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Morris Water Maze overtraining increases the density of thorny excrescences in the basal dendrites of CA3 pyramidal neurons

Eurídice Gómez-Padilla[1], Paola C. Bello-Medina[2]*, Uriel León-Jacinto[†2], Erika Orta-Salazar[2], Gina L. Quirarte[3], Víctor Ramírez-Amaya[4,5], Roberto A. Prado-Alcalá[3], Sofía Díaz-Cintra[2]*

[1]Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México 76230.

[2]Departamento de Neurobiología del Desarrollo y Neurofisiología. Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, México 76230.

[3]Departamento de Neurobiología Conductual y Cognitiva. Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, México 76230.

[4]Instituto de Investigación Médica Mercedes y Martín Ferreyra INIMEC-CONICET-UNC, Friuli N° 2434, Córdoba, Argentina 5016.

[5]Maestría en Nutrición Humana, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México 76230.

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*To whom correspondence may be addressed.

E-mail addresses:

Gómez-Padilla Eurídice: didis72@hotmail.com
León-Jacinto Uriel: yoldi@unam.mx
Bello Medina Paola Cristina: crismedi@gmail.com
Orta-Salazar Erika: erikaortabio@yahoo.com.mx
Prado-Alcalá Roberto Agustín: prado@unam.mx
Quirarte Gina Lorena: ginaqui@unam.mx
Ramírez-Amaya Víctor: vramirezamaya@immf.uncor.com
Díaz-Cintra Sofía: yoldi@unam.mx

Highlights

- Stubby, thin, and mushroom dendritic spines ratio did not change, regardless of amount of training
- A significant increase in thorny excrescences density was detected in overtrained rats
- Spatial water maze overtraining induces an increased density of MF–TE connections

ABSTRACT

The hippocampus plays a fundamental role in spatial learning and memory. Dentate gyrus (DG) granular neurons project mainly to proximal apical dendrites of neurons in the CA3 *stratum lucidum* and also, to some extent, to the basal dendrites of CA3 pyramidal cells in the *stratum oriens*. The terminal specializations of DG neurons are the mossy fibers (MF), and these huge axon terminals show expansion in the CA3 *stratum oriens* after the animals undergo overtraining in the Morris Water Maze task (MWM). However, to our knowledge there are no reports regarding the possible changes in density of post-synaptic targets of these terminals in the basal dendrites of CA3 neurons after overtraining in the MWM. The purpose of this work was to study the density of thorny excrescences (TE) and other dendritic spine types (stubby, thin, and mushroom) in the CA3 *stratum oriens* in animals overtrained in the MWM for three consecutive days and in animals trained for only one day. Seven days after MWM training, the animals were sacrificed, and their brains removed and processed for rapid Golgi staining to visualize the different types of dendritic protrusions. Our results revealed that the relative quantity of stubby, thin, and mushroom dendritic spines did not change, regardless of amount of training. However, a significant increase in the density of TE was detected in the overtrained animals. These results strongly suggest that spatial water maze overtraining induces an increased density of MF–TE connections, which might be functionally relevant for long-term spatial memory formation.

Keywords: Overtraining; Morris Water Maze; hippocampus; CA3; dendritic spines; thorny excrescences.

1. Introduction

It is well accepted that anatomical and physiological changes in synaptic connections, known as synaptic plasticity, underlie the process of memory consolidation, which allows storage of information in the central nervous system [1,2], and that activity-dependent synaptic plasticity in the hippocampus, induced by learning experiences, plays a crucial role in the encoding and storage of spatial memory [3–5].

In the hippocampal circuit, the mossy fibers (MF) originate in dentate gyrus (DG) granular neurons and their main target are the apical dendrites of CA3 pyramidal neurons in the *stratum lucidum*, and scarce MF projections are found in the basal dendrites of CA3 pyramidal neurons located in the *stratum oriens*. These MF terminals are very important for the encoding of spatial information [6,7], and their ability to activate postsynaptic cells can be higher when reaching the basal CA3 dendrites because of their proximity to the axon hillock. It is well established that overtraining in the Morris Water Maze (MWM) task induces the expansion of these MF terminals, particularly in the CA3 basal dendrites located in the *stratum oriens* [8–12]. This was consistently observed using the Timm staining method that detects zinc-containing regions, such as those in the MF terminals, and others have found similar results with different presynaptic markers such as the zinc transporter protein ZnT3, the axon-specific microtubule associated protein tau [10], and with the vesicle-associated protein synaptophysin [9]. More recently, using manganese-enhanced magnetic resonance imaging, it was confirmed that this structural plastic change also occurs in the most septal portion of the CA3 pyramidal layer [8].

The proximal dendritic segment of CA3 pyramidal neurons as well as the mossy cell dendrites exhibit a huge dendritic specialization known as thorny excrescence (TE) where the MF terminals from DG granular cells establish their synaptic contacts. One single TE can be contacted by several MF terminals [13]. These huge dendritic specializations are classified into two identifiable structures; the first one, called thorn, is a single large spine, which may show ramifications, with one large neck that contacts the dendritic shaft; and the other type is a known as cluster, which is an even larger protrusion in the CA3 proximal pyramidal dendritic shaft with multiple heads in a large an uninterrupted section of the dendrite [14].

It was demonstrated that shortly after animals had completed 8 trials of training in the MWM and 4 trials of reversal of this task, the volume and number of thorny excrescences and the area and number of postsynaptic densities increased in the *stratum lucidum* of CA3 neurons, as measured with electron microscopy, serial sections, and 3D image reconstruction analysis [15]. Notably, these structural changes were observed 15 min after the MWM training, and the MF expansion in the CA3 *stratum oriens* could be detected 7 days, but not two days after the MWM training [16]. This result suggests that off-line reactivation of the spatial experience lasting several days may be required to trigger the cellular and molecular mechanisms underlying this type of structural change. Given that the MF target TEs, it is likely that MWM overtraining inducing MF expansion in the CA3 *stratum oriens* [11] can also change the density of TEs, which would confirm the functional significance of this type of structural synaptic plasticity [17].

Other post-synaptic contacts are the dendritic spines, which have complex structure-function relationships, and their specialized cytoskeleton made of dynamic actin filaments enables them for swift morphological changes important for information processing [18]. For this reason, the study of structural and functional plasticity of these dendritic specializations after a behavioral experience can provide essential information about the mechanisms underlying learning and memory [19,20].

The dendritic spines found in most neurons are morphologically heterogeneous, traditionally classified into three main categories: stubby, thin, and mushroom [21]. These different dendritic spine shapes may have different roles in learning and

memory [22–24], and are highly susceptible to changes in shape as a result of LTP-inducing activity [25] and of learning experiences such as classical [26] and instrumental conditioning [27], and spatial learning [28]; most of these studies have focused on the hippocampal CA1 region.

Mahmmoud and colleagues [29] performed a thorough anatomical analysis in CA1 and CA3 pyramidal neurons of the different types of dendritic spines in apical and basal dendrites, after animals underwent one daily training session for 10 days in the radial arm maze and were sacrificed 6 h after the last training session. They found an increase in the density of spines, only of the mushroom type, in the basal and apical dendrites of those CA regions. It is important to note, however, that these structural changes were observed shortly after training.

A recently published work studied the effects of eight consecutive daily sessions of MWM training on the density of mushroom spines in both apical and basal CA1 and CA3 dendrites [30]. Even though in CA1 a long-lasting increase density of mushroom spines was evident, no observable changes occurred in CA3 pyramidal neurons at 5 or 25 days after training.

The study of structural changes in the dendritic shaft after spatial overtraining in the MWM task may provide essential information about the mechanisms of structural synaptic plasticity underlying the functional adaptation of the hippocampal circuitry to efficiently process spatial information [17]. Thus, the aim of the present study was to investigate whether one or three training sessions in the MWM task, modify the density of thorny excrescences and of different types of dendritic spines in the basal dendrites of pyramidal CA3 neurons in the hippocampus.

2. Materials and methods

2.1 Animals

The subjects were 31 adult male Sprague Dawley rats (250-350 g) obtained from the colony of Instituto de Neurobiología, Universidad Nacional Autónoma de México. They were individually caged with water and food *ad libitum*, and maintained in a room with 12 h of artificial light beginning at 7:00 h. The temperature of the room was 23 ± 1 °C. Training and testing were performed between 9:00 h and 14:00 h.

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The rats were randomly assigned to each group. All the experiments were approved by the Animal Ethics Committee of the Instituto de Neurobiología, Universidad Nacional Autónoma de México and were performed in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals [31].

2.2 Morris Water Maze training

Rats were handled during three min for three consecutive days before training, which was carried out in a water maze consisting of a black circular pool of 154 cm in diameter, 60 cm high, with a black platform submerged 1 cm under the surface, located in a fixed position and surrounded by several spatial cues in a dimly-illuminated room. Training was carried out as described before [12]. The tank was filled with water (25 ± 1°C) to a depth of 35 cm. Each daily session consisted of 10 trials. In each trial, the animals were introduced into the pool at one of four different starting positions around the pool that were constant for all animals in each trial. Starting positions 1 and 2, 3 and 4, and so on, had the same average distance to the platform. For this reason, we used the average latency to the platform from each pair of trials or "trial pair" for the analysis of MWM performance. After being released, the animal could freely swim for a maximum of 60 s, or until reaching the platform, where it stayed for 30 s and was then placed in a waiting cage for 30 s. If on the first trial the animal did not find the platform within 60 s, the experimenter guided it by hand. The time required for the animals to find the platform was called escape latency. Rats were distributed into four groups: trained in the MWM for one session (MWM1); trained in the MWM for three sessions (MWM3); swim control group (SC), in each of the 10 daily trials of the three sessions, these animals were released into the tank, without the platform or spatial cues, and allowed to swim for the average time that the MWM3 group swam (i.e., in sessions 1, 2, and 3, the rats swam about 36, 18, and 6 s per trial, respectively); and animals kept under identical living conditions as those of the other groups, but they never left the bioterium, except for sacrifice (BIO).

2.3 Histology

One week after MWM training randomly assigned rats from the MWM1 (n = 7) and MWM3 (n = 9) groups, and the six rats from the SC and the BIO groups were anesthetized with xylazine/ketamine (8.0 mg/kg of xylazine and 90 mg/kg of ketamine) and perfused, transcardially, with a buffered solution of 10% formalin (pH 7.4). Twenty-four hours later their brains were removed, and from each brain a 4-mm block was obtained containing the dorsal hippocampus (anteroposterior: -2.92 to - 4.20 mm with respect to Bregma; Paxinos & Watson [35]). Each block was prepared according to the rapid Golgi technique following the modification of Díaz-Cintra et al. [33]. After 13 days in the fixative solution, each block was transferred to a solution of 0.75% silver nitrate in double distilled water (vol/vol) for 12 h, washed in 50% alcohol and dehydrated in 70% (vol/vol), 96% (vol/vol), and absolute ethanol, followed by a mixture of ethanol-ether (1:1, vol/vol), and embedded in low-viscosity nitrocellulose; coronal slices were obtained at a thickness of 120 µm using a sliding microtome (Leica SR2000 R). Each slice was collected in 70% alcohol (vol/vol), dehydrated, and mounted in Entellan®.

2.4 Morphometric analysis

All the measurements were made with the use of an Optiphot-2 Nikon microscope with a 100x Plan-Apochromat® objective (1.25 NA). Thorny excrescences (thorns and clusters) were identified by an experimenter who was blind to the experimental conditions and that had been previously trained to identify both the TE structures and the types of dendritic spines in CA3 pyramidal neurons. The TE counts were made along the 25 µm closest to the soma, in the proximal segment of the basal dendrite, and the results are expressed as the mean number of thorny excrescences per 25 µm (density). A total of 361 segments were used for the TE analysis

To quantify the stubby, thin, and mushroom dendritic spines the basal dendrites of CA3 pyramidal cells were divided into three segments with respect to the soma: proximal, medial, and distal. A total of 144 complete and well-impregnated pyramidal neurons of the CA3 area in dorsal hippocampus (6 neurons x 6 rats x 4 groups) were studied in blind conditions with a total of 432 segments analysed. Each segment was

25-µm long, and in order to identify and quantify the three spine types the criteria of Harris et al. (1992) were followed [21]: thin spines in which the neck was longer than their bulbous head, mushroom spines with the largest head and short neck, and stubby spines without head or neck, showing only a small protrusion from the dendrite.

We counted the total density of spines by adding together all the stubby, thin, and mushroom dendritic spines found in the proximal, medial, and distal segments. We also measured the densities for each type of spine (Table 1). The dendritic spine ratio in each segment was calculated by counting the number of each dendritic spine type (x) found in each segment/total dendritic spines in the same segment, where (x) refers to either stubby, thin, or mushroom.

2.5 Statistical analyses

Escape latency scores obtained on the first day of training were analysed with a repeated measures two-way ANOVA (groups x trials). On the second and third sessions, where only the MWM3 group was studied, a repeated measures one-way ANOVA was used. For the histological results, regarding thorny excrescence densities, the spine density, and spine type ratio a one-way ANOVA was used. When the F-ratios were significant, they were followed by a Newman-Keuls post-hoc test.

3. Results

3.1 Morris Water Maze training. On the day-1 training session, the repeated measures two-way ANOVA of the escape latency showed significant differences among trial pairs ($F_{(4,68)} = 16.84$; $p < 0.0001$), but not among groups ($F_{(1,68)} = 1.02$; $p = 0.33$) nor for the group x trial interaction ($F_{(4,68)} = 0.43$; $p = 0.79$). The Newman-Keuls test indicated a significant decrease in the escape latency in both the MWM1 and MWM3 groups on trial pairs 2, 3, 4, and 5 as compared to trial pair 1 (p's ranging from < 0.05 to $p < 0.0001$). In the MWM3 group, the repeated measures ANOVA showed significant differences in the escape latency among trial pairs on training days 2 and 3 ($F_{(4,36)} = 18.06$; $p < 0.0001$, and $F_{(4,36)} = 6.91$; $p = 0.0003$, respectively). The Newman-Keuls test showed a significant decrease in the escape latency in the

MWM3 group on trial pairs 2, 3, 4, and 5 as compared to trial pair 1 on training days 2 and 3 (p´s ranging from < 0.01 to p < 0.0001) (Figure 1). It was also found that the last trial pair of the first day was significantly higher than the last trial pair of the third day (p < 0.05), indicating that learning improved significantly in the MWM3 group over the 3 days of training, indicative of overtraining [11].
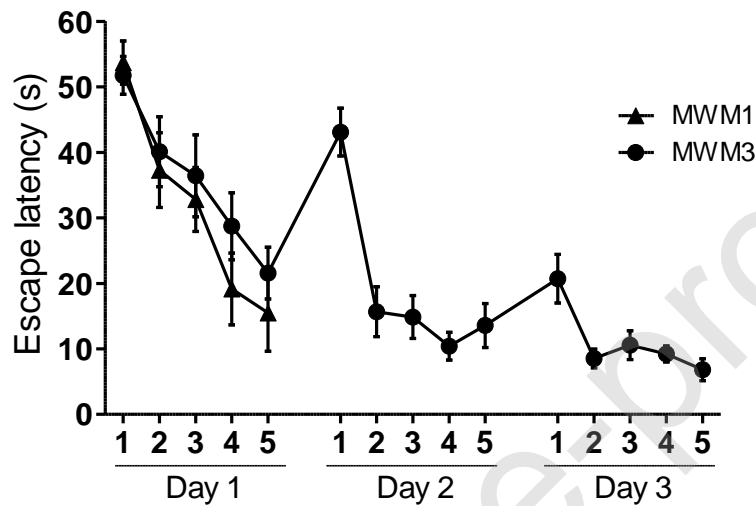

Figure 1. Escape latency (Mean ± SEM) of animals trained for one (MWM1; n = 9) or three (MWM3; n = 10) daily sessions in the Morris Water Maze task. Escape latencies on the last four trials of Day 3 were significantly lower than the last four trials of Day 1 (p < 0.05).

3.2 Morphometric analysis

Figure 2 shows a typical Golgi-stained CA3 pyramidal neuron of a rat of the BIO control group, showing thorny excrescences and stubby, thin, and mushroom dendritic spines.
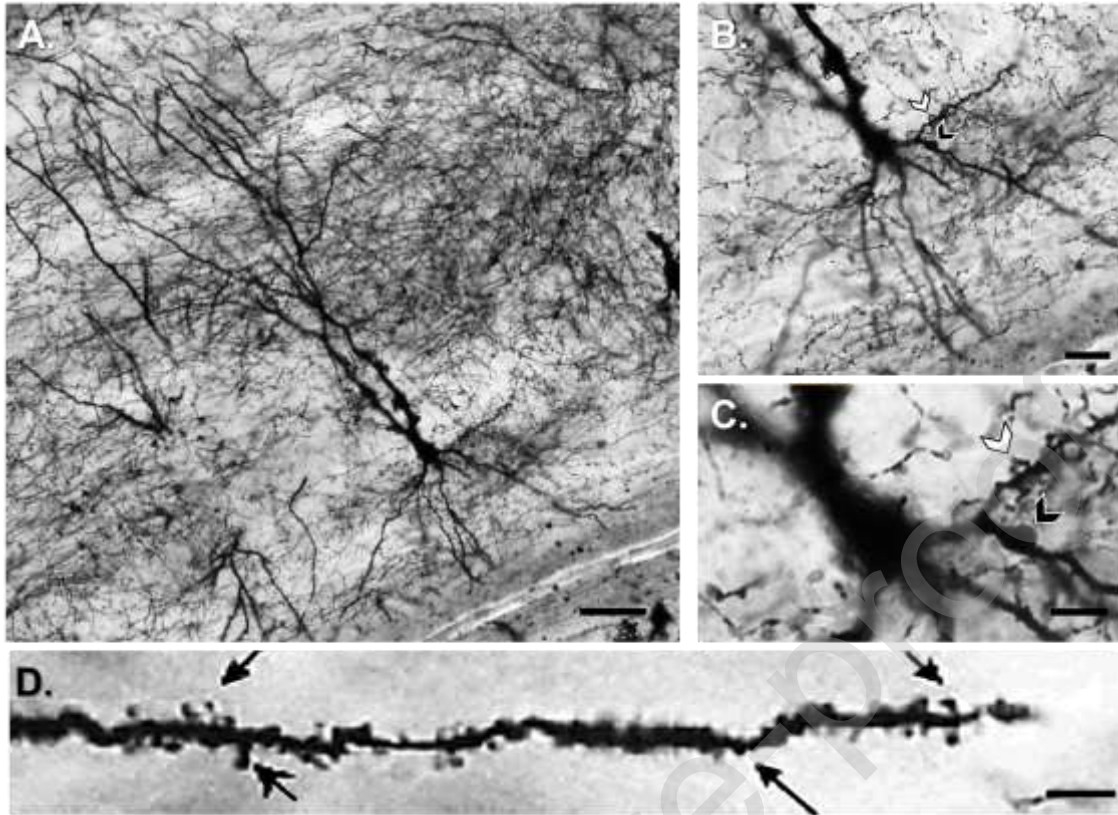
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Figure 2. Representative photomicrographs of a Golgi-stained neuron. (A) Pyramidal neuron from the CA3 field of hippocampus (20x objective, 0.40 NA), bar scale 50 μm. (B-C) Zoom-in of basal dendrites of the same neuron; the white and black arrow-heads point to a thorn and a cluster, respectively. 40x objective, 0.65 NA; bar scale 20 μm (B). 100x objective, 1.25 NA; scale bar 10 μm (C). (D) Dendritic spines from basal dendrites of a CA3 pyramidal neuron; the black arrows point to thin, stubby, and mushroom spines (100x objective, 1.25 NA); bar scale 5 μm.

3.2.1 Thorny excrescences

As described above, we distinguished between the dendritic shaft protrusions classified as thorns (single large neck with or without branches) and those classified as clusters (uninterrupted bulky protrusions with multiple heads), and the results are expressed as the density of either thorns or clusters, along the total length of the basal segments included in the analyses. The one-way ANOVA for the density of thorns (Figure 3A) in the proximal CA3 basal dendritic segment revealed no

significant differences among groups ($F_{(3,24)} = 2.91$; $p = 0.056$). Regarding the density of clusters (Figure 4B) the one-way ANOVA revealed significant differences among groups ($F_{(3,24)} = 7.18$; $p < 0.001$) and the Newman-Keuls test indicated that the MWM3 group had a significantly higher number of clusters than the other groups ($p < 0.01$ for each comparison), and there were no significant differences among the other groups.
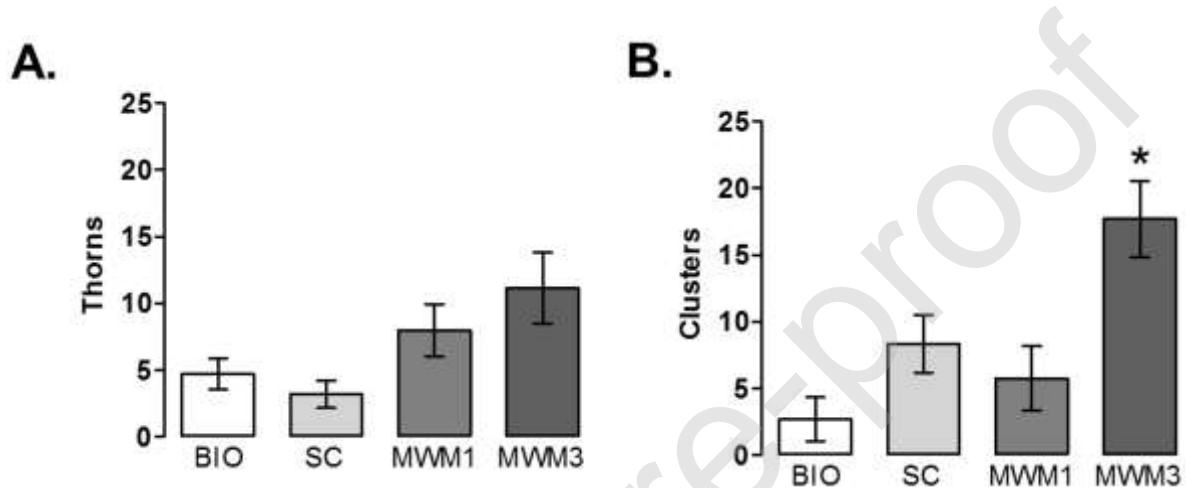

Figure 3. Mean density ($\pm$ S.E) of thorny excrescence in the proximal segment of the basal dendrites of CA3 pyramidal neurons. (A) thorns and (B) clusters in control (BIO), swim control (SC), one (MWM1), and three (MWM3) training sessions groups. n = 6 – 9 rats per group; $^*p < 0.01$ vs. the other groups.

3.2.2 Dendritic spine density

To determine whether the density of spines varies along the segments of the basal dendrite in basal conditions (BIO group), a one-way ANOVA was computed. There were significant differences among the segments ($F_{(2,15)} = 76.35$; $p < 0.0001$). The proximal segment showed a lower spine density than the medial and distal segments ($p < 0.0001$ for each comparison), and no differences between the medial and distal segments were found.

When the average spine density was calculated in each segment for every experimental condition, the one-way ANOVA showed that there are no significant differences among groups in the proximal ($F_{(3,20)} = 0.66$; $p = 0.59$), medial ($F_{(3,20)} = 0.58$; $p = 0.64$), and distal ($F_{(3,20)} = 2.05$; $p = 0.14$) segments (Figure 4A-C).
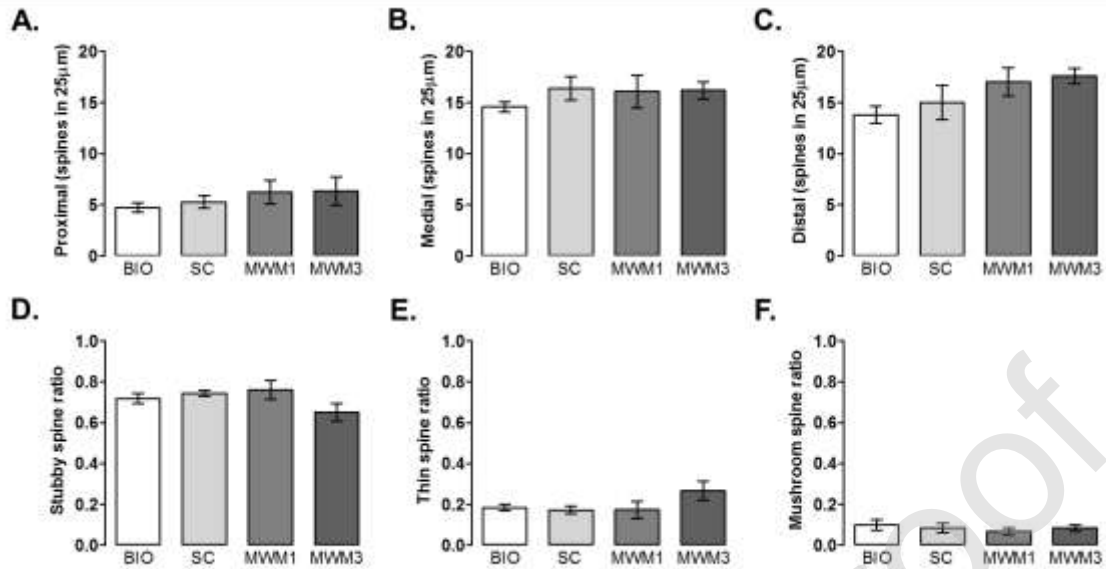
Figure 4. Dendritic spines (means ± S.E) in the basal dendrites of CA3 pyramidal neurons of groups: control (BIO), swim control (SC), one (MWM1), and three (MWM3) training sessions. There were no significant differences in spine densities among the groups in (A) proximal, (B) medial, nor in (C) distal segments. Likewise, there were no significant differences among the groups regarding (D) stubby, (E) thin, nor (F) mushroom spine ratios. n = 6 neurons per rat /6 rats per group.

3.2.3 Dendritic spine ratios

When comparing spine ratios among BIO, SC, MWM1, and MWM3, along the 75 μm comprising the three segments, the one-way ANOVA showed that there were no significant differences regarding stubby ($F_{(3,20)}$ = 1.89; p = 0.16), thin ($F_{(3,20)}$ = 1.79; p = 0.18), and mushroom ($F_{(3,20)}$ = 0.53; p = 0.67) spine ratios (Figure 4D-F). Regarding the spine ratio in each particular segment of the dendrite, the one-way ANOVA revealed no significant differences among the groups regarding stubby ($F_{(3,20)}$ = 1.93; p = 0.16), thin ($F_{(3,20)}$ = 1.66; p = 0.21), and mushroom ($F_{(3,20)}$ = 1.00; p = 0.41) spines in the proximal segment. The same was true for the medial segment, where no significant differences were found among the groups in stubby ($F_{(3,20)}$ = 2.19; p = 0.12), thin ($F_{(3,20)}$ = 2.29; p = 0.11), and mushroom ($F_{(3,20)}$ = 0.34; p = 0.79) spine ratios; by the same token, in the distal segment no significant differences were found

among the groups in stubby ($F_{(3,20)}$ = 1.45; p = 0.26), thin ($F_{(3,20)}$ = 1.73; p = 0.19), and mushroom ($F_{(3,20)}$ = 0.55; p = 0.66) spine ratios (data not shown).

A One-way ANOVA of stubby ($F_{(3,20)}$ = 1.61; p = 0.22), thin ($F_{(3,20)}$ = 1.36; p = 0.28), and mushroom ($F_{(3,20)}$ = 0.22; p = 0.88) spine density showed no differences between groups (Table 1).

Table 1. Means ± SE of total, stubby, thin, and mushroom spines found in the three 25-µm long segments of the basal dendrites of CA3 pyramidal neurons of control (BIO), swim control (SC), one (MWM1), and three (MWM3) training sessions groups

	Total spines	Stubby	Thin	Mushroom
BIO	11.08 ± 0.45	7.88 ± 0.29	2.03 ± 0.21	1.12 ± 0.29
SC	12.21 ± 1.04	9.09 ± 0.79	2.09 ± 0.26	1.03 ± 0.14
MWM1	13.10 ± 1.29	9.69 ± 0.38	2.52 ± 0.97	0.89 ± 0.25
MWM3	13.37 ± 0.44	8.74 ± 0.74	3.54 ± 0.61	1.09 ± 0.18

4. Discussion

The main finding of the present study was a significant increase in the density of thorny excrescences of the cluster type, in the proximal segment of the CA3 basal dendrites, seven days after overtraining rats in the MWM. To the best of our knowledge, this is the first report showing overtraining-induced thorny excrescence modifications in the CA3 basal dendrites. These results complement those showing that overtraining in the MWM task induces the expansion of mossy fiber terminals [10,11].

Importantly, our results point to a clear increased density of TE clusters that can be contacted by several mossy terminals [13] from a single or several DG cells. This structural change might increase the likelihood that one DG cell contacts different CA3 pyramidal neurons with axonal branch formations, where several MF terminals can reach new clusters in the CA3 basal dendrite after a spatial overtraining experience. It is noteworthy that these thorns and clusters were found only in the proximal segment of the basal pyramidal CA3 cell dendrite, which is a critical anatomical location for triggering CA3 pyramidal cell firing, since it is the closest region to the axon hillock. They are clearly closer than those reaching the proximal

CA3 apical dendrite located in the *stratum lucidum*, making the projections to the *stratum oriens* more powerful to generate activity in the CA3 pyramidal cells.

The MF-TE synapses could be considered information-processing units [13,34,35], switching between a minimal-impact "off" phase when inhibition dominates and an "on" phase when MF-TE synapses are more likely to trigger CA3 neural activity. For this reason these synaptic devices work as "conditional detonators" or discriminators [34,35], and structural plasticity in this important synaptic device plays an important role in optimizing both pattern separation and pattern completion [17], which are fundamental computations required for encoding and retrieval of memory.

Importantly, the post-synaptic structural plasticity reported here can be considered a long-term synaptic change, since it was observed 7 days after the training experience. Using sophisticated histological and imaging methods to study this important piece of the hippocampal network, a progressive expansion of MF in individual dendrites was observed with increasing age [13], and also a pronounced increase in MF complexity after months of experience in an enriched environment. Although this pre-synaptic MF change was accompanied by a modification of the post-synaptic TE, this part of the study was performed in organotypic slice cultures kept alive for several days, which precludes a direct relationship between experience and the post-synaptic TE remodeling. Nevertheless, it is important to note that they also demonstrated that the pre-synaptic MF expansion induced by a long-term enriched environment experience was preserved several months after the end of the experience [13], strongly suggesting that this type of structural modification between MF-TE is long-lasting. Previously, Stewart and colleagues [15] subjected rats to one massed session of eight trials followed, 24 h later, by four trials of reversal of the task. The animals were sacrificed 15 min after the reversal session. This experience induced a significant increase in the volume of TEs and in the number of thorns per thorny excrescence, as measured by 3D electron microscopy image reconstructions. The presynaptic anatomical modifications described by Rekart et al. [16] occurred days after MWM training, during which time the animals remained in their home cages without any further behavioral experience, thus suggesting that structural plasticity mechanisms may be driven by off-line reactivation [36,37] that induces

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recurrent rounds of synthesis and regulation of synaptic plasticity-related molecules such as Arc/Arg3.1, c-Fos, Zif268, BDNF, pCREB, pERK and pTrkB. These events could occur throughout the course of several hours or days after the animals experienced the behavioral task; the orchestrated rounds of expression and regulation of these molecules could help us understand the process of memory consolidation (for reviews see: [38–40]. It would be interesting to see how such rounds of expression of these synaptic plasticity-related molecules impact the pre- and post-synaptic structures, and whether the TEs can drive structural changes in the pre-synapsis, possibly through mechanisms similar to that of the post-synaptic release of BDNF [41].

It is also important to note that a gradual age-related growth of MF continues throughout time even in old age [13], suggesting that life-long experiences may drive this structural plasticity. Does this finding suggest that incidental life experiences may be able to increase the size of the MF-TE or only motivated life experiences can promote or maintain the MF-TE synaptic density? These questions require further research.

Even though it has been suggested that spine pruning and spine maturation are coordinated processes [42], we found that at 7 days after overtraining in the MWM task there were no significant differences in the density of dendritic spine types, nor in the ratio of the different spine types in any of the CA3 basal dendritic segments. Our current results are consistent with those found in the recently published paper by Klein et al. [30], who used a protocol with four trials in each of 8 training sessions in the MWM; the rats were sacrificed 5 or 25 days after the last training trial. They found non-significant changes in stubby, thin, and mushroom spine density, in the apical and basal CA3 dendrites.

These results contrast with those of Mahmmoud and colleagues (2015), who found a consistent and significant increase in the density of mushroom spines after 10 days of training in the radial arm maze task. Their experiment was very similar to ours, but in their study the animals were sacrificed 6 h after the last training session. It is possible then that in our study mushroom spine pruning occurred during the 7-day

rest period. This may suggest that the spine density increase in the CA3 *stratum oriens* found in the Mahmmoud et al. [29] study is a transitory change.

Others found a significant spine density increase in the apical dendrites of granular cells of the middle molecular layer of DG measured after MWM and inhibitory avoidance training; in both cases the spine density increase was found at 6 h post-training, returning to basal levels at 72 h post-training [43,44].

 It is then possible that in the present study, during those 7 days following the last spatial training experience the early structural changes are erased by pruning. Along this line, at 6 h and 24 h after inhibitory avoidance training the density of spines of dorsomedial striatal neurons gradually and significantly increased in relation to the intensity of the aversive stimulus used for training [27]. Interestingly, when distinguishing among different types of spines (stubby, thin, mushroom, branched, and multi-head), it was found that while thin spines decreased their density, mushroom spine density increased as the intensity of training was increased. According to our previous findings in CA1, these changes in the density of mushroom spines may also be maintained in the CA3 *stratum lucidum* but not in the *stratum oriens* [45]. *In vivo* time-lapse imaging in the neocortex revealed the appearance and disappearance of different postsynaptic dendritic spine subpopulations, and the frequency of these events were influenced by experience [46–48].

Finally, the lack of changes in the density of spines in the basal CA3 dendrite indicates that in this region, the overtraining experience did not produce a long-lasting increase in the amount of Schaffer recurrent collaterals. These collaterals are important for pattern completion, which is improved after the overtraining experience [17]. It is possible that structural plasticity of these collateral synapses is augmented in other CA3 regions after the spatial experience, but this proposition should wait for further research.

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Conflict of interest statement

The authors declare that they have no competing interests.

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