

Activity-based anorexia has differential effects on apical dendritic branching in dorsal and ventral hippocampal CA1

Tara G. Chowdhury · Nicole C. Barbarich-Marsteller ·
Thomas E. Chan · Chiye Aoki

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Abstract Anorexia nervosa (AN) is an eating disorder to which adolescent females are particularly vulnerable. Like AN, activity-based anorexia (ABA), a rodent model of AN, results in elevation of stress hormones and has genetic links to anxiety disorders. The hippocampus plays a key role in the regulation of anxiety and responds with structural changes to hormones and stress, suggesting that it may play a role in AN. The hippocampus of ABA animals exhibits increased brain-derived neurotrophic factor and increased GABA receptor expression, but the structural effects of ABA have not been studied. We used Golgi staining of neurons to determine whether ABA in female rats during adolescence results in structural changes to the apical dendrites in hippocampal CA1 and contrasted to the effects of food restriction (FR) and exercise (EX), the environmental factors used to induce ABA. In the dorsal hippocampus, which preferentially mediates spatial learning and cognition, cells of ABA animals had less total dendritic length and fewer dendritic branches in stratum radiatum (SR) than in control (CON). In the ventral hippocampus, which preferentially mediates anxiety, ABA evoked more branching in SR than CON. In both dorsal and ventral

regions, the main effect of exercise was localized to the SR while the main effect of food restriction occurred in the stratum lacunosum-moleculare. Taken together with data on spine density, these results indicate that ABA elicits pathway-specific changes in the hippocampus that may underlie the increased anxiety and reduced behavioral flexibility observed in ABA.

Keywords Anorexia nervosa · Exercise · Food restriction · CA1 · Dendrites

Introduction

Anorexia nervosa (AN) is a neuropsychiatric disorder characterized by excessively restricted caloric intake and abnormally high levels of physical activity. The physiological, biochemical, and behavioral aspects of the disorder are poorly understood, making treatment elusive and resulting in a high mortality rate. Despite the implicated roles of brain regions such as the hypothalamus, prefrontal cortex, nucleus accumbens, and ventral tegmental area, it is unclear whether their involvement is causative or consequential of AN (Kaye et al. 2009).

Attempts to treat AN with antidepressants, selective serotonin reuptake inhibitors, and benzodiazepines have limited effects, at best (Aigner et al. 2011; Barbarich-Marsteller et al. 2012; Ferguson et al. 1999; Kaye et al. 1998; Hay and Claudino 2012). In females, the onset of puberty is associated with mood swings and anxiety (Buchanan et al. 1992; Hayward and Sanborn 2002). AN most commonly presents in adolescent females, suggesting that changes in affect triggered by hormonal changes at puberty may be responsible for an increased vulnerability to AN (Kaye 2009).

T. G. Chowdhury · T. E. Chan · C. Aoki (✉)
Center for Neural Science, New York University,
New York, NY 10003, USA
e-mail: ca3@nyu.edu; chiye@cns.nyu.edu

N. C. Barbarich-Marsteller
Department of Psychiatry, College of Physicians and Surgeons
of Columbia University, New York, NY 10032, USA

N. C. Barbarich-Marsteller
New York State Psychiatric Institute, New York,
NY 10032, USA

Activity-based anorexia (ABA) is a bio-behavioral phenomenon that occurs in mice and rats placed on a restricted feeding schedule and given access to a running wheel. The onset of food restriction results in hyperactivity, and the combination of caloric restriction and over-exercise results in severe weight loss (Hall and Hanford 1954; Routtenberg and Kuznesof 1967; Epling and Pierce 1996; Barbarich-Marsteller et al. 2013a). Remarkably, many ABA animals continue to run throughout the period of food access, thereby promoting *voluntary* self-starvation and, if not stopped, resulting in death. This animal model exhibits many phenotypes of AN including hyperactivity (Davis et al. 1999), reduction of food intake (Gelegen et al. 2008), hypothermia (Gutiérrez et al. 2006; Gutiérrez et al. 2009), hypoleptinemia (Hebebrand et al. 2006), and increased expression of dopamine D2 receptors and brain-derived neurotrophic factor (BDNF) (Gelegen et al. 2008).

While moderate food restriction and exercise have been shown to have beneficial effects on cognition (Berchtold et al. 2010; Vaynman et al. 2004), ABA animals exhibit hyperactivity and over-exercise, which exacerbates their self-starvation. The addition of exercise results in elevated corticosterone levels in food restricted animals (Burden et al. 1993). ABA during adolescence leads to increased anxiety in adulthood (Kinzig and Hargrave 2010), and animal strains that exhibit anxiety traits have greater vulnerability to ABA (Gelegen et al. 2007, 2008).

As part of the limbic system, the hippocampus plays an important role in regulating anxiety. Local application of benzodiazepines to the dorsal hippocampus relieves anxiety, suggesting a role of GABAergic inhibition in the hippocampus in regulation of anxiety (Engin and Treit 2008). Physical exercise has been shown to promote increased growth and dendritic branching of pyramidal cells of the hippocampus by increasing levels of growth factors such as BDNF, fibroblast growth factor (FGF-2), and nerve growth factor (NGF) (Gómez-Pinilla et al. 1997; Neeper et al. 1996; Stranahan et al. 2007, 2009).

The effects of food restriction are more difficult to generalize across studies. Moderate food restriction has been shown to be associated with increased BDNF levels in the CA1 and CA3 hippocampus in adult mice (Lee et al. 2002) but decreased BDNF in dentate gyrus in adult rats (Andrade et al. 2006). Furthermore, in combination with voluntary exercise, food restriction has been shown to increase BDNF in the total CA region of hippocampus of adult female C57BL/6J mice but not A/J mice (Gelegen et al. 2008). This difference suggests a genetic basis for the responsiveness of the hippocampus to ABA.

The hippocampus has been shown to exhibit structural and functional alterations in response to stress (McEwen 1999), hormones (Woolley and McEwen 1992), and the interaction of hormones and stress (McLaughlin et al. 2010),

suggesting that it may be particularly sensitive to changes during adolescence and in females, the population among whom AN is most prevalent (DSM-IV-TR; American Psychiatric Association 2000). The dorsal and ventral regions of the hippocampus have been shown to be preferentially involved in spatial learning and anxiety, respectively (McHugh et al. 2011). In addition, pyramidal cells of the ventral hippocampus are more susceptible to the effect of glucocorticoids; total dendritic length decreases in CA3 and spine density decreases in both CA3 and CA1 in response to systemic dexamethasone (Silva-Gómez et al. 2012).

With these results in mind, the goal of the current study was to analyze the effects of ABA and of its two components separately, i.e., exercise and food restriction, on the morphology of CA1 pyramidal neurons in the dorsal and ventral hippocampus of adolescent female rats.

Materials and methods

ABA induction and behavioral controls

Sixteen female Sprague–Dawley rats (two cohorts of eight animals each) were purchased from Taconic Farms and delivered to the New York State Psychiatric Institute's animal facility on postnatal day 21 (P21). Upon arrival, animals were individually housed in the absence of males. Access to water was provided *ad libitum* throughout the study and standard rat chow was used (LabDiet, ProLab RHM 300). Rats were assigned to four experimental groups of four animals each: control (CON; *ad libitum* food access, no wheel access), ABA (ABA; 1 h per day food access, *ad libitum* wheel access), exercise control (EX; *ad libitum* food and wheel access), and food restriction control (FR; 1 h per day food access, no wheel access). The behavioral experiments were divided into two cohorts of eight animals, each including two animals from each of the four experimental groups. Beginning on P37, ABA and EX animals were each housed in a standard-sized home cage with a running wheel attached (Med Associates, Inc., St. Albans, VT, USA). Baseline wheel running in the presence of *ad libitum* food access was measured for 3 days in ABA and EX groups. Restricted food access began on day 4 for ABA and FR groups, with animals receiving unlimited access to food for 1 hour per day at the onset of the dark cycle. Body weight, food intake, and running wheel activity (where applicable) were measured daily within 20 min prior to the onset of the dark cycle. All procedures described here were in accordance with the Institutional Animal Care and Use Committees of the New York State Psychiatric Institute, Columbia University (Animal Welfare Assurance #A3007-01) and New York University (Animal Welfare Assurance #A3317-01).

Brain collection and tissue processing

Animals were euthanized on day 7 between the hours of 7–9 a.m., at the end of the light cycle. The animals were deeply anesthetized using urethane (34 %; 0.65–0.85 mL/185 g body weight, intraperitoneal injection). The animals were decapitated, and the brains were quickly removed from the skull. One hemisphere was divided along the coronal plane into two blocks of 3–4 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, USA). Coronal sections 250- μ m thick were made using a Leica VT1000M vibratome (Leica Microsystems GmbH, Wetzlar, Germany).

Sholl analysis

Golgi-impregnated neurons were filled completely such that pyramidal cell somata, apical and basal dendrites, and dendritic spines were clearly visible in the CA1 hippocampus (Fig. 1a). No differences were noted in staining quality between the different regions of the hippocampus. Sholl analysis of pyramidal cells in the CA1 region of hippocampus was conducted 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 μ m and increasing in size by increments of 20 μ m (Fig. 1b).

Cells to be traced were chosen such that their dendritic processes were not artificially broken within 200 μ m of the soma and were minimally overlapping with neighboring stained cells. The cell body and apical dendrites of cells were traced in three dimensions using Neurolucida program (MicroBrightField Inc., Williston, VT, USA) attached to an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan). Neurolucida Explorer software package was used to analyze the reconstruction of the neuron using the built-in Sholl analysis option to quantify the number of dendritic crossings made with concentric spheres around the soma center with radii increasing in increments of 20 μ m. Tracing and analysis were conducted while experimenters were blind to the identity and experimental group of the animal.

CA1 pyramidal neurons were chosen from three compartments along the septo-temporal axis of the hippocampus, namely the rostral-dorsal, caudal-dorsal, and caudal-ventral regions. Rostral-dorsal cells were taken from coronal sections approximately -2.80 to -4.30 mm from Bregma and 2.50–3.50 mm from the dorsal surface, and caudal-dorsal and caudal-ventral cells were chosen from coronal sections approximately -4.80 to -6.04 mm from Bregma, dorsal cells 2.50–3.50 mm from the dorsal surface

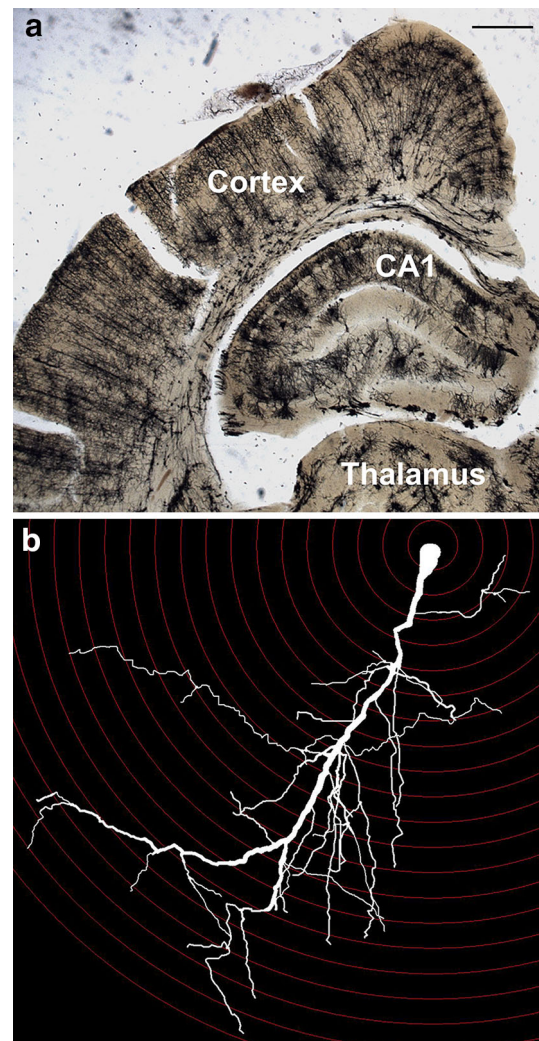


Fig. 1 Representative CA1 pyramidal cell from rostral-dorsal hippocampus traced using Neurolucida and analyzed by the Sholl method. **a** Golgi-Cox stained coronal section of rostral-dorsal hippocampus approximately -3.00 mm from Bregma; scale bar marks 1 mm. **b** Digital reconstruction of CA1 pyramidal cell traced using Neurolucida with Sholl spheres at 20 μ m intervals

and ventral cells 6.00–7.00 mm from the dorsal surface, and close to the rhinal fissure as a landmark. Sholl analysis was performed on 8 cells for each of the four groups in the rostral-dorsal, 8 cells for CON and 9 cells for ABA in the caudal-dorsal region, and 12 cells for each of the four groups in the caudal-ventral region of the hippocampus. It has been shown that subsets of cells within an anatomical nucleus, such as CA1 hippocampus, can participate independently in behaviors and that different subsets subserve different functions (Moser et al. 2008). Therefore, we have analyzed CA1 pyramidal cells independently, rather than averaging all cells within an animal.

All cells that were analyzed in the rostral-dorsal region are shown in Fig. 2, and all cells analyzed in the caudal-ventral region are shown in Fig. 3.

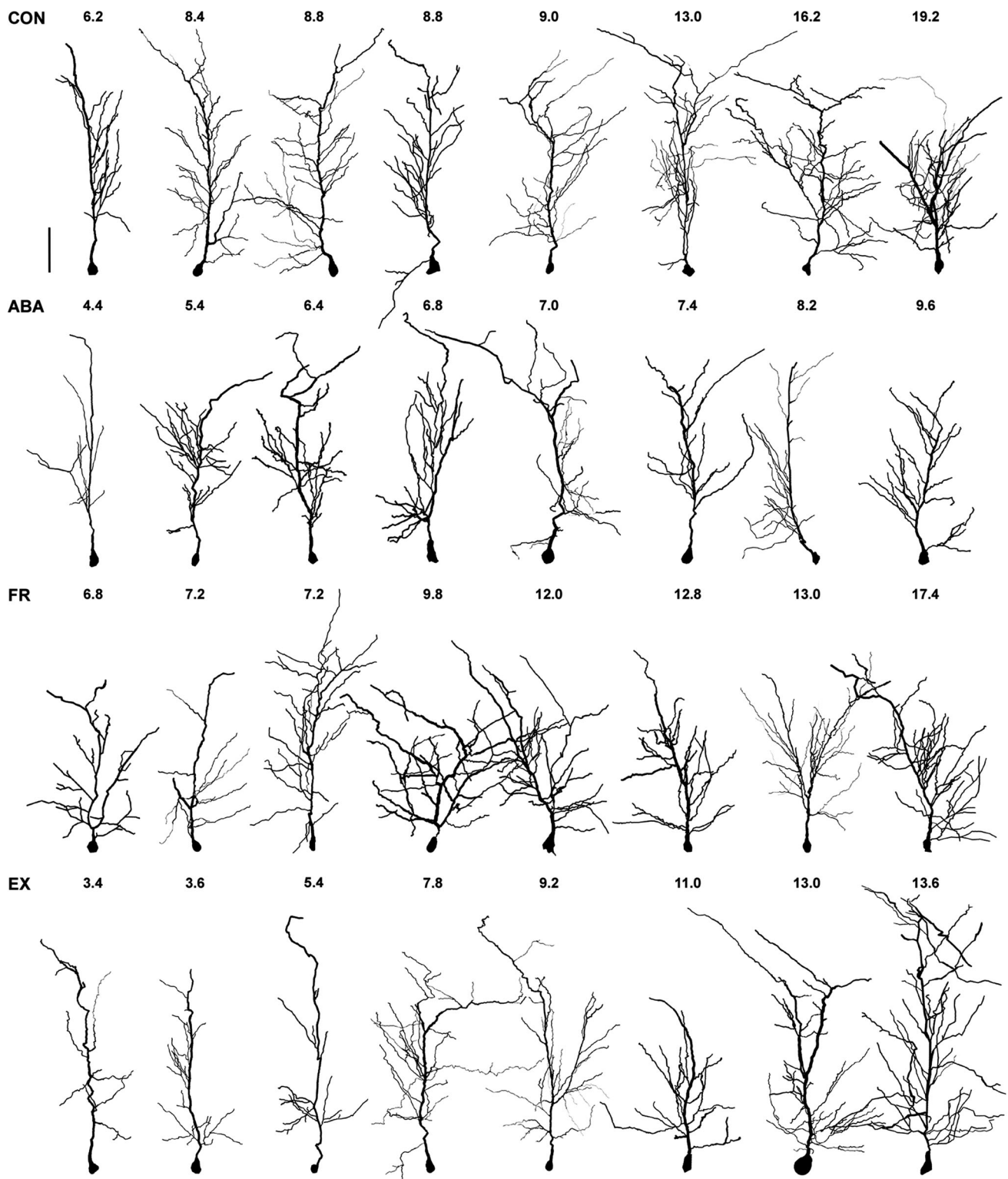


Fig. 2 NeuroLucida tracing of all cells analyzed from the rostral-dorsal sector of the hippocampal CA1. Cells were chosen in coronal sections approximately -2.80 to -4.30 mm from Bregma, 2.50 – 3.50 mm from the dorsal surface; *scale bar* shown to the left

of the top row applies to all cells and marks $100\ \mu\text{m}$. The number above each cell indicates the mean number of branches in SR resulting from Sholl analysis, and cells are arranged in order of increasing SR complexity

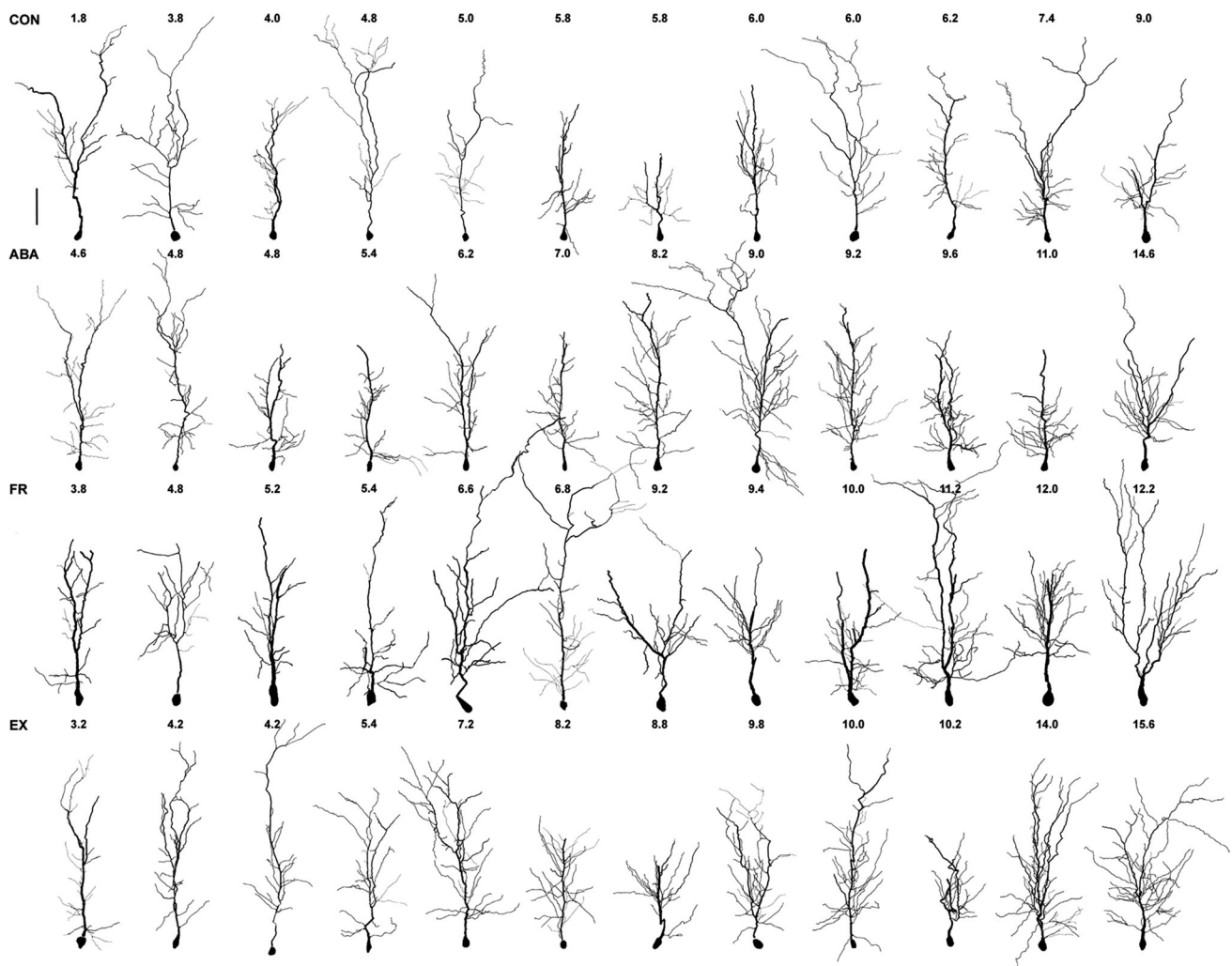


Fig. 3 NeuroLucida tracing of all cells analyzed from the caudal-ventral sector of the hippocampus. Cells were chosen in the CA1 region of the hippocampus approximately -4.80 to -6.04 mm from Bregma and 6.00 – 7.00 mm from the dorsal surface. Scale bar shown

to the left of the top row applies to all cells and marks $100\ \mu\text{m}$. The number above each cell indicates the mean number of branches in SR resulting from Sholl analysis, and cells are arranged in order of increasing SR complexity

Spine analysis

Segments of dendrites in stratum radiatum (SR) were chosen by selecting dendritic branches between 100 and $200\ \mu\text{m}$ from the soma of the same pyramidal cell. At least five dendritic segments were analyzed per brain. The dendrite section was traced with NeuroLucida software along with dendritic spines, which were categorized by spine type: filopodium, thin spine, mushroom spine, or stubby spine. NeuroLucida Explorer branched structure analysis package was used to quantify the average number of spines per $10\ \mu\text{m}$ of dendrite.

Statistical analyses

Student's *t*-test was used to compare means across two groups, as when CON and ABA Sholl analysis results

were compared at each incremental radius and in the case of spine analysis where only CON and ABA were compared. In all cases where cells from all four experimental groups (CON, ABA, FR, and EX) were being compared, one-way ANOVA was used to determine whether the condition of group had an effect on Sholl analysis results. Post hoc Tukey HSD or Fisher LSD tests were used to achieve pairwise comparisons of the individual groups. The four experimental groups can also be arranged in a two-factor design, with the factors of wheel access and food restriction being crossed to achieve four groups: CON (no wheel, no food restriction), ABA (wheel access and food restriction), FR (no wheel, food restriction), and EX (wheel access, no food restriction). Factorial ANOVA was used to examine the main effects of, and interactions between wheel access and food restriction upon cells' morphology.

Results

Body weight and wheel-running activity

Body weight loss and wheel-running activity were used as indicators of induction of ABA. These two indicators were previously reported as significantly different than control groups in a larger sample of animals (Aoki et al. 2012). As the primary goal of the current analysis was to examine dendritic branching, which requires a smaller sample size to attain sufficient power than a behavioral study, statistical analyses were not performed on behavioral variables in this small subgroup of animals. Data is presented as the range of values for each experimental group. On the last day of the experiment, the range of body weights per group was: CON 149.3–170.5 g, ABA 102.8–131.9 g, EX 142.3–170.0 g, and FR 101.1–141.7 g. Wheel-running activity ranges on the last day of the study were EX 1.3–6.3 km and ABA 1.1–13.7 km.

Differences across CON hippocampal regions

For all CON cells, the number of intersections peaked or plateaued in the range of 100–200 μm from the soma (Fig. 4a). This range of radii in the apical arbor corresponds to the SR region of the CA1. SR is a functionally distinct compartment of CA1 receiving afferents primarily but not exclusively from CA3 via Schaffer collaterals (Amaral and Lavenex 2007). Therefore, we took an average across all intersections in this range across all cells analyzed (100, 120, 140, 160 and 180 μm from soma) to compare the amount of branching specific to SR ($N = 8$ cells in rostral-dorsal and caudal-dorsal; $N = 12$ cells in caudal-ventral). We also averaged the number of intersections the dendritic arbors made in the stratum lacunosum-moleculare (SLM), 300–400 μm from the soma, which receives afferents selectively via the lateral amygdala (Pikkarainen et al. 1998), perforant path and thalamus (Çavdar et al. 2008). For the CON group, one-way ANOVA indicated a significant difference in average number of intersections in SR across the three regions [$F(2, 25) = 7.689, p = 0.003$], and post hoc comparisons indicated the pairs that were significantly different. The mean number of intersections in SR of CON hippocampus decreased along the septo-temporal axis: highest in the rostral-dorsal and lowest in the caudal-ventral region. Average intersections in SR were significantly lower in caudal-ventral cells (5.47 ± 0.94) than rostral-dorsal cells ($11.2 \pm 1.15, p = 0.0007$) and caudal-dorsal cells ($8.75 \pm 1.15, p = 0.037$). One-way ANOVA indicated no significant difference in intersections in SLM in the three regions [$F(2, 22) = 1.291, p = 0.29$].

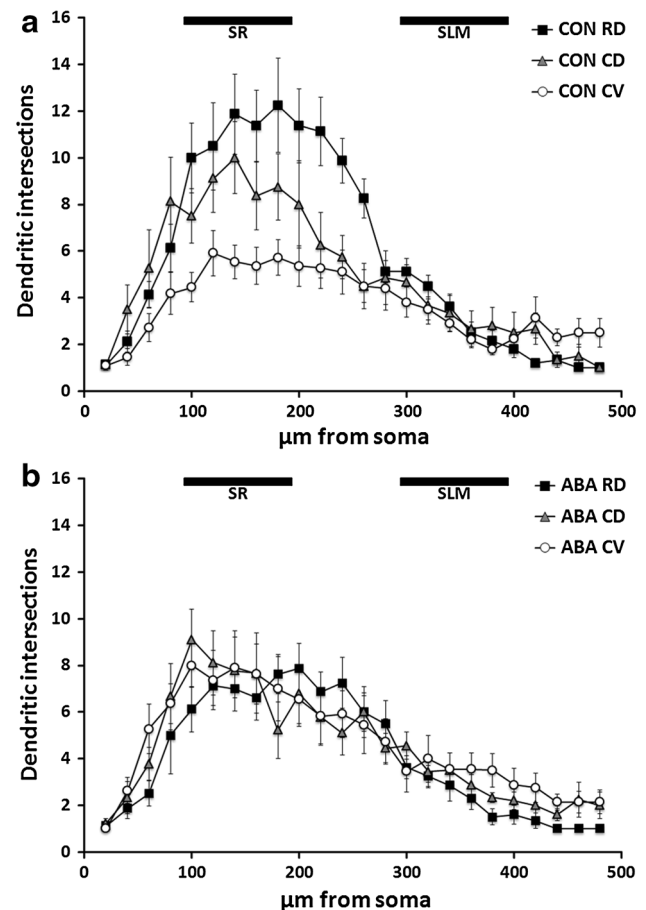


Fig. 4 Sholl analysis of apical dendrites of pyramidal cells in CA1. **a** The number of intersections with Sholl spheres centered at the soma of CON rats' rostral-dorsal (RD), caudal-dorsal (CD), and caudal-ventral (CV) CA1 is plotted against radius of the sphere as mean \pm standard error ($N = 8$ cells for RD and CD, 12 cells for CV). **b** The number of intersections with Sholl spheres centered at the soma of ABA rats' RD, CD, and CV CA1 is plotted against radius of the sphere as mean \pm standard error ($N = 8$ cells for RD, 9 cells for CD, 12 cells for CV)

Differential effects of ABA in dorsal and ventral hippocampus resulting in regional collapse

Total length of apical dendrites in the rostral-dorsal region was significantly less in ABA ($2659 \pm 224 \mu\text{m}$) than CON [$4104 \pm 427; t(14) = 2.996, p = 0.01$], a 35 % loss of dendritic length in the 8 days of the experiment. Atrophy in dendritic length was found across the layers, and was statistically significant in SR, with a 39 % loss [CON: 1580 ± 245 ; ABA: $962 \pm 88; t(14) = 2.368, p = 0.03$] but not in SLM [CON: 664 ± 80 ; ABA: $441 \pm 75; t(14) = 2.036, p = 0.06$].

The decrease in dendrite length was accompanied by a reduction in apical branching specifically in SR. Relative to CON, ABA rostral-dorsal CA1 pyramidal apical dendrites had significantly fewer dendritic intersections with Sholl

spheres at 100 [CON: 10.0 ± 1.5 ; ABA: 6.1 ± 1.0 ; $t(14) = 2.168$, $p = 0.05$], 140 [CON: 11.8 ± 1.7 ; ABA: 7.0 ± 0.9 ; $t(14) = 2.477$, $p = 0.03$], 160 [CON: 11.4 ± 1.5 ; ABA: 6.6 ± 1.0 ; $t(14) = 2.637$, $p = 0.02$], and 220 [CON: 11.1 ± 1.5 ; ABA: 6.9 ± 0.9 ; $t(14) = 2.500$, $p = 0.03$] μm , from the soma (Fig. 4). The average number of intersections in SR was significantly smaller in ABA (6.90 ± 0.57) than in CON [11.2 ± 1.59 ; $t(14) = 2.548$, $p = 0.02$].

While the average number of intersections in SLM was less in ABA than CON (CON: 3.59 ± 0.23 ; ABA: 2.79 ± 0.36), the groups did not differ significantly [$t(14) = 1.873$, $p = 0.08$].

The decreased number and length of SR dendrites in ABA was not accompanied by a compensatory increase in spine density. In fact, spine density in CON (12.26 ± 1.62 spines per $10 \mu\text{m}$) was greater than in ABA, but the difference was not significant [7.82 ± 1.18 ; $t(6) = 2.214$, $p = 0.07$].

In the caudal-dorsal hippocampus, there was no effect of ABA on the branching of CA1 pyramidal cells at any radius (data not shown).

In the caudal-ventral region, Sholl analysis data showed the opposite effect of ABA: total apical dendritic length, though not statistically significant, was greater in ABA ($3569 \pm 382 \mu\text{m}$) than in CON [2720 ± 229 ; $t(22) = 1.904$, $p = 0.07$], a 31 % increase. The increased length in ABA was strongly significant in SR [CON: 773 ± 84 ; ABA: 1312 ± 131 ; $t(22) = 3.456$, $p = 0.002$], while dendritic length in SLM did not differ [CON: 528 ± 90 ; ABA: 477 ± 104 ; $t(21) = 0.370$, $p = 0.7$]. Spine density in SR did not differ between the groups [CON: 12.98 ± 1.83 spines per $10 \mu\text{m}$, ABA: 13.96 ± 2.11 ; $t(6) = 1.932 \pm 0.353$, $p = 0.7$].

The differences in dendritic length were accompanied by concomitant changes in branching, with ABA having a greater number of intersections than CON at 40 [CON: 1.42 ± 0.34 ; ABA: 2.83 ± 0.55 ; $t(22) = 2.203$, $p = 0.04$], 60 [CON: 2.58 ± 0.57 ; ABA: 5.75 ± 1.04 ; $t(22) = 2.659$, $p = 0.01$], 80 [CON: 4.08 ± 0.82 ; ABA: 7.75 ± 1.12 ; $t(22) = 2.637$, $p = 0.02$], 100 [CON: 4.58 ± 0.60 ; ABA: 8.08 ± 0.88 ; $t(22) = 3.285$, $p = 0.003$], and 380 [CON: 2.00 ± 0.30 ; ABA: 3.86 ± 0.70 ; $t(15) = 2.717$, $p = 0.02$] μm from the soma (Fig. 4). The average number of intersections in SR was significantly greater in ABA (7.87 ± 0.87) than in CON [5.47 ± 0.53 ; $t(14) = 2.356$, $p = 0.03$], while the average number of intersections in SLM did not differ.

In contrast to the CON group, one-way ANOVA across regions in ABA showed no significant effect of hippocampal region on the amount of dendritic branching in CA1 pyramidal cells in SR or in SLM [ABA SR: $F(2, 26) = 0.233$, $p = 0.8$; ABA SLM: $F(2, 26) = 0.353$, $p = 0.7$]

(Fig. 4b). The decrease in branching in the rostral-dorsal and the increase in branching in the caudal-ventral regions resulted in cells from all three regions collapsing into an intermediate shape similar to those in the CON caudal-dorsal region.

Effects of exercise and food restriction

In order to determine the extent to which the independent variables of exercise and food restriction contributed to the ABA effects found in the rostral-dorsal and caudal-ventral regions, Sholl analysis was carried out in neurons from animals that were exposed to a running wheel only (EX) or food restriction alone (FR). Since no difference was observed between CON and ABA, the caudal-dorsal region was not explored in EX and FR groups.

In the rostral-dorsal region, the main effect of exercise was significant such that exercise caused a decrease in dendritic length in SR [$F(1, 28) = 6.015$, $p = 0.02$], while the main effect of food restriction was found to cause a decrease in dendritic length in SLM [$F(1, 28) = 5.232$, $p = 0.03$] (Fig. 5).

In the caudal-ventral region, a significant difference was found across all groups in the length of dendrites in SR [CON: 772 ± 84 , ABA: 1312 ± 131 , FR: 1255 ± 119 , EX: 1336 ± 181 ; $F(1, 44) = 3.977$, $p = 0.01$]. In addition, the main effect of exercise was significant such that exercise caused an increase in total dendritic length [$F(1, 44) = 5.862$, $p = 0.02$] and length in SR [$F(1, 44) = 5.398$, $p = 0.02$]. The main effect of food restriction was found, again, to influence the SLM, such that food restriction caused a decrease in dendritic length in SLM [$F(1, 43) = 4.340$, $p = 0.04$] (Fig. 6).

Discussion

Large-scale morphological changes occur after puberty

This study demonstrates that robust plasticity of the dendritic structure of pyramidal cells of the hippocampal CA1 persists after puberty. Studies of neuronal development have concentrated on sensory cortices, and a large body of evidence exists describing dendritic remodeling following sensory deprivation during the critical period that is well before puberty (Valverde 1967; Killackey and Leshin 1995; Fagiolini et al. 1994; Antonini et al. 1999; Hensch and Stryker 2004). While the morphology of sensory cortices develops and stabilizes before puberty (Huttenlocher 1990), functional neuroimaging studies suggest that cognitive maturation, via the development of higher-order association cortices that subservise cognitive functions, is a prolonged process that extends through adolescence

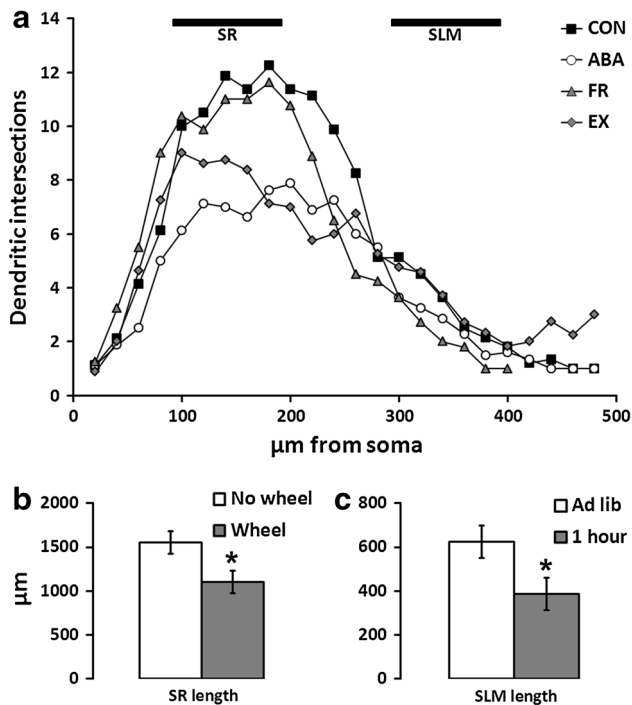


Fig. 5 Sholl analysis of apical dendrites of pyramidal cells in the caudal-dorsal CA1 of CON, ABA, FR, and EX. **a** The mean number of intersections with Sholl spheres centered at the soma of CON, ABA is repeated from Fig. 4, along with FR and EX groups. **b** The main effect of exercise on SR length is a decrease in length in ABA and EX groups (*Wheel*) relative to CON and FR groups (*No wheel*). **c** The main effect of food restriction on SLM length is a decrease in length in ABA and FR groups (*1 hour*) relative to CON and EX groups (*Ad lib*). * $p < 0.05$

(Gogtay et al. 2004; Casey et al. 2005). Similarly, subcortical regions, such as the striatum, to which association cortices project, develop with similar delay (Casey et al. 2005). Studies have shown that CA1 pyramidal cells exhibit rapid changes in spine density in response to gonadal hormone modulation in adulthood (Woolley and McEwen 1992). These changes happen on the time course of hours to days, but do not result in large-scale dendritic branch remodeling. Chronic stress has been shown to cause much more profound changes in the branch structure of hippocampal pyramidal cells, but these changes happen over weeks and were studied in adult brains (McKittrick et al. 2000). Our results show that the apical dendritic structure of CA1 pyramidal cells is modifiable during a period following puberty onset. Moreover, these changes in dendritic structure occur very rapidly; ABA evokes a measureable change over a period of just 4 days. While it is known that ovarian hormones affect hippocampal structure and function, we did not monitor estrus cycling in this experiment. It has been shown that pubescent female Sprague–Dawley rats (P35–41) exhibit only partial cycling (Hodes and Shors 2005). Moreover, calorically restricted

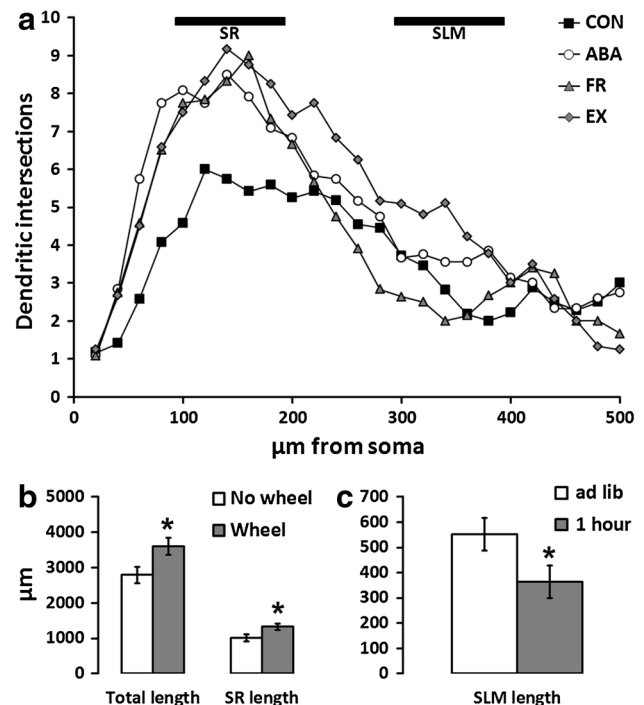


Fig. 6 Sholl analysis of apical dendrites of pyramidal cells in caudal-ventral CA1 of CON, ABA, FR, and EX. **a** The mean number of intersections with Sholl spheres centered at the soma of CON, ABA is repeated from Fig. 4, so as to facilitate direct comparison with data from the FR and EX groups. **b** The main effects of exercise on total and SR length are increases in length in ABA and EX groups (*Wheel*) relative to CON and FR groups (*No wheel*). **c** The main effect of food restriction on SLM length is a decrease in length in ABA and FR groups (*1 hour*) relative to CON and EX groups (*Ad lib*). * $p < 0.05$

rodents lose the cycling of their ovarian hormones, with increased time spent in diestrus (Nelson et al. 1985; Riddle et al. 2013). It is possible that the onset of cycling of hormones is responsible for the patterning of the regional differences in CON hippocampus—increased SR branching from the temporal to septal pole—and that the regional collapse observed in ABA hippocampus is caused by the lack of cycling hormones. Thus, adolescence is a period during which relatively rapid, large-scale cellular changes can occur in response to environmental manipulation, at least in the hippocampus, and may include other mechanisms of plasticity including gliogenesis (Barbarich-Marsteller 2013b), neurogenesis (Barbarich-Marsteller, unpublished data), cell death, and mRNA or protein expression.

Atrophy in rostral-dorsal hippocampus

The rostral-dorsal hippocampus CA1 showed the effect of decreased branching in SR in response to ABA and as a main effect of exercise. This suggests that wheel running is driving the change in rostral-dorsal SR. In the rostral-dorsal

SLM, however, food restriction drives the main effect of decreased branching and length. Thus, we suggest that food restriction and exercise are influencing regions of the hippocampus that receive distinct inputs. Synaptic inputs via the perforant path and Schaffer collaterals may be playing independent roles in remodeling pyramidal cell apical arbors, shaping their firing structure, and, ultimately, influencing behavior. In addition to the loss of dendritic length, ABA resulted in a non-significant decrease in spine density. This indicates that the loss of dendritic branching was not compensated for by an increase in spine density, but rather may be compounded by a decrease in spine density, thereby yielding a dramatic loss of excitatory inputs to these cells.

Hypertrophy in caudal-ventral hippocampus

In contrast to the rostral-dorsal region, the ventral CA1 showed large changes in SR in response to all three experimental manipulations—exercise, food restriction as well as the ABA disease model. Interestingly, the effect was in the opposite direction to that seen in the rostral-dorsal hippocampus. The result that the ventral region was sensitive to all three manipulations is consistent with the literature suggesting that the ventral region of the hippocampus is more susceptible than the dorsal region to changes in response to stress hormones (Silva-Gómez et al. 2012). However, in contrast to the results of that same study, we showed that the stressor of food restriction yielded increased dendritic branching of the ventral region, while the work by Silva-Gomez indicated decreased branching in the ventral region. This may be due to the difference in nature and duration of the stress, with theirs being 5 days, simulated by acute intraperitoneal injections of dexamethasone and ours being only for 4 days but generated by 23 h of food deprivation. In addition, their study was conducted in adult male rats, while our study was intentionally designed to study the effect of food restriction and exercise on adolescent females, as they may be particularly vulnerable to environmental stressors. Interestingly, in the caudal-ventral SLM, the combined effect of food restriction and exercise suggests that in ABA, the two factors are counteracting one another to prevent a change relative to CON.

Differential effects in SR and SLM

Dendrites serve to integrate synaptic inputs, and increased branching suggests the integration of a larger number of inputs. In the case of CA1 pyramidal cells, the dendrites integrate information arriving from several different sources, so remodeling of dendrites in a particular layer will serve to give more or less importance to inputs arriving by

a particular pathway. Dendritic remodeling in response to monocular deprivation is restricted to layer IV (Valverde 1967) and is correlated to a loss of excitatory afferents to this layer. Assuming a similar mechanism, the changes in dendritic branching in the SR of CA1 may be a response to changes in the activity of afferents to this compartment. If so, then after ABA, the reduced dendritic length in SR of rostral-dorsal CA1 may be caused by reduced activity from the neighboring CA1 pyramidal cells and CA3 pyramidal cells, or by increased activity in inhibitory interneurons in this region. Conversely, the increased dendritic branching and length in the SR of ventral CA1 as a result of food restriction, exercise, and ABA may be caused by increased afferent activity in SR, arising from the entorhinal cortex or basal nucleus of the amygdala (Pikkarainen et al. 1998), in addition to the local CA1 and CA3 pyramidal cells. The food restriction-induced decrease in SLM dendritic length may, similarly, result from decreased excitatory input from the lateral nucleus of the amygdala (Pikkarainen et al. 1998) or the midline thalamic nuclei (Su and Bentivoglio 1990).

The effect of dendritic branch remodeling on the activity of the cell depends on the density of inhibitory and excitatory synapses onto the dendrites. In both the SR and SLM, inputs upon dendritic branches (that is, excluding the shafts of apical dendrites) are greatly dominated by the excitatory types (Megias et al. 2001). While the direction of change in dendritic branching was the opposite across the rostral-dorsal to caudal-ventral sectors of the hippocampus, the direction of change was consistent across the SR and SLM: both the SR and SLM exhibited atrophy in the rostral-dorsal hippocampus of ABA animals and both strata exhibited hypertrophy in the caudal-ventral hippocampus of ABA animals. Therefore, it is likely that the down-regulation of branching in the rostral-dorsal SR following ABA induction reflects a decreased number of excitatory inputs and, thereby, decreased excitability of the hippocampal neurons there. Conversely, the increased branching of the caudal-ventral SR following ABA induction could reflect increased excitability of the hippocampal neurons there.

While the SLM remodeled coherently with the SR following ABA induction, the extent of change was relatively modest for the SLM. This suggests that the ABA-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.

In contrast to the dorsal versus ventral differences in the overall effects seen within brains of ABA animals, the change evoked by food restriction in the SLM was consistently in the direction of atrophy, regardless of the hippocampal septo-temporal (dorso-ventral) position. Perhaps

the inputs to the SLM, from entorhinal cortex or amygdala, carry information specifically about food availability or the stress associated with food deprivation that directs the animal's foraging behavior, which results in increased wheel running.

Functional implications

The hippocampus mediates both anxiety and learning, and these functions have been shown to be differentially distributed along its septo-temporal axis. Dorsal hippocampal lesions have been shown to result in spatial learning and memory impairments and ventral lesions result in reduced anxiety (Bannerman et al. 2002; Pothuizen et al. 2004). Therefore, the differential morphological effects of ABA along the septo-temporal axis of the hippocampal pyramidal cells we report here suggest that ABA may evoke multifaceted changes in hippocampus-dependent behavioral tasks. Atrophy in the SR of cells in the rostral-dorsal hippocampus may accompany reduced performance on spatial learning tasks (Chang et al. 2006), while hypertrophy in the caudal hippocampus SR may correlate with increased expression of anxiety. Our future studies will include behavioral experiments to test these hypotheses.

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