewer number of dendritic intersections with Sholl spheres, relative to those of singly-housed controls [Figure 4f; $F(1,70) = 3.082$, $p = .084$]. The average number of intersections in the SR was significantly smaller in the pair-housed group (3.0 ± 0.26) than in singly-housed controls [3.806 ± 0.29; Figure 4g; $t(70) = 2.088$, $p = .04$]. There was also a trend for decreased average dendritic lengths in the ventral SR of pair-housed animals (431.87 ± 43.77 μm), compared with singly-housed controls [520.13 ± 37.14 μm; Figure 4h; $t(70) = 1.529$, $p = .13$]. No difference was found in the number of intersections or dendritic lengths in the SLM of the ventral CA1 between the pair-housed group (intersections: 1.724 ± 0.17; length: 298.9 ± 21.16 μm) and singly-housed controls [intersections: 1.434 ± 0.18; Figure 4i, $t(70) = 1.167$, $p = .25$; length: 281.1 ± 32.69 μm; Figure 4j, $t(46) = 0.475$, $p = .64$].

3.7 | Lamina-specific effects of pair housing during adolescence on dendritic spine density of the dorsal and ventral hippocampal CA1 neurons

Spine density analysis was conducted to study the effects of pair housing during adolescence on overall spine density and proportion of mature spines. Examples of dendritic segments from the SR of the dorsal hippocampus of singly-housed and pair-housed animals are shown in Figure 5a and b, respectively. SR of the dorsal and ventral hippocampal CA1 showed no difference between pair-housed and singly-house groups in terms of spine density (Figure 5c and g) or the proportion of spines that were mature (Figure 5d and h). On the other hand, in SLM of both dorsal and ventral hippocampal CA1, spine density was significantly increased among neurons in the pair-housed group, compared with neurons from the singly-housed group [dorsal: Figure 5e, $t(34) = 3.28$, $p = .002$; ventral: Figure 5i, $t(29) = 2.22$, $p = .03$]. No difference between groups was found regarding the proportion of spines that were mature in SLM of dorsal or ventral hippocampal CA1 (Figure 5f and j).

4 | DISCUSSION

To the best of our knowledge, this is the first systematic study of the development of pyramidal neurons in the hippocampal CA1 of female rats during adolescence. By sampling neurons at multiple time points, we were able to capture global retractions and growth spurts during adolescence that would have been missed with fewer time points. We demonstrate that during adolescence, the dendritic arbor and spines of pyramidal cells in the dorsal and ventral hippocampal CA1 are remodeled differently. Figure 6 summarizes the structural changes of dendritic lengths, branching complexity, and spine density, ultimately providing an estimate of spine number (spine density × dendritic length) in the dorsal and ventral CA1 neurons from early-, mid-, and late-adolescence.

The dorsal hippocampi of singly-housed female rats exhibit transient global retractions during early- to mid-adolescence, followed by protracted maturation, evident as a 55% growth spurt of dendritic branching and 49% growth spurt in total dendritic lengths between P50 and P55. The transient decrease of dendritic branching from P35 to P50 is consistent with a previous finding that the total number of dendritic spines decreases during adolescence from P35 to P49 in female rats (Yildirim et al., 2008).

CA1 pyramidal cells of the ventral hippocampi of singly-housed female rats exhibit earlier, transient lamina-specific growth during adolescence, since dendrites of increase in branching in the SR between the adolescent ages of P44 and P50, then retract toward late adolescence/early adulthood (P55), with no concomitant change in the SLM. These findings of growth, followed by retraction of dendritic complexity in SR of the ventral hippocampus from mid- to late-adolescence, are consistent with our previous finding (Chowdhury et al., 2014b).

4.1 | Dendrites spines of hippocampal CA1 neurons exhibit developmental changes that are different for the dorsal versus ventral sectors

Dendritic spine density and maturity in the hippocampal CA1 also vary with age. Dendritic spines in the dorsal hippocampus follow a predictable pattern. They mature with age but do not change in density. This suggests transitioning of existing spines from newly formed immature types to relatively mature stable types from P35 to P44 toward P55. However, because of the transient decrease in dendritic branching and lengths at mid-adolescence (P50), total spine number in SR is estimated to also undergo a transient decrease at this time. What pivotal event may be associated with mid-adolescence is an interesting and important question for future studies.

The observed constant spine density during adolescence differs from the pruning in spine density reported previously for adolescent female mice (Afroz et al., 2016). We focused on the apical dendritic tree of the pyramidal neurons, while Afroz and colleagues pooled the segments from both basal and apical dendrites and did not differentiate measurements in the dorsal versus ventral hippocampus. The difference between the two studies suggests that the relative influence of different afferent inputs on basal and apical dendritic trees may change during adolescent development, with pruning occurring within the basal dendrites (part of data of Afroz et al.,) and no pruning of apical dendrites (our data).

In the ventral hippocampus, dendritic spines change during adolescence in a less predictable way. Dendritic density increases during late adolescence, but there is also a transient enrichment of mature spines during mid-adolescence. These findings suggest the addition of immature spines

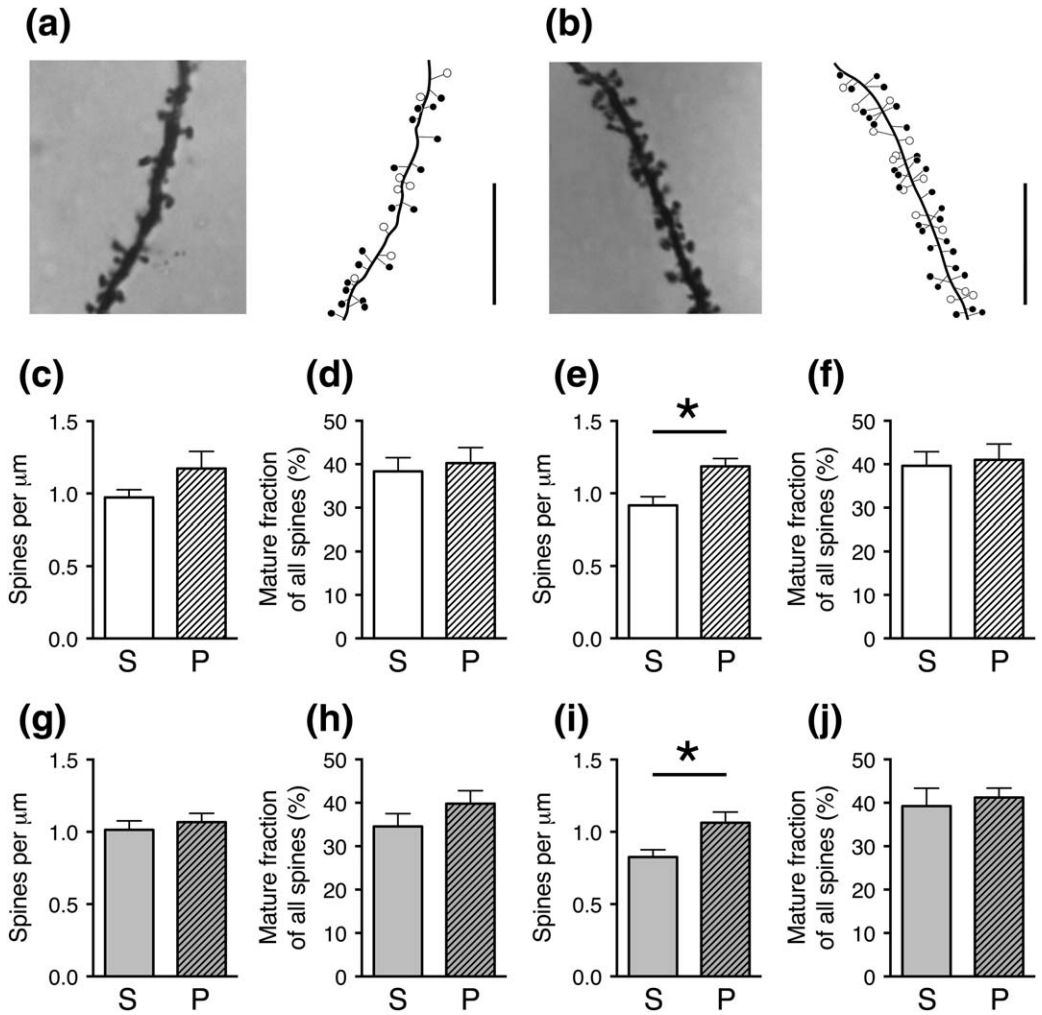

FIGURE 5 Pair housing during adolescence increases dendritic spine density in the SLM of dorsal and ventral hippocampal CA1. Representative projection of Golgi-stained apical dendritic fragments in the SLM of the dorsal hippocampus from the singly-housed control [(a) left panel] and from the pair-housed group [(b) left panel], created by joining portions in different planes of focus. In right panels (a) and (b), Neurolucida tracings of the same dendritic fragments are shown. Filled circles represent spines categorized as mature (mushroom or stubby spines). Empty circles represent spines categorized as immature (thin spines or filopodia). Calibration bar represents 10 μm. Density and proportion of mature spines in the SR and the SLM were calculated for each dendritic fragment within each group. In both dorsal and ventral hippocampal CA1, total spine density (mature + immature) was not different between the groups in the SR [dorsal: panel (c); ventral: panel (g)], but was greater in the SLM of pair-housed animals [dorsal: panel (e); ventral: panel (i)]. The proportion of spines that were mature was not significantly different across the groups in the SR [dorsal: panel (d); ventral: panel (h)] or the SLM [dorsal: panel (f); ventral: panel (j)]. Bar graphs represent means + SEM (N = 16–33 fragments per group). S, singly-housed controls. P, pair-housed animals. Asterisks indicate statistically significant difference with $p < .05$ by Student's t tests

during late adolescence. Since late adolescence is when dendrites are also lengthening, there is a significant net increase of spines, including immature ones, during late adolescence. What environmental, endocrine or afferent activity factors may contribute to the late adolescent phase of spine addition in SR of the ventral hippocampus is another interesting question for future studies.

One potential factor driving spine changes in the dorsal hippocampus at mid-adolescence and in the ventral hippocampus at late adolescence could be the estrogen receptor alpha (ERα) and G-protein-coupled estrogen receptor type 1 (GPER1): both of these have been demonstrated to occur in dendritic spines of the hippocampus, including those in SR of dorsal CA1, and to participate in synaptic plasticity (McEwen et al. 2001; Waters et al., 2015). GPER1 shows a dorsal-ventral distinction in its effects on cell proliferation in the hippocampus of adult female rats (Duarte-Guterman, Lieblich, Chow, & Galea, 2015). The hippocampus expresses ERα and β from prenatal stages (Gerlach, McEwen, Toran-Allerand, & Friedman, 1983; González et al., 2007) through adulthood (González et al., 2007; Hart, Patton, & Woolley, 2001; McEwen et al., 2001; Scudiero & Verderame, 2017) and with contrasting patterns across pyramidal versus GABAergic neurons in the dorsal versus ventral sectors (Hart et al., 2001). It remains unexplored whether the expression of any of these receptors exhibit transient rises and dips across cell types or across dorsal-to-ventral sectors of the hippocampus during adolescence.

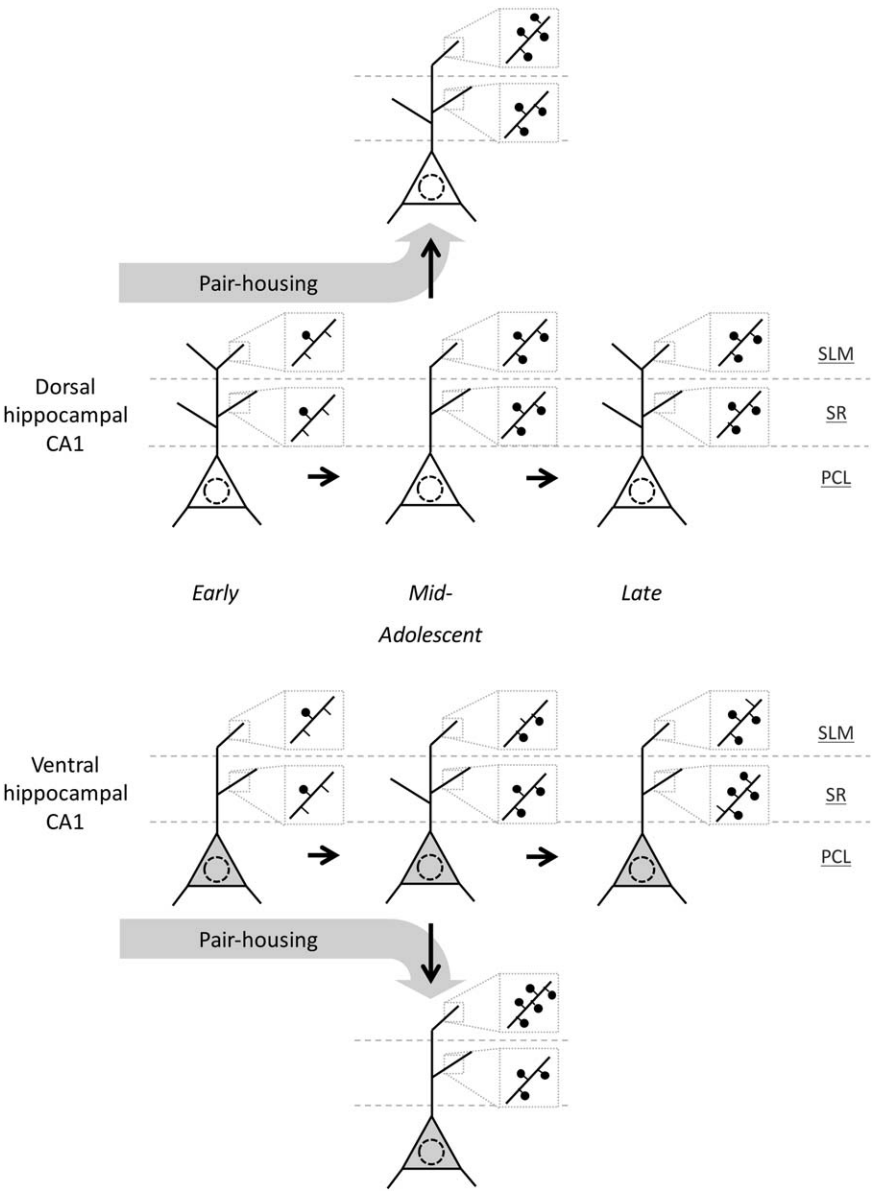

FIGURE 6 Summary of lamina-specific changes in dendrites of the dorsal and ventral hippocampal CA1 pyramidal neurons during adolescent development (right-ward black arrows) and dendritic remodeling evoked by pair housing during adolescence (up- and downward arrows). (1) Pyramidal neurons of the dorsal hippocampus transiently decrease in dendritic complexity, while pyramidal neurons of the ventral hippocampus transiently increase in dendritic complexity during adolescence. (2) Dendritic spines in the dorsal hippocampus mature with age but do not change in density. In the ventral hippocampus, dendritic spine density increases during adolescence and exhibits transient enrichment of mature spines during mid-adolescence. (3) Pair housing during early to mid-adolescence (gray arrows) increase apical dendritic branching in the dorsal hippocampus, but decrease dendritic branching in the ventral hippocampus. (4) Pair housing during adolescence increases dendritic spine density specifically in the SLM of both dorsal and ventral hippocampal CA1

4.2 | Dendrites of the dorsal and ventral hippocampal CA1 neurons exhibit transient lamina-specific growths and retractions during adolescence

The hippocampus is a component of the limbic system that plays a role in spatial learning, memory and the regulation of stress (Bannerman et al., 2003; Henke, 1990; McCormick et al., 2010; Moser, Moser, Forrest, Andersen, & Morris, 1995). The hippocampus undergoes robust development during the onset of puberty and with the introduction of the gonadal hormones (Andersen & Teicher, 2004; Zitman and Richter-Levin, 2013).

An early introduction to stress can cause structural changes in the hippocampus (Buwalda, Geerdink, Vidal, & Koolhaas, 2011; Salas-Ramirez, Frankfurt, Alexander, Luine, & Friedman, 2010) such as the size of the hippocampus (Leussis & Andersen, 2008) and dendritic remodeling of the apical CA1 pyramidal cells (Chowdhury et al., 2014a,b; Isgor, Kabbaj, Akil, & Watson, 2004). Changes in the levels of glucocorticoid receptors in the hippocampus are also associated with changes in the morphology, either increasing or decreasing the length and branching in the dendrites of the

pyramidal neurons (Andersen & Teicher, 2004; Loi, Koricka, Lucassen, & Joels, 2014; Toledo-Rodriguez & Sandi, 2011). In fact, our observations of transient growth and retraction in the dendritic branching during adolescence, with peak and dip around P44 to P50, echo the results from previous findings, namely that the stress-induced corticosterone response is lowest at P50 during adolescence (Foilb, Lui, & Romeo, 2011; Romeo, 2013).

The dorsal hippocampus forms a cortical network with the retrosplenial and anterior cingulate cortical areas that connect to other sensorimotor cortical areas and mediate cognitive processes, such as learning, memory, navigation, and exploration (Fanselow & Dong, 2010). The ventral hippocampus is connected with the amygdalar nuclei, bed nucleus of the stria terminalis, olfactory bulb, lateral septum, nucleus accumbens, and infralimbic and prelimbic areas of the medial prefrontal cortex (Adhikari, Topiwala, & Gordon, 2010; Adhikari, Topiwala, & Gordon, 2011; Fanselow & Dong, 2010). Ultimately, all of these structures innervate the hypothalamus, thus mediating motivated behaviors with strong emotional components. The ventral hippocampus receives connections from the medial division of the entorhinal cortex, which receives olfactory, gustatory, and visceral inputs (Fanselow & Dong, 2010). Thus, the transient growth and retraction of dendritic branching in the hippocampus during adolescence may contribute to the instability of emotional regulation and control of cognitive behaviors during adolescence (Geier, 2013). The U-shaped developmental trajectory of dorsal hippocampal CA1 dendrites may contribute to the reduced ability of cognitive control and increased impulsivity during mid-adolescence. The inverted U-shaped developmental curve in the ventral hippocampal CA1 dendrites may contribute to the particularly strong reactivity in the form of anxiety that adolescents exhibit in response to stress (Dahl, 2004; Romeo, 2010).

4.3 | Pair housing causes morphological changes during puberty across hippocampal regions

Our data show that pair housing during early to mid-adolescence increases apical dendritic branching of pyramidal neurons in the dorsal hippocampus, but decreases apical dendritic complexity of pyramidal neurons in the ventral hippocampus. These effects could be due to greater sensory stimulation and/or additional factors associated with pair housing. Previous studies also reported enhanced dendritic growth and branching in layer III of parietal cortex of puberty rats reared in enriched environments consisting of group housing, shelter, plastic toys, and a running wheel (Leggio et al., 2005). On the other hand, previous studies of neuronal development have concentrated on sensory cortices, and a large body of evidence exists describing dendritic remodeling following sensory deprivation during the juvenile critical period that is well before puberty (Antonini, Fagiolini, & Stryker, 1999; Fagiolini, Pizzorusso, Berardi, Domenici, & Maffei, 1994; Hensch & Stryker, 2004; Kirkwood, Lee, & Bear, 1995; Valverde, 1967). Not only has increased spine density been found in adolescent and adult rodent brains following rearing in an enriched environment (Globus, Rosenzweig, Bennett, & Diamond, 1973; Jung & Herms, 2014), but there are also reports of transient increases in spine number and in the turnover rate of spines in adulthood (Jung & Herms, 2014). Furthermore, previous studies also report that post-weaning social isolation upon males induces reductions in dendritic spine density on hippocampal pyramidal neurons (Silva-Gomez, Rojas, Juarez, & Flores, 2003), enhancements in hippocampal potassium ion channel currents (Quan, Tian, Xu, Zhang, & Yang, 2010), and alterations in hippocampal cell proliferation (McCormick et al., 2010). Our study adds to this large body of literature by showing that enrichment of adolescent females in the form of pair housing has influences on dendritic remodeling.

We have previously shown that food restriction, combined with social isolation, contributed to a dorsal–ventral distinction of changes in dendritic branching of CA1 pyramidal neurons (Chowdhury et al., 2014a). Our current results further indicate that apical dendritic structure of CA1 pyramidal neurons is modifiable during adolescence with less environmental perturbation (just isolation, no food restriction). Moreover, the direction of changes in apical dendritic structure under pair housing is the opposite between dorsal and ventral hippocampus, which may be related to the distinct pattern of connectivity of the hippocampal subregions with other brain regions, as was discussed in the previous section. Thus, the increase in dendritic branching in dorsal hippocampal CA1 may be due to increased sensory stimulation by pair housing. On the other hand, in ventral hippocampal CA1, the decrease in dendritic branching may be attributable to the stress-reducing effect of pair housing that leads to less synaptic inputs from the amygdala.

Within a single CA1 pyramidal neuron, the apical dendrites integrate information arriving from several different sources. Therefore, remodeling of dendrites in one particular layer may imply the importance of inputs arriving by that particular pathway, within specific laminae. The increased dendritic branching in the SR of dorsal CA1 of pair-housing animals may be caused by increased probability of these pyramidal neurons to receive inputs from neighboring CA1 pyramidal neurons and CA3 pyramidal cells. In the ventral CA1, our data indicate a decrease in dendritic branching in the SR with pair housing, which may be caused by decreased afferent activity in the SR, arising from the entorhinal cortex or basal nucleus of the amygdala (Pikkarainen et al., 1999), in addition to the neighboring CA1 and CA3 pyramidal neurons. While in the SR of the dorsal and ventral CA1 showed changes in response to pair housing, there was no difference in dendritic branching of the SLM across the rearing groups. This suggests that the pair housing-evoked plasticity was more strongly influenced by afferents arising from the CA3, neighboring CA1 cells, or local inhibition to the SR dendrites than by the entorhinal cortical inputs to the SLM. However, pair housing during adolescence increases dendritic spine density specifically in the SLM of both dorsal and ventral hippocampal CA1, which might suggest an increase in the ratio between excitation and inhibition in the SLM of both dorsal and ventral hippocampal CA1 regions that receive inputs from the entorhinal cortical areas. SLM is the recipient zone of excitatory inputs to the thalamic reuniens nucleus (Herkenham, 1978). This pathway is important for mediating the communication in the prefrontal cortex-to-hippocampus direction (Roy, Svensson, Mazeh, & Kocsis, 2017) as a return pathway to the more well-studied connection from the ventral hippocampus to the prefrontal cortex that mediate regulation of anxiety (Adhikari et al., 2010, 2011).

4.4 | Closing remarks

In this study, we showed that the dorsal and ventral hippocampi of singly-housed female animals exhibit layer-specific changes through development, evident as substantial and dynamic remodeling within the 20 days spanning early-, mid-, and late- adolescence (P35–P55). These findings indicate that pyramidal cells in the dorsal and the ventral hippocampal CA1 are remodeled profoundly and differently in ways that might contribute to variability in emotional regulations and cognitive controls during adolescence. We also show that pair housing with another female from P36 to 44 significantly increases branching in the SR of dorsal hippocampus and reduces the branching in the SR of ventral hippocampus in female rats, which suggests that pair housing during adolescence elicits pathway-specific changes in the hippocampus that may underlie the behavioral benefits observed in social housing. Taken together with data on spine density, these results strengthen the hypothesis that the hippocampus is particularly sensitive to experience-dependent environmental changes during adolescence. Previous study indicated that the adolescent trait of impulsivity is normalized by enriched rearing during adolescence (Adriani et al., 2006). Our previous data also suggest a dorsal–ventral distinction of changes in dendritic branching elicited by food restriction stress after puberty (Chowdhury et al., 2014a), and food restriction experience during adolescence is beneficial for cognitive performances in early adulthood (Chowdhury, Fenton, & Aoki, 2017, Under review). To understand how pair housing and the anatomical changes in the SR and SLM of the dorsal and ventral hippocampal CA1 contribute to behaviors along the developmental trajectory of adolescence, it is necessary to examine how neuroanatomical changes in afferent and efferent pathways of the hippocampus CA1 impact function. Our future studies will incorporate electrophysiology with behavioral testing at early-, mid-, and late-adolescence to explore how functional changes in circuits are involved in the hippocampus-dependent behaviors.

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CONFLICT OF INTEREST

We declare no conflict of interest in relation with the work described.

ORCID

Chiye Aoki http://orcid.org/0000-0003-4010-9425

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