Moderate exercise prevents the cell atrophy caused by hypothyroidism in rats

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Adult-onset hypothyroidism is associated with an increase in cell atrophy of the hippocampal pyramidal neurons. Physical exercise implies diverse actions on the neural tissue that promote neuron proliferation and survival. The beneficial effects of exercise seem to be inversely linked to its intensity, so that strenuous exercise has reduced protective effects. In this study we evaluated the capacity of a moderate forced-exercise routine to counteract the neurodegenerative effects of a hypothyroid condition induced during adulthood. Simultaneously with a chronic anti-thyroid chemical treatment, a group of rats was forced to walk in a motorized wheel for 30 min daily five times a week. In four weeks of treatment the rats developed a plain hypothyroid condition that in non-exercised rats was accompanied by a marked increase in the number of atrophic cells in all CA regions of the hippocampus. The forced-exercise treatment did not counter the development of hypothyroidism and its signs, but it did prevent almost completely the associated neuronal damage in all CA regions. The forced exercise also improved the cognitive function in a spatial-learning test. These results indicate that moderate exercise has the potential to prevent the structural and functional deficits associated with a hypothyroid condition.

Key words: hypothyroidism, hippocampus, neural atrophy, neuroprotection, moderate exercise

INTRODUCTION

Thyroid function is essential for an adequate development of the central nervous system but also for the maintenance of nervous function during the adult life (Sala-Roca et al., 2008). Adult-onset hypothyroidism causes mood and cognition impairment in patients (Rivas and Naranjo, 2007; Pilhatsch, 2011) and in animal models (Chaalal et al., 2014). These alterations are attributed to hippocampal damage, for hypothyroidism is associated with cell damage of the pyramidal neurons of the hippocampus in both rats (Alva-Sánchez et al., 2009a) and humans (Cooke et al., 2014). Hypothyroid rats show reductions in the total weight of the hippocampus and in the volume on the Ammon's horn (Madeira et al., 1992), which is consistent with an increase in the proportion of atrophic neurons in this region (Alva-Sánchez et al., 2009b; Khordad et al., 2018) and a reduction of the number of pyramidal cells particularly in CA1 (Madeira et al., 1992). Along with this, dendritic arborization and dendritic spine density are also reduced in the pyramidal cells of CA1 and CA3 (Sala-Roca et al., 2008). At the biochemical level, hypothyroidism involves increased production of proinflammatory cytokines in the hippocampus (Nam et al., 2018), along with increased oxidative stress in the pyramidal neurons (Torres-Manzo et al., 2018). Thus thyroid hormones (THs) are needed for the maintenance of the hippocampal morphology and function during adulthood (Alva-Sánchez et al., 2009b).

Physical activity is generally considered beneficial for brain health and cognitive function in normal subjects. Exercising implicates positive effects for the nervous tissue, such as increased blood flow, increased synapse plasticity and increased neurogenesis, particularly in the hippocampus (Cooper et al., 2018). Several studies have shown that exercise promotes the recovery of the

hippocampal function after various forms of neural insult (Kim et al., 2011; Cechetti et al., 2012; Winocur et al., 2014; Klein et al., 2016). However, these positive effects seem to be rather hormetic, implying that the beneficial effects are seen under low-intensity exercise programs, while high-intensity, strenuous exercise may have negative effects on the hippocampal function (Soya et al., 2007; Okamoto et al., 2015). Intense physical activity produces various forms of physiological stress that induce responses involving the hypothalamus-pituitary-adrenal (HPA) and the hypothalamus pituitary-thyroid (HPT) axes (Mastorakos and Pavlatou, 2005). Exercise intensity is directly related to glucocorticoid secretion (Stranahan et al., 2008), and glucocorticoids are known to affect hippocampal structure and function in various ways. Elevated glucocorticoid levels induce apoptosis, decrease neuronal survival and inhibit neurogenesis in the hippocampus, thus resulting in decreased hippocampal volume (Sandi and Pinelo-Nava, 2007). Yet glucocorticoid activity is required for adequate neural cell proliferation (Gkikas et al., 2017). According to this, adult hippocampal neurogenesis and synaptic plasticity are increased after moderate exercise not rising the blood levels of glucocorticoids, whereas it dampens if the subjects are forced to exercise so intensely as to increase plasma corticosterone (Shih et al., 2013; Okamoto et al., 2015). This suggests an inverse relationship between exercise intensity and neuronal proliferation and survival in the hippocampus, probably involving glucocorticoid secretion.

On this basis, we wanted to test the effects of exercise in hypothyroid subjects. Besides its actions on the nervous tissue, exercise can also affect the thyroid function, but the direction of this effect is not clear. Increased serum TSH and T4 levels have been described after swimming (Deligiannis et al., 1993) and treadmill (Huang et al., 2004) exercising sessions, but decreased plasma T3 and T4 were found after high-intensity cycling (Ciloglu et al., 2005; Kilic, 2007). Therefore, moderate exercise seems to stimulate the thyroid axis whereas high-intensity exercise seems to blunt it. Although glucocorticoid and T3 plasma levels are inversely affected during intense exercise in rats and humans, this relation does not seem to be a cause-effect one (Neto et al., 2013). It is thus possible that moderate exercise, not involving glucocorticoid upsurge, would stimulate thyroid activity and reduce the adverse effects of thyroid deficiency. Since hypothyroidism affects muscle metabolism (Bloise et al., 2018) and increases its fatigability (Roy et al., 2003), the potential of modest exercising to counteract the cognitive outcomes under these circumstances is a particularly pertinent question. In the present study we assessed the effect of a moderate exercise routine on the development of a hypothyroid condition induced by antithyroid drugs in rats, and evaluated the effect of this exercise protocol on the integrity and function of the population of pyramidal cells of the hippocampus.

METHODS

Male Wistar rats aged three months (300–360 g) were used, that were divided in four experimental groups according to the subsequent treatments: no-antithyroid, no-exercise (EUT), no-antithyroid with exercise (EUT+EXE), antithyroid treatment, no-exercise (HYPOT) and antithyroid treatment with exercise (HYPOT+EXE) (n=6 each). All rats were housed in individual cages in a temperature (23 ± 1°C) and light (light: dark 12:12 h) controlled room. Food (powdered laboratory rodent chow 5001, PMI) and tap water were freely available for the entire experiment. All the experimental procedures were conducted in accordance with the NIH Guidelines for the care and use of laboratory animals and in compliance of the Mexican Laws on the matter (NOM-062-Z00-1999). Every effort was made to minimize the number and potential suffering of the experimental subjects.

The treatments began after a 1-week habituation period (Fig. 1). Two of the groups were treated for the generation of hypothyroidism by administering methimazole (60 mg/kg·day, Sigma) and propyl-thiouracyl (PTU, 15 mg/kg·day, Sigma), both dissolved in the drinking water, for the following 4 weeks (Roy and Mugesh, 2006; Alva-Sánchez et al., 2012). The concentrations of the drugs were adjusted weekly according to the average daily water consumption. The other two groups received no treatment and were used as euthyroid controls.

Simultaneously to the antithyroid treatment a group of each thyroid condition was subjected to a forced exercise routine, walking for 30 min/day beginning three hours after lights off, in a motorized wheel (Omnialva, Mexico) daily five days a week (Monday to Friday). The speed was set at 5 m/min, which corresponds to a moderate exercise protocol that does not increase lactate and glucocorticoids in rats (Soya et al., 2007; Shih et al., 2013). At the end of this phase, each of the four groups had received a different combination of treatments: euthyroid without exercise (EUT group), euthyroid with exercise (EUT+EXE group), hypothyroid without exercise (HYPOT group) and hypothyroid with exercise (HYPOT+EXE group). Colonic temperature and body weight were measured regularly during the treatments.

Blood levels of thyroid hormones and corticosterone were measured both before the beginning and at the end of the treatments. Blood samples were taken from the tail tip of the rats, centrifuged to obtain the serum and stored at -20°C until its use. Hormone concentrations were determined by means of commercial

immunodetection kits: total T3 and T4 (DRG International) and corticosterone (Ann Arbor Assays).

Behavioral tests

After 4-week treatments the rats were subjected to behavioral tests to evaluate anxiety and spatial learning, 1 day apart.

Dark/light box test: to evaluate anxiety we used a plastic box divided in two chambers (60 × 45 × 40 cm each), one white and the other black, connected by a bridge of white color (30 × 25 × 18 cm). Each animal was put into the light bridge and allowed to explore freely for 5 min. The time spent in each chamber and the number of crossings between compartments were recorded (Costall et al., 1989).

Barnes maze test: the spatial learning was evaluated in a maze consisting of an elevated (108 cm from the floor) white circular platform (120 cm in diameter) containing 18 holes (9 cm in diameter) distributed equidistantly around the periphery. Only one of the holes was connected to a black escape box not visible from over the platform. The tests were conducted in an isolated room with visual signals and intense lighting.

In the first session (habituation phase) the rat was put in the center of the platform and allowed to explore freely until it got into the scape box. In the following 4 days (acquisition phase) the rats were exposed 5 min daily to the maze with the scape box at the same position. Latency was recorded as the time spent to find the scape box, and errors as the number of non-escape holes explored by the rat. Lastly, after 2 successive days with no exposition, the rats were exposed again to the maze with the escape box removed (memory phase). In this case latency was measured as the time elapsed before the rat's first approach to the target hole, and the errors as the number of holes visited by the rat before the target hole (Harrison et al., 2006).

Quantification of neuronal damage in the hippocampus

One day after the end of the behavioral tests, the rats were deeply anaesthetized and perfused intracardially with 0.9% saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.2. Brains were removed from the skull, dehydrated, and embedded in paraffin. Coronal brain sections (7 μm) were obtained.
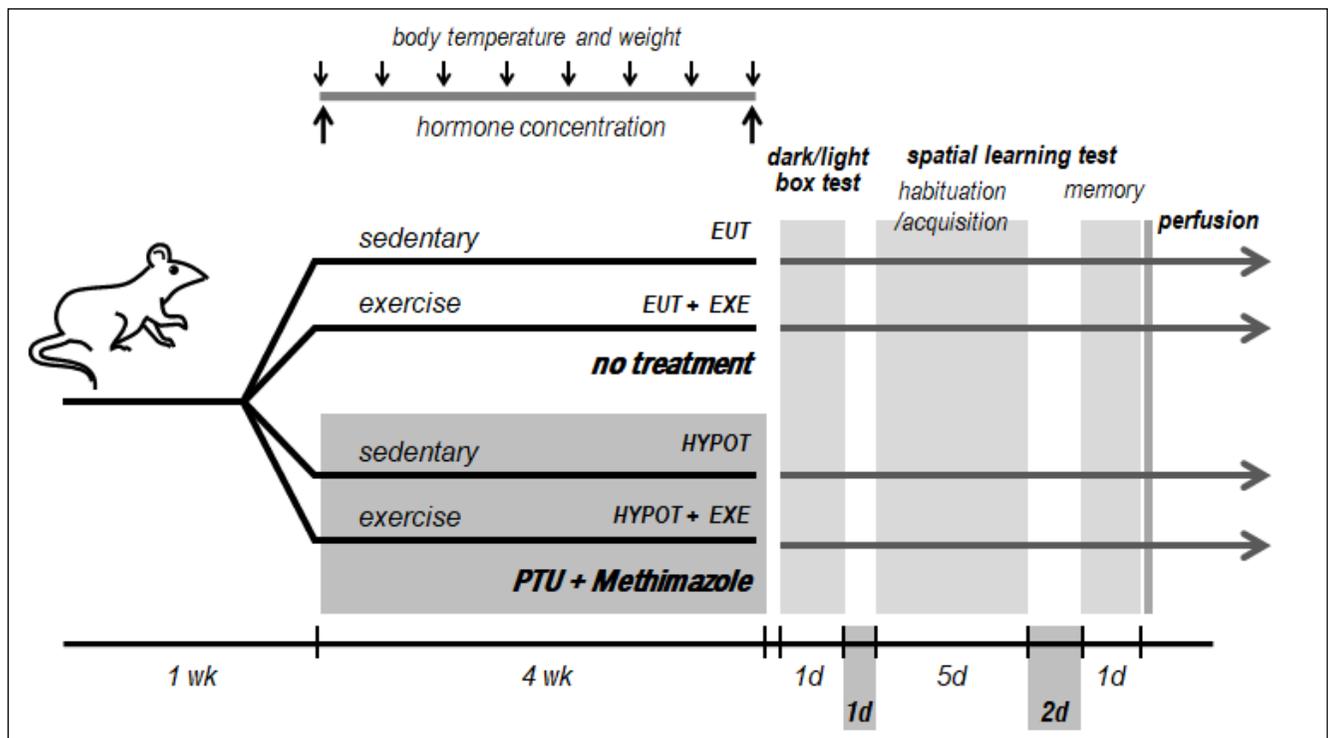

Fig. 1. Timeline of the procedures used in this study. Rats were either subjected to an antithyroid treatment (PTU+Methimazole; HYPOT groups) or not (no treatment, EUT groups) and a simultaneous moderate-exercise protocol (exercise, EXE groups) or not (sedentary) for 4 weeks. Body temperature, body weight and blood concentrations of corticosterone and thyroid hormones were measured at different times as indicated by the arrows. After the treatment two behavioral tests were performed sequentially in all rat groups: a single-session dark/light box test and a spatial-learning Barnes maze test that consisted of a 5-day habituation/acquisition phase and a single-session memory phase, 2 days apart. Rats were then sacrificed by intracardial perfusion to obtain the brains.

Paraffin sections were rehydrated and the slides were immersed in freshly prepared toluidine solution (1% in distilled water).

The hippocampal CA1, CA2, CA3 and CA4 regions were distinguished by morphological criteria. Under light microscopy (Zeiss) the pyramidal neurons were counted in each region differentiating between normal and atrophic cells by an experimenter blind to the treatment. A cell was considered atrophic if its morphology was altered exhibiting cytoplasmic hyperchromasia and nuclear material loss (Fig. 2). By means of an ocular micrometer scale at a magnification of 500x (40x objective, 10x ocular, a tube factor of 1.25), the number of neurons per cubic millimeter was calculated.

Statistical comparisons

Results are presented as means ± SEM. Data on colonic temperature, body weight, plasma T3, T4, corticosterone, the activity in the dark/light box and the escape latency and errors in the spatial learning test were analyzed by means of three-way ANOVA tests followed by Newman-Keuls *post hoc* tests if applicable. Neuron counts were analyzed by a three-way ANOVA and the number of atrophic cells was by analyzed by a two-way ANOVA and a Newman-Keuls *post hoc* test for each region. The significance level was set at 0.05 in all cases.

RESULTS

The values of body weight, body temperature and hormone levels are presented in Table I. The antithyroid treatment caused a significant reduction of both T4 ($F_{1,40}$=101.5, P<0.001) and T3 ($F_{1,40}$=17.87, P<0.001), as compared to the non-treated controls (EUT). Body weight ($F_{1,40}$=41.3; P<0.001) and colonic temperature (P<0.001 by *post hoc* Newman-Keuls test) were also re-

duced, not only in comparison to the non-treated controls, but also relative to the values of the same rats before the treatment (P<0.001).

The moderate forced-exercise treatment had little impact on the variables of both euthyroid and hypothyroid rats. In spite of its thyroid-stimulating effect, exercise did not modify significantly the body weight gain, the body temperature nor the thyroid hormone levels in euthyroid rats. Neither did it prevent the development of a hypothyroid condition in antithyroid-treated rats, for the values of plasma hormones, body weight and temperature of this group were similar to those of the non-exercised hypothyroid rats.

Importantly for the purpose of this study, the plasma levels of corticosterone were not modified significantly by the exercise or the antithyroid treatment, thus suggesting that neither the hypothyroid condition nor the exercise caused significant stress in these rats.

Behavioral tests

The rats were subjected to a dark/light box test to assess the levels of anxiety. The results are shown in Fig. 3. All four groups of rats spent significantly more time in the dark (average 78.3% of total time) than in the light compartment ($F_{1,40}$=299.4, P<0.001). There was no difference between groups in any of these measures, thus suggesting that the antithyroid and the exercise treatments did not affect the levels of anxiety of the animals. The number of crossings between the compartments was also similar between groups (average 0.72 ± 0.2; data not shown), thus indicating that the treatments did not modify significantly the physical ability nor the motivation of the rats to explore.

The rats were also subjected to a spatial learning test in a Barnes maze. The results on scape latency are shown in Fig. 4. The non-treated rats showed a consistent reduction of the time to enter the scape box, from

Table I. Effects of moderate forced-exercise routine on thyroid status and corticosterone levels.

	EUT		EUT+EXE		HYPOT		HYPOT+EXE	
	Basal	4th week	Basal	4th week	Basal	4th week	Basal	4th week
Body weight (g)	350±7.1	380±11.8	350±5.8	364±3.3	362.2±4.6	305.7±8.9*	356±5.8	289.3±3.4*
Colonic Temperature (°C)	38.1±0.1	38.4±0.1	38.0±0.2	38.4±0.2	38.6±0.1	37.9±0.1*	38.2±0.1	37.6±0.2*
T4 (µg/dL)	15.1±0.8	14.0±1.6	14.8±0.6	15.6±1.3	14.4±1.0	2.9±0.3*	14.3±0.6	2.4±0.4*
T3 (ng/dL)	28.6±1.5	23.6±1.6	27.9±3.0	31.0±3.0	28.5±2.2	14.0±2.4*	24.6±2.2	16.0±2.0*
Corticosterone (pg/mL)	8.7±0.3	9.5±0.2	8.4±0.4	8.8±0.4	9.0±0.6	9.0±0.5	9.4±0.3	8.4±0.7

Data are means ± s.e.m. of n=6. Data were analyzed by a three-way ANOVA and a Newman-Keuls *post hoc* tests for each variable.
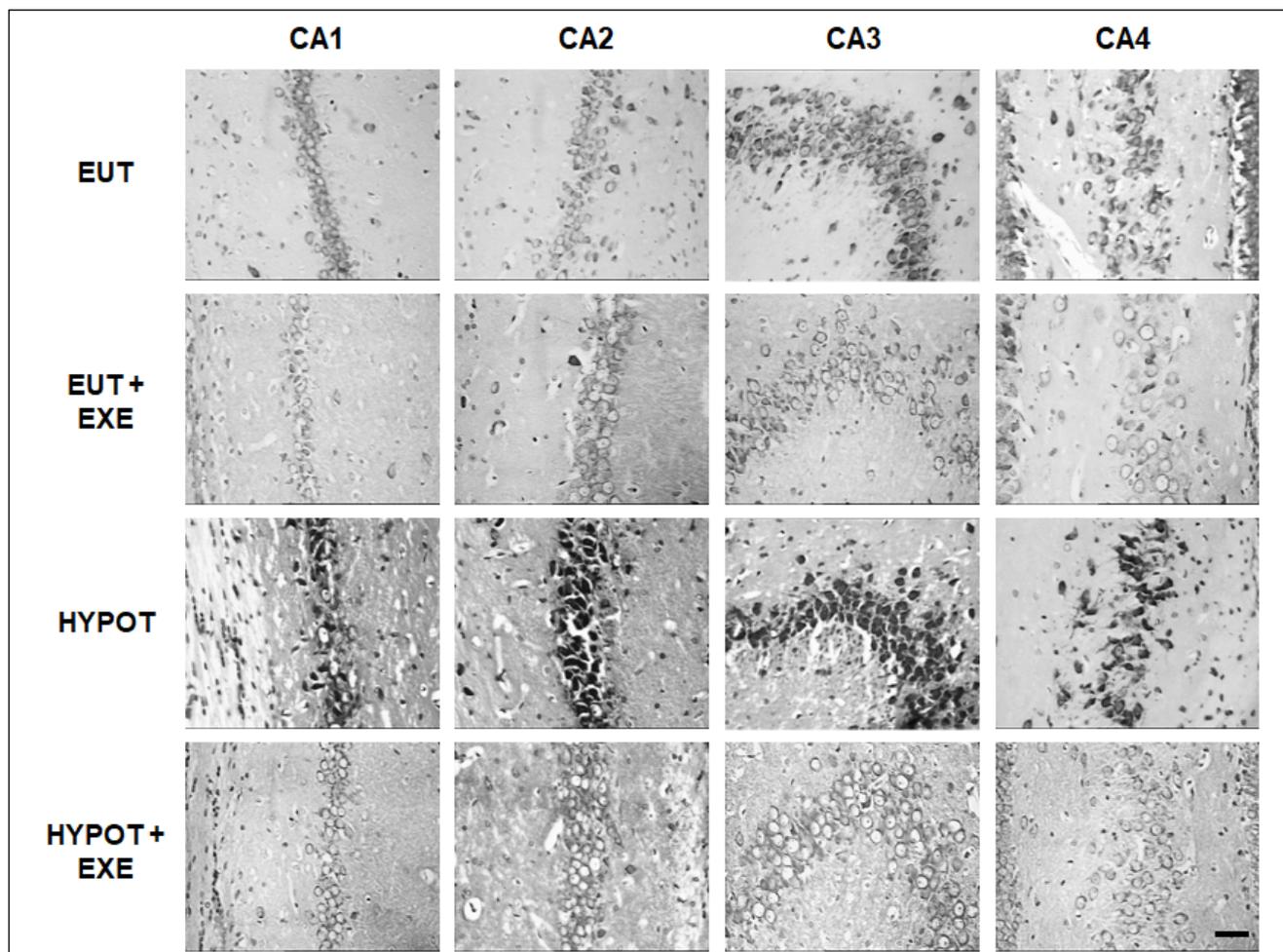*: P<0.05 *vs.* euthyroid group at the same time point.

Fig. 2. Effects of thyroid status and moderate exercise routine on hippocampal pyramidalneuron morphology in the adult rat. Representative micrographs of the different regions of the Ammon´s horn (CA 1 to 4) of euthyroid non-exercised (EUT), euthyroid exercised (EUT+EXE), hypothyroid non-exercised (HYPOT) and hypothyroid exercised (HYPO+EXE) adult male rats. All CA regions of the HYPOT group show neural damage characterized by high prevalence of atrophic neurons, whereas neural damage is significantly reduced in exercised hypothyroid rats (HYPOT+EXE). Scale bar=50 μm.

the first (day 1) to the last (day 5) day of the acquisition phase. The scape latency was similar in the memory phase, 2 days after the last exposition (day 8). Neither the exercise nor the induction of hypothyroidism did modify significantly these tendencies so that all groups showed similar performance in reducing the escape latency through the acquisition phase and retaining this trough the memory phase (Fig. 4). A three-way ANOVA of these data found a significant reduction of latency along the sessions as a global effect ($F_{2,60}$=3.43, P<0.05), but no effect of the thyroid condition or the exercise. All interactions were non-significant.

Fig. 5 shows the average number of errors made by the rats in finding the escape box of the Barnes maze. In spite of a tendency to decrease along the acquisition phase in most groups, the number of errors showed no consistent effect of reduction attributable to learning. Instead, a three-way ANOVA of these data revealed a global reducing effect in the number of errors due
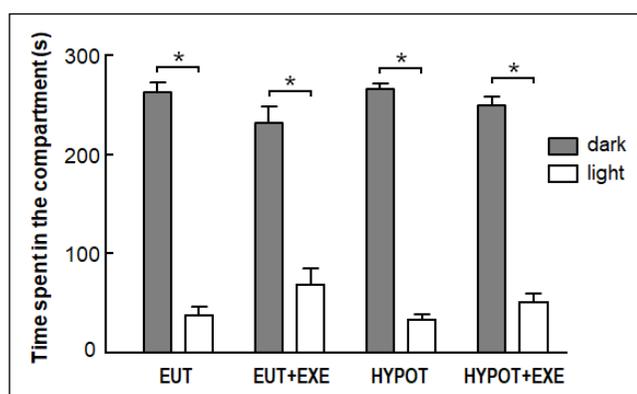

Fig. 3. Activity in the light-dark box of rats that were either subjected to an antithyroid treatment (HYPOT) or not (EUT) and simultaneously to a forced moderate exercise routine (EXE) during the previous 4 weeks. The data are the time lengths the rats spent in each compartment of a light-dark box, expressed as means + S.E.M. per treatment group (n=6). Data were compared by a 3-way ANOVA test. *: P<0.05 between the two compartments.

to exercise ($F_{1,60}$=4.04, P<0.05), irrespective of the thyroid condition. The thyroid condition caused no significant difference in any case; its most evident effect was a larger intra-group variability in all phases, particularly the memory phase (day 8) of the test.

Pyramidal cell counts

The number of hippocampal pyramidal neurons was measured in the rats of all four groups. The results are shown in Fig. 6. The total cell number was greater in CA1 and lower in CA4 ($F_{3,80}$=2378.03; P<0.001). Within each region there was no significant change of the neuron counts due to the antithyroid or the exercise treatments (Fig. 6A).

Based on the morphology of the cells (altered morphology with evident changes in cellular volume or nuclear material loss, indicating an atrophic status; Fig. 2) the neuronal damage was evaluated in each region of the hippocampus. A certain number of atrophic cells (average 22.9%) was found in every CA region in control non-treated rats. The hypothyroid non-exercised rats (HYPOT group) showed a percentage of neuronal damage significantly greater in all CA regions compared to the euthyroid rats (average 2.62 times larger; $F_{1,20}$=155.69 for CA1; $F_{1,20}$=1238.83 for CA2; $F_{1,20}$=1429.55 for CA3; $F_{1,20}$=1176.47 for CA4; P<0.001 in all cases) (Fig. 6B).

Exercising did not increase the neuron counts in any region of the hippocampus, but as a general effect it reduced the number of atrophic cells. In euthyroid rats the exercise reduced slightly the counts of atrophic cells in CA1 (26.1% reduction; P=0.012 by Newman-Keuls *post hoc* test) but had no effect in all other CA regions. In contrast, the hypothyroid rats had the number of atrophic cells markedly reduced by forced exercise in all four CA regions (50.1% reduction of damage in CA1, 56.6% in CA2, 43.8% in CA3 and 50.0% in CA4, relative to the hypothyroid non-exercised group; P<0.001 by Newman-Keuls *post hoc* test, in all cases). In CA1 and CA4 the effect of exercise completely abolished the neuronal damage caused by hypothyroidism, so that the number of atrophic cells was similar to that of euthyroid non-exercised controls. In CA2 and CA3 this effect was weaker, so that the atrophic cell counts of these regions were still higher than the euthyroid control (average 43.4% greater damage than controls, t_{10}=10.38 for CA2 and t_{10}=13.13 for CA3; P≤0.001 in both cases), yet much smaller than the hypothyroid non-exercised group (50.2% lower damage, P<0.001 by Newman-Keuls *post hoc* test) (Fig. 6B).

DISCUSSION

In adult individuals, a hypothyroid condition implies reduced neurogenesis and increased number of atrophic cells in the hippocampus, and this seems to be related to some degree of cognitive impairment and mood disturbance. Physical exercise has positive
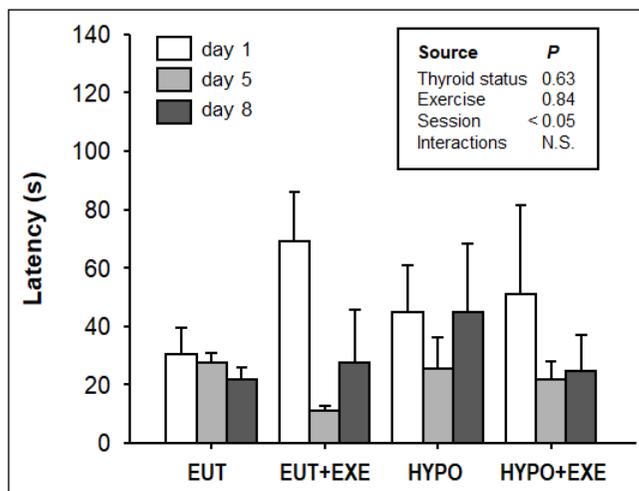

Fig. 4. Spatial learning of rats that were either subjected to an antithyroid treatment (HYPOT) or not (EUT) and simultaneously to a forced moderate exercise routine (EXE) during the previous 4 weeks. The data are the time lengths the rats spent to enter the escape box in a Barnes maze in each of the 3 phases of the test: habituation (day 1), acquisition (day 5) and memory (day 8). Data are means ± S.E.M. of each treatment group (n=6). The inbox shows the results of a 3-way ANOVA test of these data. N.S: not significant.
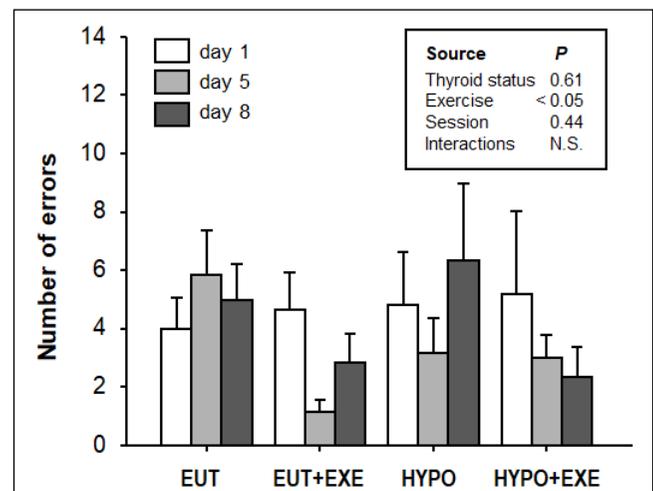

Fig. 5. Spatial learning of rats that were either subjected to an antithyroid treatment (HYPOT) or not (EUT) and simultaneously to a forced moderate exercise routine (EXE) during the previous 4 weeks. The data are the number of errors (exploration of non-target holes) made by the rats before entering the escape box in a Barnes maze, in each of the 3 phases of the test: habituation (day 1), acquisition (day 5) and memory (day 8). Data are means ± s.e.m. of each treatment group (n=6). The inbox shows the results of a 3-way ANOVA test of these data. N.S: not significant.

effects in diverse aspects of the nervous physiology and may thus improve the cognitive function. Besides, exercise also has the potential to improve the thyroid condition by stimulating thyroid activity, thus reducing the negative outcomes of hypothyroidism. In this work we evaluated the capacity of a moderate exercise routine to counter the neuronal and cognitive effects of hypothyroidism in adult rats. We induced a hypothyroid condition by blocking simultaneously the synthesis of thyroid hormones and the peripheral T4 deiodination with a mixture of antithyroid drugs methimazole and PTU (Taurog and Dorris, 1989; Yoshihara et al., 2019). As a result, the antithyroid-treated rats had reduced levels of both T4 and T3, along with reduced colonic temperature and body weight. These results are consistent with previous reports (Alva-Sánchez et al., 2012; Chang et al., 2014) and indicate that this treatment resulted in the rats having a plain hypothyroid condition. Simultaneous to the antithyroid treatment, we subjected the rats to a forced exercise routine. Exercise can have positive or negative effects on the neuron populations of the hippocampus depending on its intensity (Shih et al., 2013; Okamoto et al., 2015), an effect attributable to the activation of the hypothalamus-pituitary-adrenal axis. Forced exercise can also induce anxiety-like behaviors independently of corticoid stimulation (Leasure and Jones, 2008). Since hypothyroidism affects muscular function (Bloise et al., 2018), we employed a moderate forced-exercise routine suitable for hypothyroid rats and aimed at not increasing the activity of the HPA axis and other markers of stress. At the end, this exercise program (5 m/min, 30 min/day, 5 days/week) did not modify the plasma corticosterone levels, the body temperature and weight or the thyroid activity, nor did it elevate the behavioral index of anxiety. This indicates that our protocol is appropriate for that purpose. In this condition we analyzed the structure of the population of pyramidal neurons in the four CA regions of the Ammon's horn of the hippocampus. Previous reports have shown that hypothyroidism is associated with massive neuronal damage in this region, seemingly involving glutamatergic activity (Alva-Sánchez et al., 2009a). In accordance, we found a dramatic increase in the counts of atrophic cells in all CA regions of the hypothyroid rat hippocampi.

The main finding of this study is that moderate exercise could prevent the neuronal damage caused by hypothyroidism. In some regions of the hippocampus the damage was completely abolished (i.e., CA1 and CA4) while it was markedly reduced in others (by 50% in CA2 and CA3). Of note, this effect was observable only as a suppression of the increased damage associated with hypothyroidism, for the counts of atrophic cells in euthyroid rats (i.e., the basal neurodegenerative rate) were barely affected. This indicates that exercise counteracts some of the negative actions of hypothyroidism.

Exercise could affect the thyroid activity in several ways, including systemic and local actions. For instance, exercise involves stimulation of the thyroid gland (Huang et al., 2004), and this could counteract in some degree the inhibitory action of the antithyroid drugs. Exercise is also known to stimulate D2 deiodinase activity in some tissues (Bocco et al., 2016) thus increasing T3 supply at the cell level. In this study the exercise program was applied throughout the antithy-
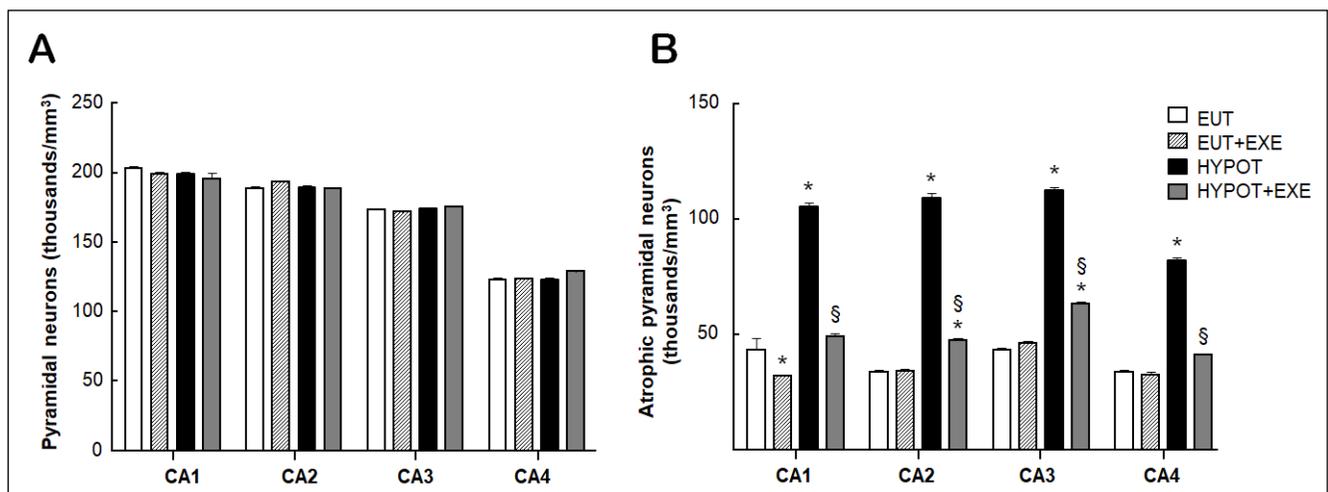

Fig. 6. Pyramidal neuron counts in the four CA regions of the hippocampus of rats that were either subjected to an antithyroid treatment (HYPOT) or not (EUT) and simultaneously to a forced moderate exercise routine (EXE) during 4 weeks. (A): Number of total pyramidal cells per CA region. (B): Number of atrophic pyramidal neurons in each region after each treatment combination. Data are means + s.e.m. of n=6. Data were compared by a 2-way ANOVA test for each region. *: $P<0.01$ *vs.* EUT group; §: $P<0.01$ *vs.* the HYPOT group.

roid treatment, so that thyroid-stimulating effects of exercise could have prevented the development of the hypothyroid condition. This was not the case, for exercise did not rise the thyroid hormones in the blood of the antithyroid-treated rats at the end of the treatment, nor did it restore their thyroid-dependent functions, such as body temperature and weight (Alva-Sánchez et al., 2012). This indicates that the reduction in neurodegenerative rate in exercised rats is not attributable to the recovery of thyroid activity; exercise seems to exert a neuroprotective effect upon the neural tissue.

The hippocampus is a highly proliferative region in adults, and hippocampal neurogenesis is compromised in numerous pathological conditions (Montero-Pedrazuela et al., 2006; Guzman-Marin et al., 2008; Tozuka et al., 2009; Mu and Gage, 2011). Exercise induces cell proliferation (Uda et al., 2006), and exercising has been shown to counteract the reduction in cell proliferation in variety of conditions (Shin et al., 2013; Winocur et al., 2014; Klein et al., 2016; Tapia-Rojas et al., 2016). Nevertheless, the present results suggest that in hypothyroid adult rats the exercise does not affect cell proliferation primarily, for the total cell counts were not modified in any region of the hippocampus. Instead, exercise seems to promote cell survival. This is consistent with previous observations that neural deterioration produced during hypothyroidism involves cell damage rather than reduced proliferation (Alva-Sánchez et al., 2014), and that forced exercise (as in this case) have effects mainly on cell survival while voluntary exercise affects cell proliferation (van Praag et al., 1999).

Among its neural effects, exercise is known to increase the levels of brain-derived neurotrophic factor (BDNF; Wrann et al., 2013). BDNF is a neurotrophin that regulates apoptosis in the nervous tissue (Marosi and Mattson, 2014). Thyroid hormones are necessary for neurotrophin gene expression (Gilbert et al., 2016) and it has been proven that hypothyroidism decreases BDNF levels in the hippocampus (Shafiee et al., 2016). BDNF is known to promote hippocampal cell plasticity and survival by diverse ways deriving from the activation of its receptor tropomyosin-related kinase B (TrkB). Upon binding to TrkB, BDNF causes activation of the mitogen-activated protein kinase (MAPK), the phospholipase C-gamma, and the phosphatidylinositol-3 kinase (PI3-K) cascades. While some of these actions lead to increased synaptic plasticity and LTP facilitation, the expression of the antiapoptotic effector Bcl-2 (Almeida et al., 2005) and the activation of the signaling kinase mTOR (Smith et al., 2014) reduce cell death. This mechanism is thus a strong candidate to explain the changes in neuron survival we found in this study. Previous reports show that the reduction in BDNF levels due to hypothyroidism is modest (i.e., less than 10%

change), so the question remains of whether this effect could entirely explain the pronounced increase in cell atrophy (more than 100%) that we found in this study. Moreover, it has been proposed that in order to cause BDNF up-regulation in the rat, the amount of exercise must exceed a certain threshold (Shen et al., 2001). The estimated distance travelled by the rats in this study (150 m/day) is far below that threshold (500 m/day). This suggests that other mechanisms beside BDNF could be implicated in this neuroprotective effect. This subject would benefit from studies directed to address the participation of BDNF and its receptor TrkB in the hypothyroid brain.

Spatial learning is considered a functional correlate of hippocampal performance (Eichenbaum and Cohen, 2001). In spite of a clear-cut difference in thyroid hormone levels and a dramatic increase in neuron atrophy, the hypothyroid rats in this study showed no difference in the learning performance with respect to euthyroid controls. The time spent (latency) to find the scape hole of the maze was equally reduced in euthyroid and hypothyroid rats along the training sessions. The only cognitive effect associated to hypothyroidism was a slightly higher intra-group variability of the number of errors made by the rats in finding the escape hole, which could be indicative of a heterogeneous effect of hypothyroidism in cognitive performance. Previous studies employing different models of hypothyroidism in adult rats have yielded conflicting results (Montero-Pedrazuela et al., 2006; Alzoubi et al., 2009; Bárez-López et al., 2017), thus suggesting that adult-onset hypothyroidism may affect cognitive performance in a heterogeneous way probably dependent on the prior life story of the individual. In contrast with the thyroid status, the exercise treatment had a clear global effect (i.e., irrespective of whether rats were hypothyroid or not) of reducing the number of errors after conditioning to the maze. This implies that exercise has a general effect of improving learning and memory in both euthyroid and hypothyroid conditions. Exercise also reduced the intra-group variability of the hypothyroid rats, so that the data dispersion of this group was similar to that of the euthyroid controls. These results are in line with the previously reported effects of exercise on cognitive performance (Hillman et al., 2008) and indicate that exercise may help reverting some functional deficits associated with a hypothyroid condition.

In summary, the present study demonstrates that the induction of hypothyroidism to adult rats results in a significant increase in the number of atrophic cells in all CA regions of the hippocampus. This could be associated to a variable degree of cognitive impairment in a spatial-learning test. The rise in hippocampal

cell atrophy is prevented by a moderate, not stressing forced-exercise routine that also regularizes the cognitive functions to values similar to those of euthyroid subjects. The effect of exercise is not mediated by stimulation of thyroid activity nor promotion of local neurogenesis, but instead it seems to involve enhanced cell survival most probably linked to the expression of neurotrophic factors. This last possibility deserves further investigation.

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