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

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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 & Claudino, 2011). AN most commonly presents in adolescent females, suggesting that hormonal changes at puberty may be responsible for an increased vulnerability to AN (Kaye, 2009). In females, the onset of puberty is associated with mood swings and anxiety (Buchanan et al., 1992; Hayward and Sanborn, 2002).

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, 2012). 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 (Gutierrez et al., 2006; 2009), hypoleptinemia (Hebebrand et al., 2006), and increased expression of dopamine D2 receptors and Brain-derived neurotrophic factor (BDNF) (Gelegen et al., 2008).

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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). Additionally, 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) (Gomez-Pinilla et al., 1997; Neeper et al., 1996; Stranahan et al., 2007; Stranahan et al., 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 alter structurally in response to stress (McEwen, 1999), hormones (Woolley and McEwen, 1992), and the interaction of hormones and stress (McLaughlin, 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; APA, 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). Additionally, 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-Gomez et al., 2012).

With these results in mind, the goal of the current study was to analyze the effects 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 hour per day food access, *ad libitum* wheel access), exercise control (EX; *ad libitum* food and wheel access), and food restriction control (FR; 1 hour 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 P37, ABA and EX animals were housed in a standard-sized home cage with a running wheel attached (Med Associates, Inc., St. Albans, VT). 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 one hour per day at the onset of the dark cycle. Body weight, food intake, and running wheel activity (where applicable) were measured daily within twenty minutes 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 7–9AM, 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 and 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 Neuro Technologies, Ellicott City, MD, USA). Coronal sections 250 μ m thick were made using a Leica VT1000M vibratome (Leica Microsystems GmbH, Wetzlar, Germany).

Sholl analysis

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 to 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 to 3.50 mm from the dorsal surface and ventral cells 6.00 to 7.00 mm from the dorsal surface, and close to the rhinal fissure as a landmark.

Cells to be traced were chosen such that their dendritic processes were not artificially broken within 200 μ m of the soma and 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.

Spine analysis

Segments of dendrites in stratum radiatum (SR) were chosen by selecting dendritic branches between 100–200 μm from the soma of the same pyramidal cell. Segments of dendrites in stratum lacunosum-moleculare (SLM) were chosen between 300–400 μm from the soma. 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 density of spines per unit length 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 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 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.

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.

Analysis of rostral-dorsal CA1 pyramidal cells

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 (Figure 1a). No differences were noted in staining quality between the different regions of the hippocampus. Sholl analysis of pyramidal cells in the rostral-dorsal CA1 region 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 μm and increasing in size in increments of 20 μm (Figure 1b).

Sholl analysis was performed on 8 cells per group in the rostral-dorsal region of the hippocampus. All cells that were analyzed are shown in Figure 2. Relative to CON, cells from ABA animals had significantly fewer dendritic intersections with Sholl spheres at 100 (CON: 10.0 ± 1.5 ; ABA: 6.1 ± 1.0 ; $t(14)=2.168$, $p=.05$), 140 (CON: 11.8 ± 1.7 ; ABA: 7.0 ± 0.9 ; $t(14)=2.477$, $p=.03$), 160 (CON: 11.4 ± 1.5 ; ABA: 6.6 ± 1.0 ; $t(14)=2.637$, $p=.02$), and 220 (CON: 11.1 ± 1.5 ; ABA: 6.9 ± 0.9 ; $t(14)=2.500$, $p=.03$) μm , from the soma (Figure 3a).

For all cells, the number of intersections peaked or plateaued in the range of 100–200 μm from the soma. 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 ($N = 8 \text{ cells} \times 5 \text{ radii per cell} = 40 \text{ radii}$ for each experimental group). An ANOVA was conducted to compare this value of average intersections in SR in CON, ABA, FR, and EX conditions, and the result was significant ($F(3, 156)=9.732$, $p=0.00001$). *Post hoc* comparisons using the Tukey HSD test indicated that the mean value for the CON group (11.20 ± 0.75) was significantly greater than the ABA group (6.90 ± 0.40) and the EX group (8.38 ± 0.72), but not significantly different from the FR group (10.78 ± 0.68). Additionally, the FR group differed significantly from both the ABA and EX groups (Figure 3b). A two-factor ANOVA was conducted to test the main effects of the environmental factors of exercise and food restriction. The results suggest that while there is a main effect of exercise in the SR of rostral-dorsal CA1 ($F(1, 156)=26.422$; $p<0.00001$), which is to reduce dendritic branching, there is no significant effect of food restriction ($F(1, 156)=2.1249$; $p=0.15$), or an interaction between the two factors ($F(1, 156)=0.6489$; $p=0.42$).

We also averaged the number of intersections the dendritic arbors made in the SLM, 300–400 μm from the soma, which receives afferents selectively via the perforant path. An ANOVA indicated a between-groups difference in the SLM ($F(3, 134)=2.8133$, $p=0.0417$). While no pairwise differences were found using the Tukey HSD test, the Fisher LSD test indicated that the value of the FR group (2.54 ± 0.35) was significantly less than that of the CON group (3.62 ± 0.28 ; $p=0.031$) and the EX group (3.69 ± 0.46 ; $p=0.025$), but not different from the ABA group (2.78 ± 0.25) (Figure 3c). A two-factor ANOVA analysis indicated a main effect of FR ($F(1, 134)=8.3915$, $p=0.0044$), which was to reduce dendritic branching, but no main effect of EX ($F(1, 134)=0.20729$, $p=0.65$), and no significant interaction of the two factors ($F(1, 134)=0.06265$, $p=0.802$).

In order to confirm that similar differences were present in dendritic length, we repeated the analysis with dendritic length in each compartment, SR and SLM. A one-way ANOVA indicated a difference in dendritic length within SR across groups ($F(3, 156)=9.4011$, $p=0.00001$), with *post hoc* analyses indicating that the CON value ($316.07 \pm 21.91 \mu\text{m}$; $N=40$) was greater than that of ABA ($192.48 \pm 14.40 \mu\text{m}$; $N=40$), and the FR value ($305.17 \pm 18.78 \mu\text{m}$; $N=40$) was greater than that of ABA. The difference between EX ($249.78 \pm 18.35 \mu\text{m}$; $N=40$) and ABA in total length in SR was just below statistical significance ($p=0.056$). One-way ANOVA of total dendritic length in SLM also indicated a difference between groups ($F(3, 146)=4.5950$; $p=0.00419$), with *post hoc* analyses indicating

that CON value (132.89 ± 11.41 ; $N=40$) was greater than FR (78.47 ± 12.38 ; $N=34$; $p=0.006748$), and EX (123.13 ± 11.71 ; $N=38$) was also greater than FR ($p=0.0435$), while ABA (92.87 ± 11.71 ; $N=38$) was not different from any other group.

Spine density in SR was measured for one dendritic section per animal, and compared across the four groups. One-way ANOVA suggested that there was no effect of group on spine density ($F(3, 12)=1.9134$, $p=0.1812$). While the result is not statistically significant, it suggests that there was not a compensatory increase in spine density in dendrites of the ABA group, as the mean value for CON (1.22 ± 0.16 spines/ μm ; $N=4$) was greater than that of ABA (0.77 ± 0.11 ; $N=4$) ($t(6)=2.257$, $p=0.0648$).

Spine density in SLM was measured for CON and ABA groups and resulted in no significant difference between the groups (CON: 0.63 ± 0.16 spines/ μm ; ABA: 0.66 ± 0.11 ; $t(6) = 0.166$, $p=0.87$).

Analysis of caudal-dorsal CA1 pyramidal cells

In the caudal-dorsal region of the hippocampus, eight cells per animal in the CON and ABA groups were traced and analyzed by Sholl analysis. CON and ABA did not differ significantly in the average number of intersections at any radius (Figure 4). Additionally, the average intersections in the SR, 100–200 μm range, did not differ significantly: CON (8 ± 1 , $N=8$), ABA (7 ± 1 , $N=9$) ($t(15)=0.626$; $p=0.541$).

Analysis of caudal-ventral CA1 pyramidal cells

All caudal-ventral cells that were analyzed are shown in Figure 5. The Sholl analysis data showed significant differences, with CON having fewer intersections than ABA at 40 (CON: 1.42 ± 0.34 ; ABA: 2.83 ± 0.55 ; $t(22)=2.203$, $p=0.038$), 60 (CON: 2.58 ± 0.57 ; ABA: 5.75 ± 1.04 ; $t(22)=2.659$, $p=0.014$), 80 (CON: 4.08 ± 0.82 ; ABA: 7.75 ± 1.12 ; $t(22)=2.637$, $p=0.015$), and 100 (CON: 4.58 ± 0.60 ; ABA: 8.08 ± 0.88 ; $t(22)=3.285$, $p=0.0034$) μm from the soma (Figure 6a). Again, estimates of branching in SR were obtained by averaging values in the range of 100–180 μm from the soma in each group. A one-way ANOVA indicated a significant effect of group on the number of intersections in SR ($F(3, 236)=8.1048$, $p=0.00004$). *Post hoc* comparisons using the Tukey HSD test indicated that the average value for the CON group (5.47 ± 0.32 ; $N=60$) was significantly lower than the values for ABA (7.87 ± 0.49 ; $N=60$), FR (8.05 ± 0.49 ; $N=60$), and EX (8.4 ± 0.55 ; $N=60$) (Figure 6b). A two-factor ANOVA indicated significant main effects of both exercise ($F(1, 236)=8.5610$, $p=0.0038$) and food restriction ($F(1, 236)=4.7574$, $p=0.0302$), both of which were to increase dendritic branching. Additionally, there was a significant interaction of exercise and food restriction ($F(1, 236)=10.996$, $p=0.0011$).

Again, the SLM layer was compared across groups by averaging the number of intersections from 300–400 μm from the soma. There was a significant effect of group on intersections in SLM ($F(3, 187)=10.656$, $p<0.00001$). *Post hoc* comparisons indicated significant differences between CON (2.85 ± 0.20 ; $N=54$) and EX (4.63 ± 0.33 ; $N=49$), FR (2.41 ± 0.27 ; $N=39$) and ABA (3.67 ± 0.35 ; $N=49$), and FR and EX (Figure 6c). A two-factor ANOVA showed main-effects of both environmental factors: exercise ($F(1, 187)=26.331$, $p<0.00001$), which

increased dendritic branching, and food restriction ($F(1, 187)=5.5759, p=0.019$), which decreased branching, but not an interaction between the factors ($F(1, 187)=0.76128, p=0.38405$).

We conducted an analysis of total dendritic length in SR and SLM compartments of caudal-ventral hippocampus, and these results are similar to the results of the branching patterns. A one-way ANOVA indicated a difference in dendritic length within SR across groups ($F(3, 236)=12.473, p<0.00001$), with *post hoc* analyses indicating that the CON value ($154.51\pm11.08 \mu\text{m}$) was significantly less than that of ABA ($262.32\pm14.80 \mu\text{m}; p=0.00001$), FR ($250.94\pm14.77 \mu\text{m}; p=0.000042$), and EX ($267.26\pm18.71; p=0.000008$). No significant differences were found between the ABA, FR, and EX groups in caudal-ventral SR. In the SLM, there was also a significant effect of group on dendritic length ($F(3, 202)=7.9678, p<0.00005$). *Post hoc* comparisons indicate significant differences between CON (105.74 ± 9.21) and FR ($66.59\pm6.85; p=0.0197$), FR and ABA ($106.10\pm10.13; p=0.0189$), and FR and EX ($133.31\pm10.17; p=0.000014$).

Spine density analysis was again performed in CON and ABA groups, and showed no significant difference between the groups in SR (CON: $1.71\pm0.28, N=6$; ABA: $1.35\pm0.21, N=4$; $t(8)=0.914, p=0.387$). In SLM, mean spine density is higher in ABA (0.753 ± 0.030 spines/ $\mu\text{m}, N=4$) than CON ($0.577\pm0.072, N=4$), but this difference did not come to statistical significance ($t(6)=2.249, p=0.065$).

Differences across hippocampal regions

We used the same data already described to test the effect of hippocampal region on the structure of CA1 pyramidal cells. A one-way ANOVA of the CON data in SR indicated a significant difference in average number of intersections across the three regions ($F(2, 137)=30.598, p<0.00001$). *Post hoc* comparisons indicated that all three pairs are significantly different. Average intersections in SR were higher in rostral-dorsal cells (11.2 ± 0.75) than caudal-dorsal ($8.75\pm0.61, p=0.0076$) and caudal-ventral ($5.47\pm0.32, p=0.000022$) cells; and caudal-dorsal values were greater than caudal-ventral values ($p=0.000052$). The same tests were run for the CON SLM, and the ANOVA indicated no significant differences between the groups ($F(2, 119)=2.7073, p=0.0708$).

In contrast to the CON groups, one-way ANOVA in the ABA data show no significant effect of hippocampal region on the amount of dendritic branching in CA1 pyramidal cells in SR or in SLM (ABA SR: $F(2, 142)=0.82672, p=0.43957$; ABA SLM: $F(2, 121)=2.1109, p=0.12557$).

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, and the development of higher-order association cortices that subserve cognitive functions, is a prolonged process that extends through adolescence (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 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 four days. Thus, adolescence is a period during which relatively rapid, large-scale cellular changes can occur, at least in the hippocampus, and may include other mechanisms of plasticity including gliogenesis (Barbarich-Marsteller, 2013), neurogenesis (Barbarich-Marsteller, in preparation), 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 as well as EX. 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. Thus, we suggest that food restriction and exercise are influencing regions of the hippocampus that receive distinct inputs. Hippocampal activity via these two different pathways may be playing a role in the remodeling of the cells and the animals' behavior. Unlike the responses seen with gonadal hormone treatment (Woolley and McEwen, 1992), no significant change in spine density was observed. This indicates that the loss of dendritic branching was not accompanied by compensatory increases in spine density, thereby yielding a net 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 of that seen in the rostral-dorsal hippocampus. The result that the ventral region was sensitive to all three manipulations is consistent with other literature suggesting that the ventral region of the hippocampus is more susceptible than the dorsal region to changes in response to stress hormones (Silva-Gomez 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 difference 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 hours of food deprivation. Also, 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 that results in shunting inhibition of inputs to those dendrites as well as suppression of Ca^{2+} -mediated spikes (Miles et al., 1996). 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 caudal BLA (Pikkarainen et al., 1998), in addition to the local CA1 and CA3 pyramidal cells.

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 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 anterior-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 responses 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 entorhinal cortical or amygdalar input to the SLM carries information specifically about food availability or the stress associated with food deprivation that directs the animal's foraging behavior, which is translated to 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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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 $20\text{ }\mu\text{m}$ intervals

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

Fig. 3.

Sholl analysis of apical dendrites of pyramidal cells in the rostral-dorsal CA1. **a** The number of intersections with Sholl spheres centered at the soma of CON and ABA groups is plotted against radius of the sphere as mean $\pm$ standard error (N=8 cells per group), **b** The average number of intersections across spheres with radii from 100–180 μ m, corresponding to SR is plotted across all four groups (mean $\pm$ standard error; N=40 radii per group), **c** The average number of intersections across spheres with radii from 300–380 μ m, corresponding to SLM is plotted across all four groups (mean $\pm$ standard error; CON: N=39, ABA: N=36, FR: N=28, EX: N=35 radii) * p <0.05 **** p <0.0001

Fig. 4.

Sholl analysis of apical dendrites of pyramidal cells in the caudal-dorsal CA1. Cells were chosen in the CA1 region of hippocampus approximately -4.80 to -6.04 mm from Bregma and 2.50 – 3.50 mm from dorsal surface; the number of intersections with Sholl spheres centered at the soma of CON ($N=8$ cells) and ABA ($N=9$ cells) groups is plotted against radius of the sphere as mean $\pm$ standard error

Fig. 5.

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 to 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

Fig. 6.

Sholl analysis of apical dendrites of pyramidal cells in caudal-ventral CA1. **a** The number of intersections with Sholl spheres centered at the soma of CON and ABA groups is plotted against radius of the sphere as mean $\pm$ standard error (N=12 cells per group), **b** The average number of intersections across spheres with radii from 100–180 μ m is plotted across all four groups (mean $\pm$ standard error; N=60 radii per group), **c** The average number of intersections across spheres with radii from 300–380 μ m is plotted across all four groups (mean $\pm$ standard error; CON: N=54, ABA: N=49, FR: N=39, EX: N=49 radii) * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001