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Nandrolone decanoate reduces the positive effects of resistance training on cognition, anxious behavior, and hippocampal morphology in rats

Decanoato de nandrolona reduz os efeitos positivos do treinamento resistido na cognição, comportamento ansioso, e morfologia hipocampal de ratos

El decanoato de nandrolona reduce los efectos positivos del entrenamiento de resistencia sobre la cognición, el comportamiento ansioso y la morfología del hipocampo en ratas

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Abstract
AAs became popular among competitive athletes and the general population for recreational and performance purposes. The indiscriminate use of AAs occurs in supraphysiological doses and negatively affects cognition. Moreover, nandrolone decanoate (DECA) abuse may reduce the beneficial effects of resistance training (RT) on health. Objective: We aimed to investigate if DECA administration in supraphysiological doses interferes with the positive effects of RT on anxious behavior, memory, and morphology of neurons in the hippocampus of rats. Materials and Methods: Rats were randomly assigned into four groups (N= 12): Control (C); DECA (D); Trained (T) Trained+DECA (TD). EAA DECA was administered in daily supraphysiological doses of 15mg/kg during the 8 weeks of the RT protocol. All groups performed a maximum load test before and after the RT protocol; anxious behavior and memory tests were

performed, and thereafter, rats were euthanized for morphologic analyses of the hippocampus. DECA produced an anxiogenic effect, worsened cognition and reduced the thickness of the grainy layer of the DG in the hippocampus. Moreover, DECA reduced the positive effects of RT on cognition and DG neurons. This is the first study showing that suprapharmacological doses of DECA reduce the positive effects of RT on memory and the thickness of the granular layer of the DG.
Keywords: Memory; Exercise; Testosterone; Hypertrofhy; Behavior.

Resumo
Os EAA tornaram-se populares entre atletas competitivos e a população em geral para fins recreativos e de desempenho. O uso indiscriminado de EAAs ocorrem em doses suprafisiológicas e afetam negativamente a cognição. Além disso, o abuso de decanoato de nandrolona (DECA) pode reduzir os efeitos benéficos do treino de resistência (TR) na saúde. Objetivo: Pretendíamos investigar se a administração de DECA em doses suprafisiológicas interfere com os efeitos positivos da TR no comportamento, memória e morfologia dos neurónios no hipocampo dos ratos. Materiais e Métodos: Os ratos foram distribuídos aleatoriamente em quatro grupos (N= 12): Controle (C); DECA (D); Treinado (T) Treinado+DECA (TD). A EAA DECA foi administrada em doses suprafisiológicas diárias de 15mg/kg durante as 8 semanas do protocolo TR. Todos os grupos realizaram um teste de carga máxima antes e depois do protocolo TR; o comportamento ansioso e os testes de memória foram realizados, e depois, os ratos foram eutanasiados para análises morfológicas do hipocampo. O DECA produziu um efeito ansiogênico, agravou a cognição e reduziu a espessura da camada granulado da DG no hipocampo. Além disso, a DECA reduziu os efeitos positivos do TR na cognição e nos neurónios da GD. Este é o primeiro estudo que mostra que as doses suprafarmacológicas do DECA reduzem os efeitos positivos do TR na memória e a espessura da camada granular da GD.
Palavras-chave: Memória; Exercício físico; Testosterona; Hipertrofia; Comportamento.

Resumen
Los AAs se han vuelto populares entre los atletas competitivos y la población en general con fines recreativos y de rendimiento. El uso indiscriminado de AAs se produce en dosis suprafisiológicas y afecta negativamente a la cognición. Además, el abuso de decanoato de nandrolona (DECA) puede reducir los efectos beneficiosos del entrenamiento de resistencia (RT) en la salud. Objetivo: Se quiso investigar si la administración de DECA en dosis suprafisiológicas interfiere con los efectos positivos de la RT sobre el comportamiento, la memoria y la morfología de las neuronas en el hipocampo de las ratas. Materiales y métodos: Las ratas se distribuyeron aleatoriamente en cuatro grupos (N = 12): Control (C); DECA (D); Trenado (T) Trenado+DECA (TD). DECA AE se administró en dosis suprafisiológicas diarias de 15 mg/kg durante las 8 semanas del protocolo TR. Todos los grupos realizaron una prueba de carga máxima antes y después del protocolo TR; se realizaron pruebas de comportamiento ansioso y memoria, y luego las ratas fueron sacrificadas para el análisis morfológico del hipocampo. DECA produjo un efecto aniogénico, empeoró la cognición y redujo el grosor de la capa granulada de DG en el hipocampo. Además, DECA redujo los efectos positivos de TR en la cognición y las neuronas gd. Este es el primer estudio que muestra que las dosis suprafarmacológicas de DECA reducen los efectos positivos de TR sobre la memoria y el grosor de la capa granular de GD.
Palabras clave: Memoria; Ejercicio físico; Testosterona; Hipertrofia; Conducta.

1. Introduction

Anabolic-androgenic steroids (AAs) are synthetic and natural derivatives of the male hormone testosterone, which exert androgenic and anabolic effects (Bhasin, 2000; Kanayama & Pope, 2018). AAs became popular among competitive athletes and the general population for recreational and aesthetic purposes, such as physical performance, increasing muscle mass and decreasing body fat (Bhasin et al., 1996; Nieschlag & Nieschlag, 2019). The indiscriminate use of AAs occurs in supraphysiological doses, usually with doses 10 to 100 times higher than in hormone replacement therapies (Costine et al., 2010; Parkinson & Evans, 2006; Pope JR et al., 1993), leading to a number of both physical and psychological adverse effect (Kutscher et al., 2002; Su et al., 1993).

Recent studies in humans who received supraphysiological doses of AAs have reported several psychological adverse effects, including mania, hypomania, irritability, paranoia, extreme mood swings, irritability, cognitive impairment, body dysmorphic disorder, anxiety, and depression (Pagonis et al., 2006; Pope et al., 1997, 2000; Pope JR et al., 1993; Su et al., 1993). Moreover, several studies in animal models have demonstrated substantial harmful effects caused by the use of AAs in supraphysiological doses to the central nervous system (CNS) (Joksimovic et al., 2017; Novaes Gomes et al., 2014; Selakovic et al., 2017; Tanehkar et al., 2013). Administration of supraphysiological doses of AAs in animals caused an increase in anxious

and depressive behavior, cognitive impairment, and morphological and functional alterations in several brain areas, including the amygdala, prefrontal cortex, and hippocampus (Joksimovic et al., 2017; Mariotti et al., 2014; Novaes Gomes et al., 2014; Selakovic et al., 2017). In opposition to the harmful health effects caused by AAs abuses, several studies have shown the ability of physical exercise to counteract physical and mental pathophysiological processes (Cassilhas et al., 2016; De Sousa et al., 2020, 2021; Egan & Zierath, 2013). Strong evidence has shown that exercise increases anti-apoptotic markers (Joukar et al., 2017; Novaes Gomes et al., 2014) in the brain, induces neuronal proliferation (Novaes Gomes et al., 2014), increases brain-derived neurotrophic factor (BDNF) levels (Tanehkar et al., 2013), improves cerebral blood flow (Mariotti et al., 2014) and antioxidant defense in the hippocampus (Camiletti-Moirón et al., 2015). Notably, recent animal studies have found that aerobic exercise reduces several symptoms induced by the supraphysiological doses of AAs administration, such as anxiety and depressive behavior (Joksimovic et al., 2017; Selakovic et al., 2017).

However, resistance training is the main modality practiced by recreational AAs users (Camiletti-Moirón et al., 2015; Novaes Gomes et al., 2014; Pope et al., 2014), and it is unclear if this exercise modality protects the brain from the administration of supraphysiologic doses of AAs. Thus, we aimed to investigate the possible protective effects of resistance training (RT) on cognition, anxious behavior, and hippocampus morphology of rats submitted to a chronic administration of supraphysiological doses of nandrolone decanoate (DECA).

2. Methodology

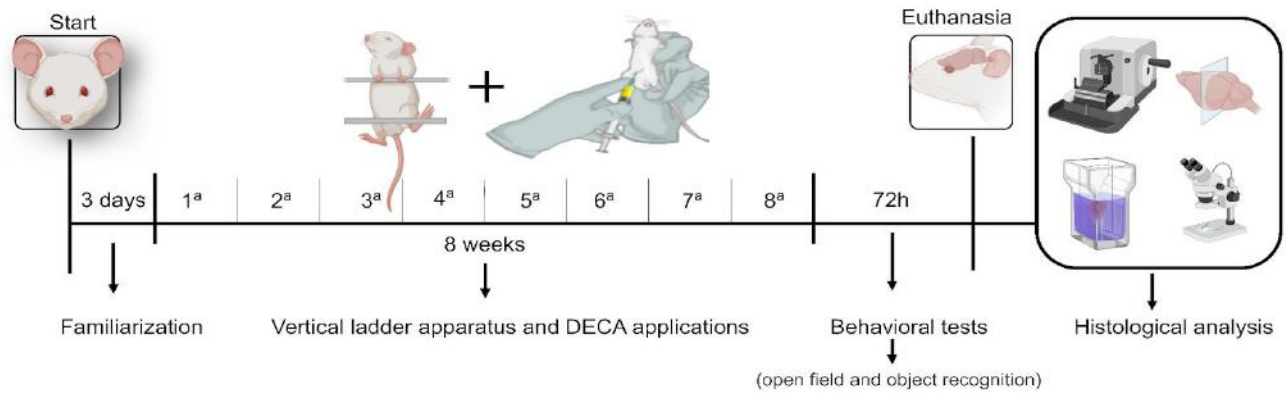
Animals and experimental conditions

Experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 1996). The experimental protocols were approved by Ethics Committee on Animal Use/Federal University of Jequitinhonha and Mucuri Valleys, Diamantina, MG, Brazil (protocol number N. 015/2016). Male Wistar rats were housed in polypropylene cages (4 rats/cage) in a controlled environment (22 ± 2 ◦C; 50% humidity; 12:12 light: dark cycle –lights turn on 6 p.m. and turn off 6 a.m.), with free access to filtered water and commercial rat chow (Nuvilab Nutrients LTDA, Colombo, PR, Brazil).

Experimental design

Sixty-day-old male Wistar rats (n=48) weighing ~250g were used. The animals were randomly assigned into four groups (n=12): Control group –untrained rats treated with vehicle (C); Training group – trained rats treated with vehicle (T); Decanoate group - untrained rats treated with DECA (D); Training + Decanoate group- trained rats treated with DECA (TD). The resistance training (RT) protocol was performed using a latter vertical apparatus according to a previous study (Cassilhas et al., 2013; Cassilhas, Lee, Fernandes, et al., 2012; Cassilhas, Lee, Venâncio, et al., 2012). Briefly, the resistance training protocol consisted of a familiarization period followed by eight weeks of training (5 times a week) with administration of DECA or vehicle. Rats received Decanoate (deca-durabolin® - DECA - 15mg/kg/day) or vehicle (saline – 0.9%) subcutaneously, 5 times a week (total of forty applications) (Hartgens & Kuipers, 2004). After the intervention period, the animals were submitted to behavioral tests (open field and object recognition) for 72 hours (Figure1).

Figure 1 - Experimental design.



Source: Authors.

Resistance training program

The animals from the RT groups were submitted to the familiarization phase at the vertical ladder apparatus. The familiarization with the apparatus was conducted over three days and consisted of three attempts of climbing. First, the animal was kept in the chamber at the top of the ladder for 60 seconds. On the first attempt, the animal was placed on the proximal portion of the ladder (35 cm from the door of the chamber). On the second attempt, the animal was positioned on the middle of the ladder (55 cm from the door of the chamber). On the third attempt, the animal was placed at the bottom of the ladder (110 cm from the door of the chamber). All animals learned to climb, and three days after familiarization, the trained groups (T and TD) were submitted to the RT for eight weeks (5 times a week – Monday to Friday). The RT consisted of eight series of climbing the ladder with progressive overload, fixed on the animal's tail. Each series averaged 8 to 12 climbing movements (repetitions). In the first two series, the overload was 50% of the animal's maximum load test (MLT); then, the load increased according to the series progression (third and fourth series: 75% MLT; fifth and sixth series: 90% MLT; seventh and octave 100% MLT). In the interval of each series, the animal rested in the chamber at the top of the ladder for 60s. (Cassilhas et al., 2013; Cassilhas, Lee, Fernandes, et al., 2012; Cassilhas et al., 2012).

Maximum load test

The maximum load test (MLT) was performed on the first day of training each week. In total, rats performed eight MLT, which defined the workload for the next exercise sessions of the week. In the first week, after the familiarization period, the animals were placed on the vertical ladder apparatus with a load corresponding to 75% of MLT (fixed on the animal´s tail). With each successful climb attempt, 30g of weight was added to the animal´s tail. In the following weeks, the animal started the test with the maximum load reached in the previous week, and again 30g were added until the animal's exhaustion; then, the previous load was considered as the maximum load for the starting of the next week (Novaes Gomes et al., 2014).

Open field test

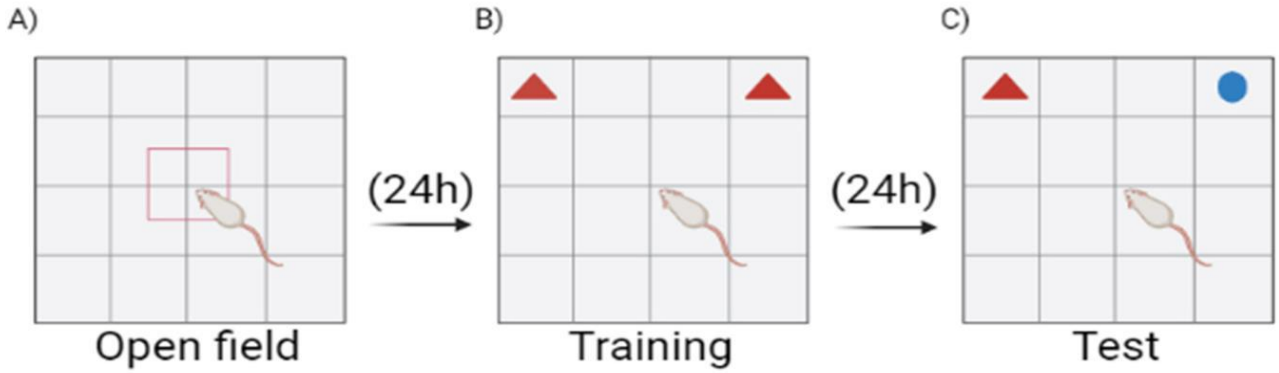
The open field test (Figure2a) was performed forty-eight hours after the last DECA administration and RT session. Each animal was placed in the center of the arena and evaluated during a 5-minute session. The rats were filmed, and later, the following parameters were manually evaluated: a) time spent in the center of the arena; b) time spent on the periphery of the

arena. The arena was completely cleaned with 70% ethanol between tests to eliminate olfactory signs, according to the works (Bourin et al., 2007; de Sousa et al., 2019; Kumar et al., 2013).

New object recognition test (NOR)

The object recognition test (NOR) was performed in three stages. In the first stage, familiarization, the Open Field test was used, where the rats were placed in the center of the box and released to explore the environment for 5 minutes (Figure 2a). In the second step, two equal objects (objects A and B) were placed in the test box (training) with the same exploration time (Figure 2b). The third step (test) occurred 24 hours later and was conducted in the same way as training, but with a new object in place of a training object (object C) (Figure 2c). To measure the learning of the animal, the percentage of time was performed exploring each object during the training and testing sessions to see if the animal actually learned the task. The analysis is performed by the formula (Time of exploration of the family object x 100 / sum of the exploration time of the two objects); then, the percentage of exploration of the familiar object is obtained. To measure the percentage of exploration time in the new object, the formula is used exchanging the familiar object for the new one (Time of exploration of the new object x 100 / sum of the time of exploration of the two objects) (Figueiredo et al., 2013).

Figure 2 – New Object recognition test. (NOR).

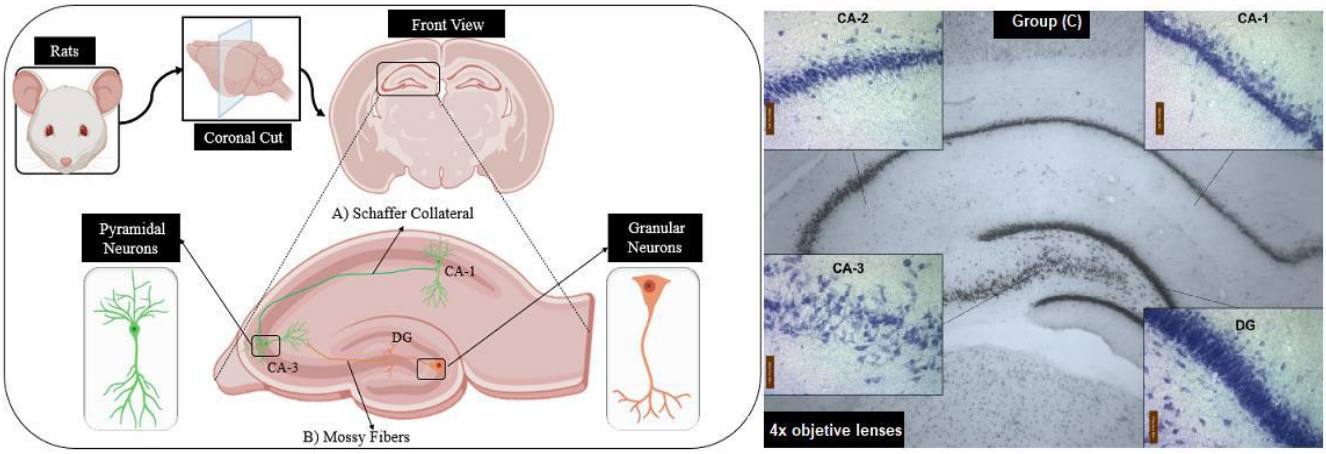


Source: Authors. Dimensions and functioning of behavioral testing areas. Dynamics of the open field and object recognition test phases.

Histology

We used an atlas of rat brain coordinates to locate the area of interest; (Interaural 5.86 mm Bregma -3.14 mm to Interaural 4.70 mm Bregma -4.30 mm) (Paxinos & Watson, 2007). The brains were sliced at 30 µm thick into a cryotomes microtome at -20°C in coronal section and transferred to a 24 well's plate in sucrose solution 30% in PBS at 4ºC (with an interval of 4 sections per well). The selected sections were mounted on gelatinized slides hydrated in Milli-Q water and stained with toluidine blue. For the evaluation and photographic documentation, we used 4 sections of hippocampus from each animal. Four images from right hippocampal areas (CA-1, CA-2, CA3- and DG) at 40× magnification was obtained using a Nikon Eclipse 200 microscope (Tokyo, Japan) (Figure 3). The thickness of layers containing neuronal perikaryal was measured with ImageJ software (ImageJ 1.53, Wayne Rasband, National Institutes of Health, USA). The measurements were manually performed using the straight-line tool.

Figure3 – Histology of the hippocampus of rats, CA1, CA2, CA3 and DG.



Source: Authors.

Statistical analysis

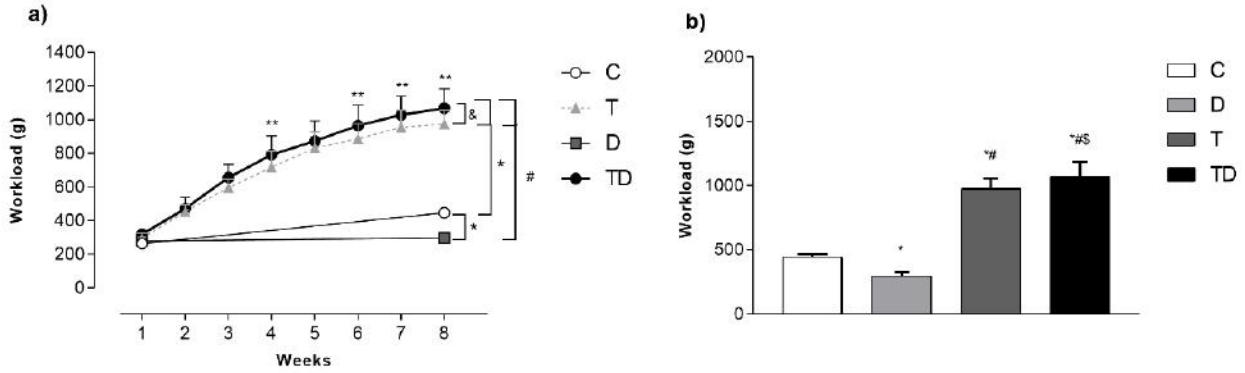
GraphPad Prism 8.0 software was used for all analyses and graphs. The Shapiro-Wilk's test was used to verify the data normality. Data are presented as mean ± standard deviation. Differences among groups were analyzed using one or two-way ANOVA for repeated measurements followed by Tukey's post hoc test. The significance level was established at 5%.

3. Results

Maximum load

Figure 4a presents the data of the maximum load tests over the RT intervention; no differences in the MLT were observed among groups in the first week of RT (p>0.05). From the 4th to 8th week, MLT was significantly higher in the trained groups compared with the untrained groups (p>0.05) and, notably, it was still higher in the TD group compared with the T group (p>0.05). Figure 4b shows the MLT results from all groups in the last week (8th week). D group presented the lowest MLT score, while the TD group had the highest one, and the MLT score was higher in the T group compared to both C and D groups (C: 446,9±19,5, D: 297,4±29,1, T: 974,9±82,2, TD: 1069,4±114,6 g; p < 0.05).

Figure 4 - Workload in weeks and pre and post workload after intervention.

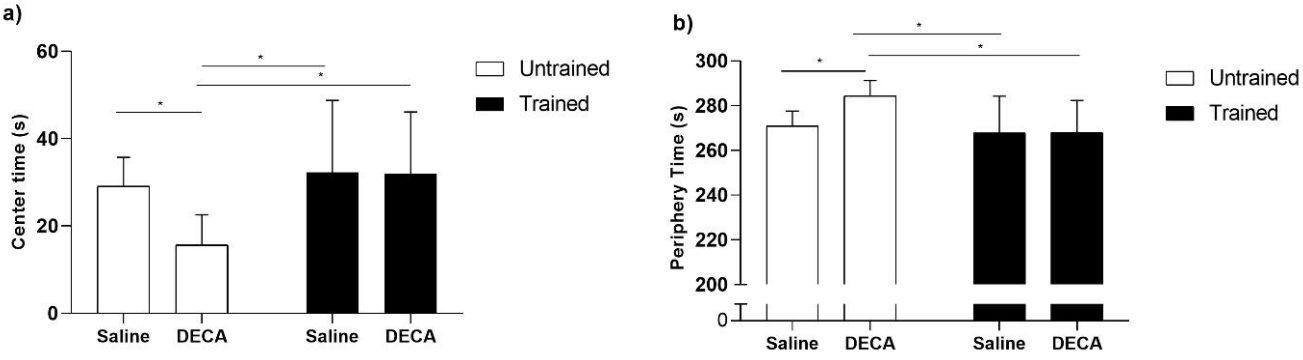


Effects of resistance training for 8 weeks (a) and training workload after 8 weeks (b). Anova one way or two way followed by Tukey test; * indicates difference in relation to the control group (C) p<0.05; # indicates difference in in relation to the deca group (D) p<0.05; and $ indicates difference between the trained groups p<0.05. Source: Authors.

Anxious behavior

Figure 5 shows data from the open field test. The D group presented the highest levels of anxious behavior (represented by the lowest time in the center of the open field and the highest time in the periphery of the open field) among groups. Notably, the T and TD groups had lower levels of anxious behavior (represented by the higher time in the center of the open field and the lower time in the periphery of the open field) than the D group (D *vs* C: 15.65±6.91 *vs* 29.07±6.68 sec, *p*= 0.0491, D *vs* T: 15.65.65±6.91 *vs* 32.29±16.51 sec, *p* =0.0096, **FIG 5a**; D *vs* C: 284.3±6.91 *vs* 270.9±6.68 sec *p*=0.0096, D *vs* T: 284.3±6.91 *vs* 267.7±16.51 sec *p=0.0096*; **FIG 5b**).

Figure5 – Open field test.

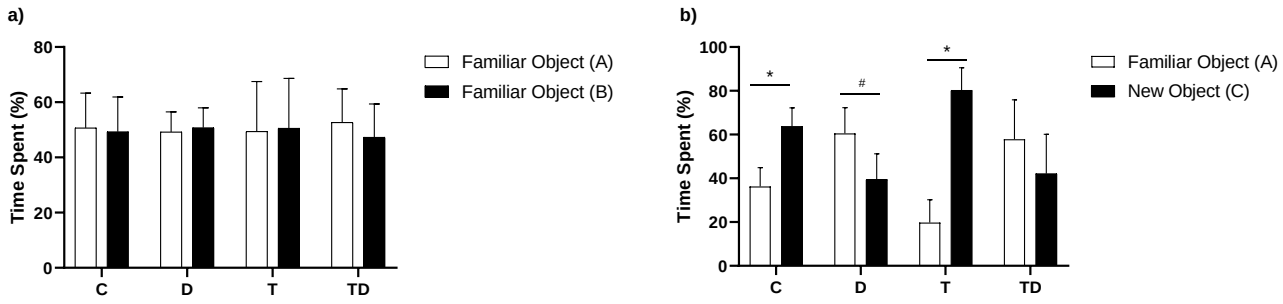


Effects of resistance training on the anxious behavior of Wistar rats obtained by the open field test. One Way Anova followed by Tukey's test. * Indicate statistical difference between groups. P<0.05. Source: Authors.

New object recognition test (NOR)

Figure 6 shows cognition data from NOR test. In the exploration of both objects A and B during NOR training, all groups showed similar results for both objects A and B (C: object A- 50.71±12.61 %, object B- 49.29±12.61 %, p>0.05; D group: object A - 49.25±7.23 %, object B-50.75±7.23 %, p>0.05; T group: object A- 49.43±18.08 %, object B- 50.57±18.08 %, p>0.05; TD group: object A - 52.72±12.07 %, object B - 47.28±12.07 sec, p>0.05; Figure 6A). In the NOR test (Figure 6B), the D group did not learn the task and explored more the familiar object (A) than the new one (C) (D group: familiar object A - 60.55±11.73 *vs* new object C - 39.44±11.73 %, p=0.0047; C group: familiar object A - 36.31±8.52 % *vs* new object C - 63.69±8.52, p<0.0001). On the other hand, the T group learned the task and explored the new object (C) more than the familiar object (A) (T group: new object C - 80.17±10.36 *vs* familiar object A - 19.83±10.36%, p<0.0001); however, the TD group did not learn the task, showing no preference for either object A or C (TD group: familiar object A - 57.87±18.02 *vs* new object C - 42.13±18.02 %, p<0.0832).

Figure 6 – NOR training and testing.

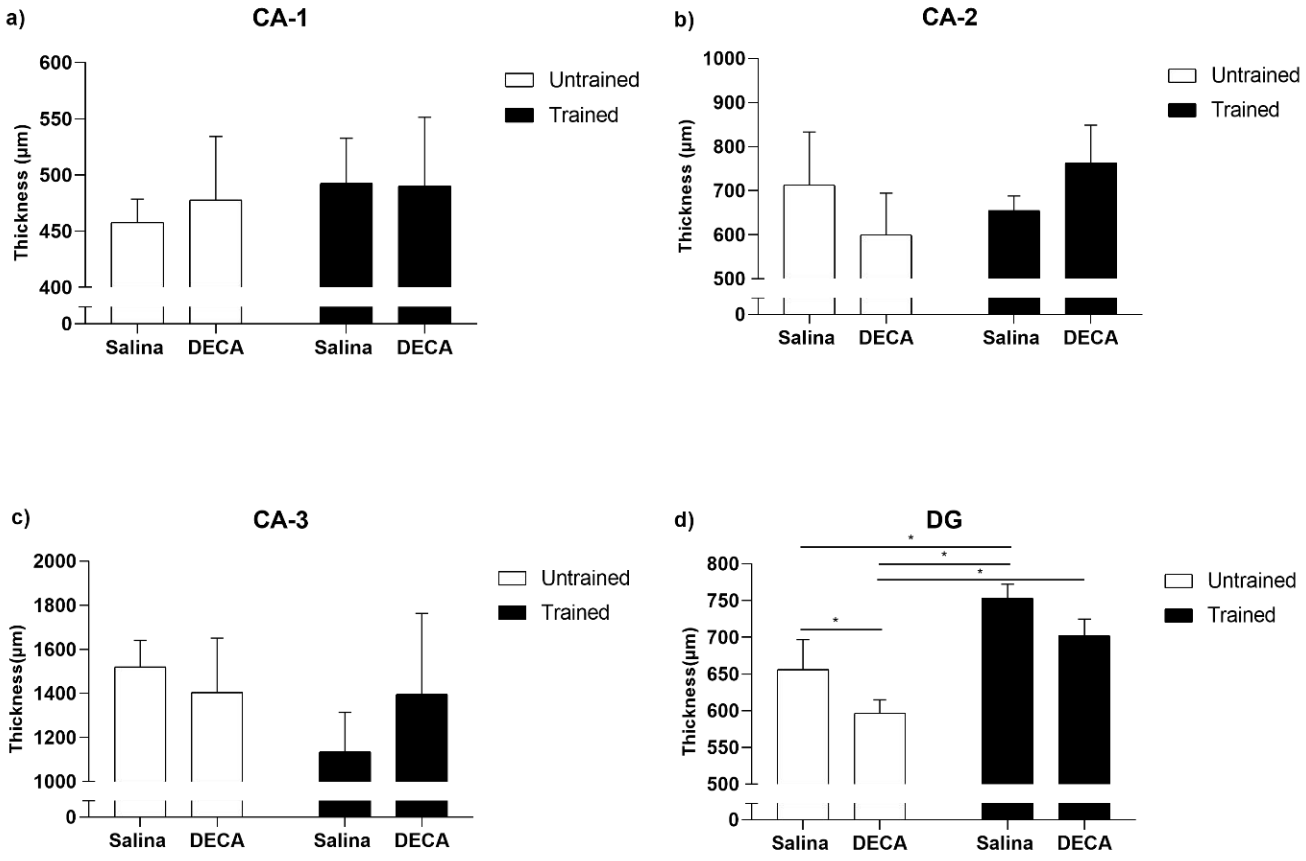


Effects of resistance training on memory of Wistar rats obtained by the new object recognition test. t test was used for intra-group comparison. * Indicate statistical difference intra-groups. P<0.05. Source: Authors.

Hippocampus morphology

Figure 7 shows the results of the morphology in the right hippocampus, evaluated by the thickness (μm) of the pyramidal layer of the CA-1, CA-2 and CA-3 areas and the granular layer of the DG after the intervention period. The thickness of CA-1, CA-2 and CA-3 did not differ between the groups p>0.05 (Figure 7a-c). The D group presented the lowest thickness of grainy layer of the DG area. The RT increased the thickness of the grainy layer of the DG (T vs. C group, p<0.05) and DECA administration did not change this positive effect of RT (TD vs. T group, p>0.05); however, when compared with C group, DECA administration abolished the increase in the thickness of the grainy layer of the DG induced by RT (T *vs.* C, p<0.05; TD *vs.* C, P>0.05) (Figure 7d).

Figure7 – Histology CA1, CA2, CA3 and DG.



Effects of resistance training in morphology in the right hippocampus in CA-1(a), CA-2(b), CA-3(c) and DG(d). Two Way Anova followed by Tukey's test. * Indicate statistical difference between groups. P<0.05. Source: Authors.

4. Discussion

In the present study, we investigated if chronic administration of DECA would reduce the protective effects of RT on anxiety, cognition, and morphology of the hippocampus in rats. We found that chronic supraphysiological doses of DECA reduced the positive effects of RT on cognition and the granular cell layer of the hippocampus DG area.

The deleterious effects AAs abuse are related to the dosage (often 10 to 100 times higher than the therapeutic dosage), which is usually administrated into several repeated courses, or "cycles", over weeks to months (Hartgens & Kuipers, 2004). This abuse also involves the simultaneous use of different classes and categories of AAS to increase the anabolic response and decrease the side effects (Costine et al., 2010; Kanayama et al., 2009; Kanayama & Pope, 2018; Kutscher et al., 2002; Parkinson & Evans, 2006). However, this simultaneous use of different AAs at supraphysiological doses may induce several side effects (Bhasin et al., 2021; Kanayama & Pope, 2018).

The positive effects of physical training on health depend on the exercise modality (e.g., running, resistance training, or swimming), exercise intensity, and duration (Bompa & Haff, 2012; Fleck & Kraemer, 2017; Seo et al., 2014).

The increase in muscle strength is a consequence of the increase in motor unit recruitment and muscle hypertrophy (Egan & Zierath, 2013; Greising et al., 2012). The increase in muscle strength is an important adaptation for improving the quality of daily life as it allows a person to perform daily activities with less effort (Egan & Zierath, 2013; Fleck & Kraemer, 2017; McCall et al., 1996). Here we observed that animals that underwent RT had an increase in muscle strength (Figure 4).

The main clinical recommendation for the use of AAs is related to cachexia treatment (body weight and muscle mass losses) caused by diseases such as cancer, human immunodeficiency syndrome (AIDS), chronic obstructive pulmonary disease (COPD), diabetes mellitus, and aging (Tsametis & Isidori, 2018). The results obtained in the open field test (figure 5) indicate that chronic exposure to DECA produces an anxiogenic effect (decrease in time spent in the central zone and increase in time spent in the periphery zone), which is in line with other studies. SELAKOVIC et al., (2017) found that suprapharmacological doses of DECA (20 mg/kg weekly for 6 weeks) increased anxious behavior in Wistar rats. In another study, they also observed that the combined use of two different AAs (DECA and testosterone enanthate) for 6 weeks had anxiogenic effects in rats (Joksimovic et al., 2019).

Some studies have proposed that the anxiogenic effects caused by chronic abuse of AAs may be related to an increase in the expression of corticotrophin-releasing hormone (CRH) and GABAergic inhibition in the amygdala (Costine et al., 2010; Oberlander & Henderson, 2012). Other studies have found that DECA abuse changes the expression of serotonin receptors (5-HT) in several regions of the brain, including the amygdala, prefrontal cortex, hypothalamus, hippocampus, and other interconnected mood-related areas (Ambar & Chiavegatto, 2009).

Selakovic et al., (2017) found that swimming training effectively neutralized the anxiogenic effects chronic abuse of DECA in male Wistar rats. Similar results were found by Joksimovic et al., (2019). However, the main modality practiced by recreational users of AAS is RT and, to date, no studies have investigated if AAS abuse impairs the positive effects of RT on cognition, anxious behavior, and hippocampal morphology of rats. Here we firstly found that AAS suppresses the beneficial effects RT on cognition and hippocampus of rats. In the NOR test, we observed that the C and T groups learned the test, exploring more time the new object. On the hand, the D and TD groups did not learn the test, revealing that the cognitive impairment induced by DECA did not reduce if DECA is administrated simultaneously with RT. Thus, we believe that deleterious effect of DECA on the hippocampus are not protected by RT.

It is well known that RT improves brain health (Camarda et al., 2012; Cassilhas et al., 2007, 2013, 2020; Cassilhas, Lee, Fernandes, et al., 2012; Cassilhas, Lee, Venâncio, et al., 2012; Chen et al., 2017; De Sousa et al., 2020; Devanne & Allart, 2019; Gomez-Pinilla & Hillman, 2013; Hayek et al., 2019; Lefaucheur, 2019; Lista & Sorrentino, 2010) and the mechanisms involved with these beneficial effects are unclear. We have published a series of studies describing the effects of RT on cognition and neuroplasticity (Cassilhas et al., 2007, 2013, 2020; Cassilhas, Lee, Fernandes, et al., 2012; Cassilhas, Lee, Venâncio, et al., 2012; De Sousa, 2018). Since RT is the most practiced exercise by AAs abusers (Hartgens & Kuipers, 2004), it's is imperative to investigate if the positive effects of RT on brain may be reduced by AAs abuse. Here, we found that, while RT increased the thickness of the grainy layer of the DG and DECA administration reduced it. These results suggest that the RT induces neurogenesis while DECA abuse induces atrophy in hippocampus. We also revealed that RT increased the number of Ki-67-positivecells (a cell proliferation marker) in DG of hippocampus. Nevertheless, this RT effects were abolished by DECA (Novaes Gomes et al., 2014). In another study, animals submitted to 8 weeks of swimming training worse the anti-apoptotic markers and increased levels of pro-apoptotic factors in the hippocampus of rats administered with DECA (Joukar et al., 2017). In addition, running did not avoid the negative effects of DECA administration on spatial learning and memory of rats (Tanehkar et al., 2013).

The anti-anxiolytic effect caused by RT in animals exposed to AAS may occur due to its favorable regulations in the hippocampus. Thus, granular neurons of the DG establish projections for the tonsil region that determine the triggering intensity of the fear emotion, thereby controlling the state of anxiety. Although this possible neurobiological mechanism brought in our study needs to be further elucidated, we cannot rule out that the use of DECA leads to ultrastructural and biochemical changes in hippocampus neurons and TF protects against these effects, which opens the possibility for future studies to investigate with other techniques this possibility raised.

Whereas AAs abuses are most often done in different cycles and with combinations of different classes of anabolic agents, in which they can cause even more damage to users' health, further future studies should be conducted with different combinations of anabolic substances, and interspersed by periods of suppression of use, associated with physical exercise, in order to simulate different forms of abuse of AAs. Thus, it will allow the analysis of what these harmful effects would be, in order to alert the general population against the deleterious consequences of unnecessary and uncontrolled administration of AAs.

5. Conclusion

Taken together, this is the first study showing that supra pharmacological doses of DECA reduce the positive effects of RT on memory and the thickness of the granular layer of the DG. Future studies with other dosages of DECA, other types of exercise and mainly studying metabolic pathways are necessary for deepening knowledge about physical exercise and AAS.

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References

Ambar, G., & Chiavegatto, S. (2009). Anabolic-androgenic steroid treatment induces behavioral disinhibition and downregulation of serotonin receptor messenger RNA in the prefrontal cortex and amygdala of male mice. *Genes, Brain and Behavior*, *8*(2), 161–173. https://doi.org/10.1111/j.1601-183X.2008.00458.x

Bhasin, S. (2000). Testosterone Replacement and Resistance Exercise in HIV-Infected Men With Weight Loss and Low Testosterone Levels. *JAMA*, *283*(6), 763. https://doi.org/10.1001/jama.283.6.763

Bhasin, S., Hatfield, D. L., Hoffman, J. R., Kraemer, W. J., Labotz, M., Phillips, S. M., & Ratamess, N. A. (2021). Anabolic-Androgenic Steroid Use in Sports, Health, and Society. *Medicine & Science in Sports & Exercise*, *53*(8), 1778–1794. https://doi.org/10.1249/MSS.0000000000002670

Bhasin, S., Storer, T. W., Berman, N., Callegari, C., Clevenger, B., Phillips, J., Bunnell, T. J., Tricker, R., Shirazi, A., & Casaburi, R. (1996). The Effects of Supraphysiologic Doses of Testosterone on Muscle Size and Strength in Normal Men. *New England Journal of Medicine*, *335*(1), 1–7. https://doi.org/10.1056/NEJM199607043350101

Bompa, T. O., & Haff, G. G. (2012). *Periodização Teoria e Metodologia do Treinamento* (Phorte (ed.); 5ª). Phorte.

Bourin, M., Petit-Demoulière, B., Nic Dhonnchadha, B., & Hascöet, M. (2007). Animal models of anxiety in mice. *Fundamental & Clinical Pharmacology*, *21*(6), 567–574. https://doi.org/10.1111/j.1472-8206.2007.00526.x

Camarda, S. R. de A., Tebexreni, A. S., Páfaro, C. N., Sasai, F. B., Tambeiro, V. L., Juliano, Y., Barros Neto, T. L. de, Edwards, L. J., Nelson, M. E., Rejeski, W. J., Blair, S. N., Duncan, P. W., Judge, J. O., King, A. C., MacEra, C. A., Castaneda-Sceppa, C., Bos, I., De Boever, P., Int Panis, L., … De Mello, M. T. (2012). Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. *Neuroscience*, *44*(2), 1–7. https://doi.org/10.1016/j.neuroscience.2011.11.029

Camiletti-Moirón, D., Aparicio, V. A., Nebot, E., Medina, G., Martínez, R., Kapravelou, G., Andrade, A., Porres, J. M., López-Jurado, M., & Aranda, P. (2015). High-intensity Exercise Modifies the Effects of Stanozolol on Brain Oxidative Stress in Rats. *Int J Sports Med*, *36*(12), 984–991. https://doi.org/DOI: 10.1055 / s-0035-1548941

Cassilhas, R. C., Lee, K. S., Fernandes, J. C. J., Oliveira, M. G. M., Tufik, S. S., Meeusen, R., Mello, M. T. De, Gomes, F. C. F. G. N., Fernandes, J. C. J., Campos, D. V., Cassilhas, R. C., Viana, G. M., D'Almeida, V., de Moraes Rêgo, M. K., Buainain, P. I., Cavalheiro, E. A., Arida, R. M., Viana, V. A. R., Grassmann, V., … Delacour, J. (2020). The impact of resistance exercise on the cognitive function of the elderly. *Medicine and Science in Sports and Exercise*, *39*(1), 1–9. https://doi.org/10.1249/mss.0b013e318060111f

Cassilhas, R. C., Lee, K. S., Fernandes, J., Oliveira, M. G. M., Tufik, S., Meeusen, R., & De Mello, M. T. (2012). Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2011.11.029

Cassilhas, R. C., Lee, K. S., Venâncio, D. P., Oliveira, M. G. M., Tufk, S., & de Mello, M. T. (2012). Resistance exercise improves hippocampus-dependent memory. *Brazilian Journal of Medical and Biological Research*. https://doi.org/10.1590/S0100-879X2012007500138

Cassilhas, R. C., Reis, I. T., Venâncio, D., Fernandes, J., Tufik, S., & Mello, M. T. de. (2013). Animal model for progressive resistance exercise: a detailed description of model and its implications for basic research in exercise. *Motriz: Revista de Educação Física*, *19*(1), 178–184. https://doi.org/10.1590/S1980-65742013000100018

Cassilhas, R. C., Tufik, S., & Mello, M. T. de. (2016). Physical exercise, neuroplasticity, spatial learning and memory Ricardo. *International Journal of Powder Metallurgy*, *53*(1), 27–36. https://doi.org/10.1007/s00018-015-2102-0

Cassilhas, R. C., Viana, V. A. R., Grassmann, V., Santos, R. T., Santos, R. F., Tufik, S., & Mello, M. T. (2007). The impact of resistance exercise on the cognitive function of the elderly. *Medicine and Science in Sports and Exercise*, *39*(8), 1401–1407. https://doi.org/10.1249/mss.0b013e318060111f

Chen, C., Nakagawa, S., An, Y., Ito, K., Kitaichi, Y., & Kusumi, I. (2017). The exercise-glucocorticoid paradox: How exercise is beneficial to cognition, mood, and the brain while increasing glucocorticoid levels. *Frontiers in Neuroendocrinology*, *44*, 83–102. https://doi.org/10.1016/j.yfrne.2016.12.001

Costine, B. A., Oberlander, J. G., Davis, M. C., Penatti, C. A. A., Porter, D. M., Leaton, R. N., & Henderson, L. P. (2010). Chronic anabolic androgenic steroid exposure alters corticotropin releasing factor expression and anxiety-like behaviors in the female mouse. *Psychoneuroendocrinology*, *35*(10), 1473–1485. https://doi.org/10.1016/j.psyneuen.2010.04.015

De Sousa, R. A. L. (2018). Brief report of the effects of the aerobic, resistance, and high-intensity interval training in type 2 diabetes mellitus individuals. *International Journal of Diabetes in Developing Countries*, *38*(2), 138–145. https://doi.org/10.1007/s13410-017-0582-1

De Sousa, R. A. L., Caria, A. C. I., De Jesus Silva, F. M., Diniz e Magalhães, C. O., Freitas, D. A., Lacerda, A. C. R., Mendonça, V. A., Cassilhas, R. C., & Leite, H. R. (2020). High-intensity resistance training induces changes in cognitive function, but not in locomotor activity or anxious behavior in rats induced to type 2 diabetes. *Physiology & Behavior*, *223*(June), 1–7. https://doi.org/10.1016/j.physbeh.2020.112998

de Sousa, R. A. L., de Lima, E. V., da Silva, T. P., de Souza, R. V., Figueiredo, C. P., Passos, G. F., & Clarke, J. R. (2019). Late Cognitive Consequences of Gestational Diabetes to the Offspring, in a New Mouse Model. *Molecular Neurobiology*, *56*(11), 7754–7764. https://doi.org/10.1007/s12035-019-1624-0

De Sousa, R. A. L., Rocha-Dias, I., de Oliveira, L. R. S., Improta-Caria, A. C., Monteiro-Junior, R. S., & Cassilhas, R. C. (2021). Molecular mechanisms of physical exercise on depression in the elderly: a systematic review. *Molecular Biology Reports*, *48*(4), 3853–3862. https://doi.org/10.1007/s11033-021-06330-z

Devanne, H., & Allart, E. (2019). Boosting brain motor plasticity with physical exercise. *Neurophysiologie Clinique*, *2018*, 2018–2020. https://doi.org/10.1016/j.neucli.2019.01.003

Egan, B., & Zierath, J. R. (2013). Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metabolism*, *17*(2), 162–184. https://doi.org/10.1016/j.cmet.2012.12.012

Figueiredo, C. P., Clarke, J. R., Ledo, J. H., Ribeiro, F. C., Costa, C. V., Melo, H. M., Mota-Sales, A. P., Saraiva, L. M., Klein, W. L., Sebollela, A., De Felice, F. G., & Ferreira, S. T. (2013). Memantine Rescues Transient Cognitive Impairment Caused by High-Molecular-Weight A Oligomers But Not the Persistent Impairment Induced by Low-Molecular-Weight Oligomers. *Journal of Neuroscience*, *33*(23), 9626–9634. https://doi.org/10.1523/JNEUROSCI.0482-13.2013

Fleck, S. J., & Kraemer, W. J. (2017). Fundamentos do treinamento de força muscular. In *Fundamentos do treinamento de força muscular [recurso eletrônico] / Steven J. Fleck, William J. Kraemer ; tradução: Jerri Luis Ribeiro, Regina Machado Garcez ; revisão técnica: Ronei Silveira Pinto, Matheus Daros Pinto. – 4. ed. – Porto Alegre : Artmed, 2*.

Gomez-Pinilla, F., & Hillman, C. (2013). *The Influence of Exercise on Cognitive Abilities - Comprehensive Physiology*. https://doi.org/10.1002/cphy.c110063.The

Greising, S. M., Gransee, H. M., Mantilla, C. B., & Sieck, G. C. (2012). Systems biology of skeletal muscle: fiber type as an organizing principle. *WIREs Systems Biology and Medicine*, *4*(5), 457–473. https://doi.org/10.1002/wsbm.1184

Hartgens, F., & Kuipers, H. (2004). Effects of androgenic-anabolic steroids in athletes. *Sports Medicine*, *34*(8), 513–554. https://doi.org/10.2165/00007256-200434080-00003

Hayek, L. El, Khalifeh, M., Zibara, V., Assaad, R. A., Emmanuel, N., El-ghandour, R., Nasrallah, P., Bilen, M., Ibrahim, P., Younes, J., Haidar, A., Barmo, N., Jabre, V., Stephan, J. S., Sleiman, S. F., Emmanuel, N., Karnib, N., El-ghandour, R., Nasrallah, P., … Sleiman, S. F. (2019). *Lactate mediates the effects of exercise on learning and memory through SIRT1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF)*. https://doi.org/10.1523/JNEUROSCI.1661-18.2019

Joksimovic, J., Selakovic, D., Jovicic, N., Mitrovic, S., Mihailovic, V., Katanic, J., Milovanovic, D., & Rosic, G. (2019). Exercise Attenuates Anabolic Steroids-Induced Anxiety via Hippocampal NPY and MC4 Receptor in Rats. *Frontiers in Neuroscience*, *13*. https://doi.org/10.3389/fnins.2019.00172

Joksimovic, J., Selakovic, D., Matovic, M., Zaletel, I., Puskas, N., & Rosic, G. (2017). The role of neuropeptide-Y in nandrolone decanoate-induced attenuation of antidepressant effect of exercise. *PLOS ONE*, *12*(6), e0178922. https://doi.org/10.1371/journal.pone.0178922

Joukar, S., Vahidi, R., Farsinejad, A., Asadi-shekaari, M., & Shahouzehi, B. (2017). Ameliorative Effects of Endurance Exercise with Two Different Intensities on Nandrolone Decanoate-Induced Neurodegeneration in Rats: Involving Redox and Apoptotic Systems. *Neurotoxicity Research*, *32*(1), 41–49. https://doi.org/10.1007/s12640-017-9705-1

Kanayama, G., Hudson, J. I., & Jr, H. G. P. (2009). Long Term Psychiatric and Medical Consequences of Anabolic Androgenic Steroid Abuse. *Drug and Alcohol Dependence*, *98*(617), 1–12. https://doi.org/10.1016/j.drugalcdep.2008.05.004.Long-Term

Kanayama, G., & Pope, H. G. (2018). History and epidemiology of anabolic androgens in athletes and non-athletes. *Molecular and Cellular Endocrinology*, *464*, 4–13. https://doi.org/10.1016/j.mce.2017.02.039

Kumar, V., Bhat, Z. A., & Kumar, D. (2013). Animal models of anxiety: A comprehensive review. *Journal of Pharmacological and Toxicological Methods*, *68*(2), 175–183. https://doi.org/10.1016/j.vascn.2013.05.003

Kutscher, E. C., Lund, B. C., & Perry, P. J. (2002). A Review for the Clinician. *Sports Med*, *32*(5), 285–296.

Lefaucheur, J. P. (2019). Boosting physical exercise with cortical stimulation or brain doping using tDCS: Fact or myth? *Neurophysiologie Clinique*, *2018*, 20–23. https://doi.org/10.1016/j.neucli.2019.01.002

Lista, I., & Sorrentino, G. (2010). Biological mechanisms of physical activity in preventing cognitive decline. In *Cellular and Molecular Neurobiology* (Vol. 30, Issue 4, pp. 493–503). https://doi.org/10.1007/s10571-009-9488-x

Mariotti, R., Fattoretti, P., Malatesta, M., Nicolato, E., Sandri, M., & Zancanaro, C. (2014). Forced mild physical training improves blood volume in the motor and hippocampal cortex of old mice. *The Journal of Nutrition, Health & Aging*, *18*(2), 178–183. https://doi.org/10.1007/s12603-013-0384-1

McCall, G. E., Byrnes, W. C., Dickinson, A., Pattany, P. M., & Fleck, S. J. (1996). Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *Journal of Applied Physiology*, *81*(5), 2004–2012. https://doi.org/10.1152/jappl.1996.81.5.2004

Nieschlag, E., & Nieschlag, S. (2019). The history of discovery , synthesis and development of testosterone for clinical use. *European Society of Endocrinology*, *180*(6), 201–212.

Novaes Gomes, F. G., Fernandes, J., Vannucci Campos, D., Cassilhas, R. C., Viana, G. M., D'Almeida, V., de Moraes Rêgo, M. K., Buainain, P. I., Cavalheiro, E. A., & Arida, R. M. (2014). The beneficial effects of strength exercise on hippocampal cell proliferation and apoptotic signaling is impaired by anabolic androgenic steroids. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2014.08.009

Oberlander, J. G., & Henderson, L. P. (2012). Corticotropin-Releasing Factor Modulation of Forebrain GABAergic Transmission has a Pivotal Role in the Expression of Anabolic Steroid-Induced Anxiety in the Female Mouse. *Neuropsychopharmacology*, *37*(6), 1483–1499. https://doi.org/10.1038/npp.2011.334

Pagonis, T. A., Angelopoulos, N. V., Koukoulis, G. N., & Hadjichristodoulou, C. S. (2006). Psychiatric side effects induced by supraphysiological doses of combinations of anabolic steroids correlate to the severity of abuse. *European Psychiatry*, *21*(8), 551–562. https://doi.org/10.1016/j.eurpsy.2005.09.001

Parkinson, A. B., & Evans, N. A. (2006). Anabolic Androgenic Steroids: A Survey of 500 Users. *Medicine & Science in Sports & Exercise*, *38*(4). https://journals.lww.com/acsm-msse/Fulltext/2006/04000/Anabolic_Androgenic_Steroids__A_Survey_of_500.6.aspx

Paxinos, G., & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates* (I. Academic Press (ed.); 6th ed.).

Pope, H. G., Gruber, A. J., Choi, P., Olivardia, R., & Phillips, K. A. (1997). Muscle Dysmorphia: An Underrecognized Form of Body Dysmorphic Disorder. *Psychosomatics*, *38*(6), 548–557. https://doi.org/10.1016/S0033-3182(97)71400-2

Pope, H. G., Gruber, A. J., Mangweth, B., Bureau, B., DeCol, C., Jouvent, R., & Hudson, J. I. (2000). Body Image Perception Among Men in Three Countries. *American Journal of Psychiatry*, *157*(8), 1297–1301. https://doi.org/10.1176/appi.ajp.157.8.1297

Pope, H. G., Kanayama, G., Athey, A., Ryan, E., Hudson, J. I., & Baggish, A. (2014). The lifetime prevalence of anabolic-androgenic steroid use and dependence in Americans: Current best estimates. *The American Journal on Addictions*, *23*(4), 371–377. https://doi.org/10.1111/j.1521-0391.2013.12118.x

Pope JR, H. G., Katz, D. L., & Hudson, J. I. (1993). Anorexia nervosa and "reverse anorexia" among 108 male bodybuilders. *Comprehensive Psychiatry*, *34*(6), 406–409. https://doi.org/https://doi.org/10.1016/0010-440X(93)90066-D

Selakovic, D., Joksimovic, J., Zaletel, I., Puskas, N., Matovic, M., & Rosic, G. (2017). The opposite effects of nandrolone decanoate and exercise on anxiety levels in rats may involve alterations in hippocampal parvalbumin–positive interneurons. *PLOS ONE*, *12*(12), e0189595. https://doi.org/10.1371/journal.pone.0189595

Seo, D. Y., Lee, S. R., Kim, N., Ko, K. S., Rhee, B. D., & Han, J. (2014). Humanized animal exercise model for clinical implication. *Pflugers Archiv European Journal of Physiology*, *466*(9), 1673–1687. https://doi.org/10.1007/s00424-014-1496-0

Su, T. P., Pagliaro, M., Schmidt, P. J., Pickar, D., Wolkowitz, O., & Rubinow, D. R. (1993). Neuropsychiatric effects of anabolic steroids in male normal volunteers. *JAMA*, *269*(21), 2760–2764. http://www.ncbi.nlm.nih.gov/pubmed/8492402

Tanehkar, F., Rashidy-Pour, A., Vafaei, A. A., Sameni, H. R., Haghighi, S., Miladi-Gorji, H., Motamedi, F., Akhavan, M. M., & Bavarsad, K. (2013). Voluntary exercise does not ameliorate spatial learning and memory deficits induced by chronic administration of nandrolone decanoate in rats. *Hormones and Behavior*, *63*(1), 158–165. https://doi.org/10.1016/j.yhbeh.2012.10.003

Tsametis, C. P., & Isidori, A. M. (2018). Testosterone replacement therapy: For whom, when and how? *Metabolism*, *86*, 69–78. https://doi.org/10.1016/j.metabol.2018.03.007