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Effects of Chronic Administration With High Doses of Testosterone Propionate on Behavioral and Physiological Parameters in Mice With Differing Basal Aggressiveness

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The effects of testosterone propionate, an anabolic-androgenic steroid, on the behavior displayed during a social encounter by gonadally intact male mice were investigated. Animals were distributed into three groups according to their attack latency in a pre-screening test (high-, moderate-, and low-attacking mice) and each group received weekly injections of 60 or 120 mg/kg of testosterone or sesame oil for 10 weeks. Behavioral tests were then carried out. Afterwards, organs were weighed and blood samples collected in order to obtain hormonal data. Treatment had a differential impact on attack in the three groups of animals. Only the high-attacking testosterone-treated mice showed lower total duration of attack than their controls. Those that received 60 mg/kg spent more time exhibiting exploratory behaviors. As an index of the anabolic activity of the drug, all testosterone-treated mice had heavier kidneys and, as an index of the androgenic activity of testosterone propionate, they had heavier seminal vesicles, lighter testes, and showed higher testosterone levels in a dose-dependent way than their controls. Hence, the effect of treatment on peripheral physiological parameters was similar in all three groups whereas behavioral effects differed depending on basal aggressiveness, considered a characteristic of coping style. Aggr. Behav. 29:173–189, 2003. © 2003 Wiley-Liss, Inc.

Key words: anabolic-androgenic steroids; basal aggressiveness; testosterone; androgenic target organs; anabolic target organs

INTRODUCTION

There is wide agreement with regard to the pro-aggressive action of androgens, based on several decades of research on the effects of testosterone administration in castrated mammals. This influence is modulated by factors such as previous experience, genetics, and

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area of the agonistic encounter. The effect of testosterone derivatives (anabolic-androgenic steroids) on aggression in non-castrated male rodents has also been explored in order to confirm that the increased aggression and violent behavior described in humans is a consequence of the abuse of these compounds. The repeated administration of a single anabolic-androgenic steroid induces the appearance of dominant behavior [Bonson et al., 1994; Bonson and Winter, 1992] and a slight increase in threats [Lumia et al., 1994] in male rats. Works have presented contradictory results in mice. Single anabolic-androgenic steroids [Martínez-Sanchis et al., 1996, 1998a; Sandnabba et al., 1994] or combined in a mixture [Ahima and Harlan, 1992; Bronson, 1996; Clark et al., 1995; Menard et al., 1995; Moya-Albiol et al., 1997] have no effects, enhance, or even diminish aggression after chronic or acute treatment. Among factors which could explain these contradictory findings, such as the doses employed and the duration of treatment, the role of individual differences is one of the less explored.

Anabolic-androgenic steroids are sometimes abused in competitive and non-competitive sports, which can lead to a complex abuse syndrome. In a previous study [Martínez-Sanchis et al., 1998b], we analyzed the effects of moderate doses of abuse (from 3.75 up to 30 mg/kg) of testosterone propionate on agonistic behavior in male mice. After the administration of testosterone for ten weeks, treated mice displayed shorter latencies of threats than control mice in the last of three encounters. Subsequently, the total sample was divided into two groups (high- and low-attacking mice) depending on the attack latencies shown in the first encounter. Statistical analyses were recalculated. The former results were replicated only in high-attacking animals. Martínez-Sanchis et al., 1998a examined this aspect in another experiment. High- and low-attacking mice were selected prior to being treated with a single high dose of testosterone propionate (60 mg/kg). The results confirmed that only high-attacking animals were affected by the treatment, and displayed a lower frequency of aggressiveness than their controls.

Since the doses self-administered by abusers are often between 10 and 100, and even up to 1000-fold higher than the therapeutic one (1 mg/kg) [Lukas, 1996; Wadler and Hainline, 1989], in the present work 60 and 120 mg/kg per week were injected to mimic doses used by heavy abusers. These doses are similar to others which induced the appearance of dominance in intact male rats [Bonson et al., 1994; Bonson and Winter, 1992]. The main aim of the present study was to elucidate the effects of chronically-administered supraphysiological dosages of anabolic-androgenic steroids on behavioral and peripheral physiological measures in male mice, in light of their previous levels of aggressiveness. Thus, the effects of a long-term administration (10 weeks) of high doses of testosterone propionate (one of the most abused anabolic-androgenic steroids) on agonistic behavior were evaluated in intact male mice selected on the basis of their attack latency. Blood samples were taken to study the relationship between changes in aggression and hormonal levels (testosterone and corticosterone) resulting from the treatment. The role of corticosterone was also considered in this study because both hormonal functions have been influenced by common factors such as social status and stress, and the relationship between them seems complex and is still unclear [Hayashi and Moberg, 1987; Norman, 1993].

Finally, in order to evaluate the anabolic and androgenic potency of testosterone propionate separately, body, liver, kidney, seminal vesicles, preputial glands, and testes weights were recorded. We hypothesized, firstly, that the long-term treatment with high doses of anabolic-androgenic steroids would induce changes in offensive behaviors and these changes would be greater in the most aggressive animals. Secondly, it would cause

physiological effects characterized by androgenic inhibition, anabolic enhancement, and hepatotoxic features typical of abuse.

METHODS

Animals

A total of 245 OF-1 outbred male mice purchased from Iffa Credo Laboratories (France) were used in the experiment. Animals were six weeks old on arrival at the laboratory. Seventy animals were individually housed in $20 \times 10 \times 13$ cm plastic cages and used as experimental animals. The remainder were housed in groups of five in $21.5 \times 21.5 \times 15$ cm cages and used as 'standard' opponents in the social encounter tests. They were rendered temporarily anosmic by intranasal lavage with a 4% zinc sulfate solution (PANREAC, Montplet and Esteban S.A., Barcelona, Spain) 1 day before testing. Anosmic mice were employed as 'standard' opponents because they elicit attack but never initiate such behavior [Parmigiani and Brain, 1983). All animals were subjected to a 12-h light/dark cycle (lights off 0730-1930h, local time) with constant temperature ($20.5 \pm 0.5^{\circ}$C). Food (PANLAB S.L., Barcelona, Spain) and water were available ad libitum.

All animals were acquired and cared for in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drug

The experimental animals were injected intramuscularly once a week for 10 weeks with drug or vehicle in a volume of 0.1 ml [Lumia et al., 1994]. Each of them was always injected the same day of the week alternating between the left and right legs. Sesame oil was injected in controls and was utilized for dissolving testosterone propionate in adequate volume (commercial name Testex Leo 250 Prolongatum; Laboratorios Byk Leo S.L., Jaén, Spain) in order to provide two doses: 60 and 120 mg/kg.

Social Encounter Test

Each experimental animal participated twice in the social encounters: once after three weeks of isolation (screening test) and once after ten weeks of treatment (behavioral test). Encounters between an experimental mouse and an anosmic opponent were carried out in a neutral area ($59 \times 29 \times 32.5$ cm transparent glass cage) illuminated by a red light (40 W) for ten minutes. They were preceded by a minute of adaptation in which the animals were separated by a plastic partition. All social encounters started at the 2nd h of the animal's dark phase and were recorded with a video camera positioned in front of the test cage.

The social encounters were assessed using an ethological technique based on a computerized observational procedure [Brain et al., 1989]. The behavior of the experimental animals was classified into 11 broad categories, each of which included a collection of different behavioral postures and elements. The names of categories and their constituent elements are as follows: (1) Body care (abbreviated groom, self-groom, wash, shake, scratch); (2) Digging (dig, kick dig, push dig); (3) Non-social exploration (explore, rear, supported rear, scan); (4) Exploration from a distance (approach, attend, circle, head orient, stretched attention); (5) Social investigation (crawl over, crawl under, follow, groom, head groom, investigate, nose sniff, sniff, push past, walk around); (6) Threat (aggressive groom, sideways

offensive, upright offensive, tail rattle); (7) Attack (charge, lunge, attack, chase); (8) Avoidance/Flee (evade, flinch, retreat, ricochet, wheel, startle, jump, leave, wall clutch); (9) Defensive/Submissive (upright defensive, upright submissive, sideways defensive); (10) Sexual behavior (attempted mount, mount); (11) Immobility (squat, cringe). A detailed description of all elements can be found in Brain et al. [1989] and Martínez et al. [1986].

Analysis of the videotapes involved assessment of the behavior of only the experimental animals. This analysis was performed by a trained observer who was unaware of the experimental group to which each animal belonged. The computer program provides data on the frequency (number of occurrences of each category), total duration (accumulated time spent in each category) and latency of each category in the social encounter.

No Sexual behavior, Avoidance/Flee, Defensive/Submissive, and Immobility were recorded, thus data from these categories were not presented in the results section.

Procedure

Three weeks after arrival, when mice were 9 weeks old, experimental animals were prescreened for aggressive behavior in a social encounter for 10 minutes. The attack latency allowed us to classify animals into three groups based on their levels of aggression: high-, moderate- and low-attacking mice. High-attacking animals showed attack latencies below or equal to percentile 33 ($\leq$198.84 seconds), low-attacking subjects had attack latencies above or equal to percentile 66 ($\geq$420.48 seconds) and, moderate-attacking mice displayed attack latencies above percentile 33 and below percentile 66 ($>$198.84 and $<$420.48 seconds). Afterwards, they were randomly allocated to one of nine subgroups (n = 7–9) each receiving one of the two testosterone doses or sesame oil, according to the following configuration: high-attacking mice (0, 60 or 120 mg/kg of testosterone), low-attacking animals (0, 60 or 120 mg/kg of testosterone), moderate-attacking subjects (0, 60 or 120 mg/kg of testosterone). Subjects were injected and weighed once a week after the screening test, from the fifth to the fourteenth week after arrival. Twenty-four hours after the last injection a second social encounter was conducted. The interval between the injection and the test was selected based on the literature [Fujioka et al., 1986; Korpela and Sandnabba, 1998; Lynch and Story, 2000; Lumia et al., 1994; Martínez-Sanchis et al., 1996; Prezant et al., 1993]. Twenty-four hours after the behavioral test, experimental animals received a lethal dose of Pentothal (Pentothal sódico, Laboratorios Abbott S.A., Madrid, Spain) to withdraw blood samples by cardiac puncture. Body organs of 57 animals (testes, liver, seminal vesicles, preputial glands, and kidneys) were removed and weighed on a precision balance (Digital Scale LE-2000, Letica S.A., Barcelona, Spain). Time restrictions did not allow us to utilize the total sample, although all subgroups were equally represented. Additionally, liver samples were analyzed for the existence of hepatotoxicity. Thus, the P450 cytochrome dependent system, which includes the P450 cytochrome enzyme, the co-enzyme NADPH cytochrome c reductase and P450 cytochrome dependent mono-oxygenase enzymes, was tested.

Hormone Assay

Blood samples were prepared by centrifugation to separate the serum, which was frozen (-80°C) until assay. Testosterone and corticosterone analyses were carried out in the Central Research Unit, Faculty of Medicine, University of Valencia (Valencia, Spain) using Diagnostic Products Radioimmunoassay Test Kits for testosterone and corticosterone (Diagnostic Products Corporation, Los Angeles). The commercial kits used for these

determinations were Coat-A-Count Total Testosterone and Coat-A-Count Rat Corticosterone. In testosterone determination, the sensitivity was 4 ng/dl. The intra- and interassay variation coefficients were 6.1% and 7.3%, respectively. In corticosterone determination the sensitivity was 5.7 ng/ml, the intra- and interassay variation coefficients being 4.3% and 5.8%, respectively. Testosterone (nmol/l) and corticosterone (ng/l) values were the mean of duplicate determinations.

Statistical Analysis

Repeated-measures analysis of variance (test × treatment × group) were carried out for the behavioral data. Physiological data (organ and body weight, and hormonal levels) were analyzed by analysis of variance (ANOVA) for the main effects and their interactions. Newman-Keuls test was used for 'post hoc' pairwise comparisons. When appropriate, Spearman or Pearson correlations were used to examine the relationship among offensive behaviors and testosterone levels. Values of p equal or less than 0.05 were considered significant. All statistical analyses were carried out using SPSS statistical package.

RESULTS

Behavioral Categories

In accordance with the hypothesis previously mentioned, the effects of the interaction between the levels of aggressiveness and treatment on behavior will be detailed first. Secondly, results referring to behavioral effects of treatment itself independent of the degree of aggression will be described. Thirdly, they will be followed by the effects of levels of aggression and, finally, the influence of experience ('test' effect) independent of experimental manipulation will be reported. Mean and standard error of total duration, frequency and latency of behavioral categories, in screening and final test, displayed by high-, moderate-, and low-attacking mice which received testosterone propionate or sesame oil are presented in Table I. Overall analyses (Fisher's Fs) will be found in Table II and post-hoc tests in the text. The majority of the effects were found in the parameter of total duration and some effects in frequency and latency of the categories, all of them indicated in the text.

Behavioral Effects of Treatment in Animals Differing in Aggressiveness

Non-social exploration and attack were the behavioral categories significantly affected by treatment and levels of aggression, although the direction of the effects are different depending on the case (Fig. 1 for overall analyses see Table II, columns of 'Test × Group × Treatment' and 'Group × Treatment' effects).

With regards to *non-social exploration*, the high-attacking group was the only one which showed significant differences among doses, animals treated with 60 mg/kg of testosterone spending more time in non-social investigation than controls. These testosterone-treated animals also showed increases in the final test with respect to the screening ($F2, 22 = 5.45$, $p < 0.01$), which contrasts with those decreases exhibited by the other animals.

With respect to *attack*, treatment effects were also significant only in the high-attacking group (for 'Treatment': $F2, 20 = 10.35$, $p < 0.001$; for 'Test × Treatment': $F2, 20 = 5.55$, $p < 0.01$). Testosterone-treated groups did not increase their duration of attack as much as

Table I. Mean and Standard Error (in Brackets) of the Total Duration (TD), Frequency (FR) and Latency (LT) of Behavioral Categories, in Screening and Final Test, Displayed by Low, Moderate and High Attacking Mice Which Received Testosterone Propionate (60 or 120 mg/kg) or Sesame Oil

		High-Aggressive						Intermediate Aggressive						Low-Aggressive					
Group Treatment		Sesame oil		60 mg/kg PT		120 mg/kg PT		Sesame oil		60 mg/kg PT		120 mg/kg PT		Sesame oil		60 mg/kg PT		120 mg/kg PT	
Test		First	Final	First	Final	First	Final	First	Final	First	Final	First	Final	First	Final	First	Final	First	Final
Attack	TD	62.91	156.59	35.79	62.82	37.14	91.04	28.95	67.29	37.67	110.20	39.68	79.49	3.63	86.51	2.45	48.01	5.42	37.71
		(15.05)	(13.18)	(5.95)	(14.31)	(8.31)	(11.71)	(6.02)	(17.24)	(13.89)	(39.94)	(10.55)	(11.97)	(2.64)	(20.79)	(1.29)	(14.10)	(3.53)	(13.04)
	FR	24.89	20.22	21.57	14.43	18.86	14.14	11.75	11.88	11.00	9.71	12.22	15.00	2.57	17.14	1.63	7.75	2.38	8.63
		(5.06)	(1.55)	(2.84)	(3.29)	(2.41)	(1.56)	(2.32)	(2.42)	(2.76)	(0.94)	(2.63)	(2.25)	(2.09)	(4.73)	(0.80)	(1.99)	(1.24)	(3.22)
	LT	125.58	50.53	105.90	56.94	113.16	44.63	308.78	138.01	348.21	154.96	266.63	86.64	576.54	153.07	576.60	252.07	530.22	291.29
		(17.50)	(21.89)	(24.42)	(12.34)	(20.03)	(12.95)	(19.56)	(68.53)	(34.50)	(48.75)	(19.94)	(22.28)	(14.55)	(81.45)	(13.48)	(79.66)	(29.44)	(66.84)
Threat	TD	79.51	66.51	93.79	88.83	63.92	60.38	45.24	69.58	75.28	97.33	53.33	80.35	20.88	73.88	12.61	69.72	16.59	93.05
		(13.68)	(10.14)	(12.30)	(14.44)	(6.49)	(10.04)	(8.79)	(10.13)	(8.04)	(12.90)	(12.24)	(13.17)	(8.89)	(17.27)	(6.11)	(16.79)	(5.75)	(20.01)
	FR	52.00	29.00	56.00	37.29	49.29	28.43	28.13	27.00	38.71	29.71	38.11	34.33	8.86	32.43	6.38	20.38	10.50	25.13
		(7.58)	(3.13)	(7.40)	(5.79)	(3.90)	(4.64)	(4.58)	(4.74)	(6.60)	(3.80)	(7.05)	(5.48)	(3.76)	(9.26)	(2.99)	(4.83)	(2.89)	(4.85)
	LT	78.89	12.52	66.42	6.34	57.50	17.04	201.76	51.40	210.16	33.57	148.82	51.04	326.40	110.90	449.53	195.66	297.80	125.16
		(14.92)	(4.33)	(20.44)	(1.73)	(10.41)	(4.53)	(40.75)	(42.45)	(53.36)	(22.57)	(23.62)	(20.38)	(72.53)	(83.14)	(54.63)	(71.39)	(50.07)	(52.95)
Social	TD	53.93	10.13	59.86	23.71	45.98	7.66	100.45	23.69	74.61	27.85	86.02	32.02	121.65	27.01	104.81	42.35	134.43	78.30
		(9.08)	(4.26)	(12.85)	(6.98)	(10.53)	(3.66)	(15.56)	(8.71)	(15.03)	(13.78)	(12.91)	(9.86)	(14.06)	(14.04)	(8.34)	(10.33)	(31.37)	(28.35)
	FR	31.44	5.33	29.00	17.14	27.14	5.00	37.50	7.38	33.86	10.86	39.00	13.89	46.71	11.14	38.50	16.50	42.13	19.63
		(4.58)	(1.85)	(5.70)	(5.25)	(3.94)	(1.98)	(1.92)	(2.43)	(5.73)	(3.63)	(4.71)	(3.71)	(4.25)	(4.00)	(3.58)	(2.91)	(5.09)	(4.66)
	LT	7.02	145.15	14.27	53.39	16.55	208.97	21.08	21.88	14.02	62.16	14.08	25.63	22.92	19.44	18.08	41.70	13.38	62.78
		(1.57)	(67.86)	(3.15)	(32.91)	(3.74)	(105.50)	(7.40)	(12.34)	(3.14)	(39.35)	(3.78)	(13.93)	(8.48)	(6.54)	(6.77)	(19.12)	(5.17)	(49.38)
Distance	TD	41.56	47.09	46.40	44.14	58.18	55.05	38.82	48.73	42.45	52.83	36.69	48.01	29.44	48.77	53.86	43.30	39.23	54.24
		(2.64)	(4.30)	(4.68)	(3.12)	(7.19)	(6.34)	(5.84)	(8.80)	(3.96)	(4.78)	(3.40)	(3.63)	(1.39)	(7.65)	(14.26)	(6.68)	(2.27)	(7.37)
	FR	57.33	34.33	58.57	36.57	68.29	33.29	57.00	29.75	57.14	31.86	53.89	32.78	46.86	31.00	60.38	30.13	58.38	34.63
		(2.61)	(1.93)	(4.33)	(2.78)	(2.93)	(4.22)	(5.09)	(3.65)	(4.46)	(2.83)	(4.00)	(1.42)	(2.42)	(2.74)	(4.36)	(3.16)	(1.70)	(3.35)
	LT	7.55	6.81	6.30	33.31	7.81	9.83	11.19	16.53	6.16	5.56	4.53	5.24	10.23	6.30	6.65	12.77	4.79	5.26
		(1.67)	(1.65)	(2.25)	(28.10)	(2.19)	(3.13)	(7.85)	(3.59)	(1.43)	(1.72)	(0.85)	(1.54)	(3.85)	(1.50)	(1.12)	(3.47)	(1.06)	(0.82)

Nonsocial	TD	325.49	274.82	334.29	348.26	356.97	312.29	336.11	354.19	334.26	280.98	334.02	317.54	376.25	322.25	380.30	367.58	372.15	296.07
		(13.83)	(13.57)	(6.94)	(22.77)	(8.32)	(10.00)	(11.27)	(19.76)	(17.13)	(22.95)	(14.49)	(19.71)	(8.53)	(22.75)	(6.72)	(18.98)	(27.74)	(26.04)
	FR	96.11	56.00	95.71	62.43	107.14	59.14	96.00	50.88	96.00	50.00	93.22	59.11	93.00	57.14	99.63	50.50	89.75	53.38
		(6.04)	(2.65)	(5.02)	(5.51)	(5.97)	(5.28)	(5.18)	(4.85)	(6.54)	(3.82)	(6.99)	(4.71)	(4.96)	(2.69)	(3.89)	(3.90)	(5.96)	(4.54)
	LT	1.47	0.82	0.28	0.55	0.24	0.41	0.27	0.48	0.33	1.99	0.28	0.67	0.41	0.61	0.28	0.58	0.36	1.39
		(1.14)	(0.28)	(0.03)	(0.05)	(0.01)	(0.04)	(0.06)	(0.09)	(0.03)	(1.52)	(0.05)	(0.17)	(0.07)	(0.09)	(0.02)	(0.11)	(0.11)	(0.94)
Body care	TD	16.56	26.38	13.28	19.70	18.57	46.63	19.10	27.26	14.87	22.17	15.02	30.31	24.19	19.00	13.36	16.71	16.82	30.89
		(4.07)	(6.88)	(2.63)	(3.77)	(2.48)	(10.19)	(2.18)	(9.26)	(4.49)	(4.95)	(2.57)	(8.14)	(4.73)	(1.86)	(2.10)	(3.85)	(4.43)	(11.04)
	FR	15.00	10.11	12.71	9.71	16.57	14.86	14.38	9.88	12.14	8.43	13.11	13.11	15.57	8.14	10.13	7.88	11.50	12.13
		(3.01)	(1.82)	(2.78)	(2.55)	(2.72)	(1.45)	(2.12)	(1.77)	(2.71)	(1.46)	(2.26)	(2.76)	(2.40)	(1.03)	(1.53)	(1.20)	(1.90)	(3.04)
	LT	135.63	185.77	198.17	186.10	184.78	139.25	103.25	183.51	149.41	231.29	156.98	201.68	106.16	164.16	177.95	161.67	142.03	209.22
		(38.57)	(36.75)	(43.47)	(41.49)	(41.83)	(26.04)	(22.03)	(71.48)	(29.90)	(58.82)	(17.97)	(42.09)	(22.08)	(37.11)	(28.88)	(19.29)	(25.07)	(34.14)
Digging	TD	20.87	18.60	17.48	12.68	20.18	27.17	31.93	9.33	21.79	8.83	27.29	12.29	24.53	22.63	33.65	12.56	13.69	9.39
		(6.04)	(7.01)	(5.61)	(5.21)	(5.89)	(9.14)	(10.47)	(7.91)	(5.38)	(4.03)	(6.41)	(5.06)	(4.35)	(9.76)	(7.31)	(5.05)	(2.44)	(4.53)
	FR	17.22	7.22	15.14	5.71	17.29	8.29	22.50	3.38	19.86	3.86	22.89	4.44	21.14	7.43	26.38	4.38	14.50	3.38
		(4.05)	(2.21)	(3.24)	(2.21)	(5.03)	(1.55)	(5.15)	(2.35)	(4.81)	(1.71)	(4.23)	(1.54)	(2.59)	(2.52)	(5.83)	(1.34)	(3.06)	(1.49)
	LT	223.11	369.08	194.89	291.77	197.46	351.18	124.39	434.59	78.68	423.56	111.73	362.58	134.90	343.35	54.09	386.92	119.46	470.92
		(53.03)	(41.40)	(33.98)	(29.03)	(59.58)	(43.44)	(51.88)	(83.94)	(24.55)	(66.81)	(28.75)	(61.74)	(34.04)	(64.66)	(7.23)	(45.81)	(29.36)	(54.08)

Table II. Overall Analyses (Fisher's Fs) for the Total Duration (TD), Frequency (FR) and Latency (LT) of Behavioral Categories Displayed by High, Moderate and Low Attacking Mice Which Received Testosterone Propionate or Sesame Oil

	Test *x* Group *x* Treatment	Group *x* Treatment	Treatment	Test *x* Treatment	Group	Test *x* Group	Test
Attack							
TD		$F_{4,61} = 3.8^{***}$			$F_{2,61} = 11.5^{+++}$		$F_{1,61} = 78.2^{+++}$
FR					$F_{2,61} = 24.6^{+++}$	$F_{2,61} = 13.5^{+++}$	
Threat							
TD					$F_{2,61} = 6.6^{+++}$	$F_{2,61} = 6.6^{+++}$	$F_{1,61} = 28.7^{+++}$
FR					$F_{2,61} = 22.9^{+++}$	$F_{2,61} = 26.9^{+++}$	
LT					$F_{2,61} = 26.4^{+++}$	$F_{2,61} = 8.7^{+++}$	$F_{1,61} = 79.4^{+++}$
Social							
TD				$F_{2,61} = 3.9^{**}$	$F_{2,61} = 11.6^{+++}$		$F_{1,61} = 149.4^{+++}$
FR			$F_{2,69} = 3.2^{*}$	$F_{2,61} = 4.1^{**}$	$F_{2,61} = 7.2^{+}$		$F_{1,61} = 208.7^{+++}$
LT					$F_{2,61} = 3.6^{*}$	$F_{2,61} = 4.4^{**}$	$F_{1,61} = 12^{++}$
Distance							
TD							$F_{1,61} = 6.3^{**}$
FR	$F_{4,61} = 3.1^{**}$						$F_{1,61} = 368.1^{+++}$
Nonsocial							
TD	$F_{4,61} = 3.8^{***}$	$F_{4,61} = 2.7^{*}$			$F_{2,61} = 3.3^{*}$		$F_{1,61} = 22^{+++}$
FR							$F_{1,61} = 389.6^{+++}$
Body Care							
TD				$F_{2,61} = 3.3^{*}$			$F_{1,61} = 13.7^{+++}$
FR							$F_{1,61} = 10.9^{+}$
Digging							
TD				$F_{2,61} = 3.2^{*}$			$F_{1,61} = 10^{+}$
FR							$F_{1,61} = 95.3^{+++}$
LT						$F_{2,61} = 6^{***}$	$F_{1,61} = 114.6^{+++}$

$^{*}p<0.05$, $^{**}p<0.02$, $^{***}p<0.01$, $^{+}p<0.002$, $^{++}p<0.001$, $^{+++}p<0.0001$.

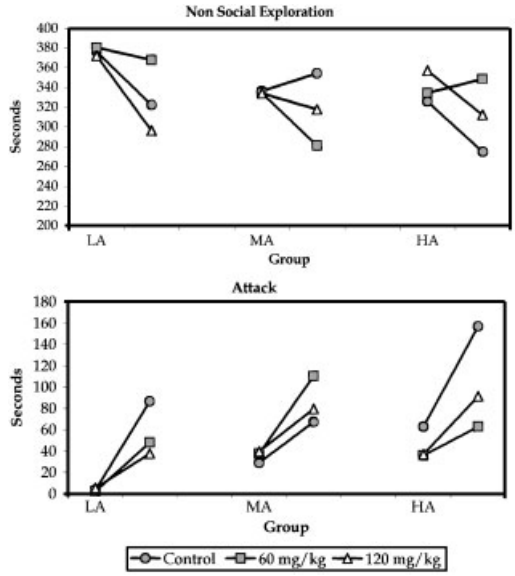

Fig. 1. Mean of the total duration (seconds) of non social exploration and attack, in screening and final test, displayed by low, moderate and high attacking mice (LA, MA and LA) which received testosterone propionate (60 or 120 mg/kg) or sesame oil (control).

did the control animals in the second test (for both, $p < 0.05$). Thus, prolonged administration of high dosages of testosterone buffered attack behavior relative to controls.

Additionally, 'Test × Group × Treatment' interaction also resulted significant in frequency of *exploration from a distance*, where the treatment differentially affected the high- and low-attacking groups. In the former, the greatest drops were found in those animals treated with 120 mg/kg of testosterone; while in the low-attacking group, they were found in mice which received 60 mg/kg of the drug ($F2, 20 = 4.43$, $p < 0.03$ and $F2, 20 = 3.63$, $p < 0.05$, respectively).

Effects of Different Doses of Testosterone

Treatment effects were found to be significant in the frequency and time spent in social investigation and, in total duration of body care and digging (Fig. 2 columns of 'Treatment' and 'Test × Treatment' effects in Table II).

In *social investigation*, 'Treatment' and 'Test × Treatment' interactions were significant. Treated groups showed fewer pronounced decreases in total duration of this category in the final rather than in the screening test when compared with controls (for all, $p < 0.0001$). Furthermore, frequency of this behavioral category also resulted significant, all groups showing less frequency in the final than in the screening test (for all, $p < 0.0001$) but treated animals exhibited social investigation significantly more frequently than controls in the final test ($F2, 69 = 3.21$, $p < 0.05$).

Additionally, *body care* increased in both treated groups while this increase was not significant in controls ($F1, 21 = 6.44$, $p < 0.02$, and $F1, 23 = 11.75$, $p < 0.002$ for 60 and 120 mg/kg of testosterone, respectively).

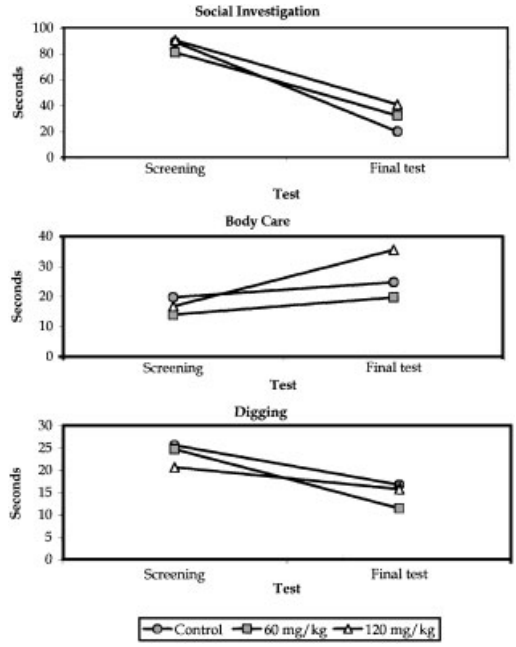

Fig. 2. Mean of the total duration (seconds) of social investigation, body care and digging, in screening and final test, displayed by mice which received testosterone propionate (60 or 120 mg/kg) or sesame oil (control).

Digging significantly decreased only in animals treated with 60 mg/kg of testosterone in the final with respect to the screening test (F1, $21 = 14.70$, $p < 0.001$).

Effects of Previous Aggressiveness

As was expected, effects of levels of aggressiveness were significant in attack and threat (Fig. 3 column of 'Group' in Table II). The more aggressive the group, the more frequency and time spent in these categories and the earlier offensive behaviors were exhibited in both baseline and final test (for all, $p < 0.05$). Furthermore, both social and non-social investigation were also significantly affected. In both cases, the low-attacking group showed significantly greater drops than those shown by the other animals. At every moment, this group spent more time in social and non-social behaviors than the others.

Apart from these main effects, 'Test × Group' interaction was significant in other parameters and behavioral categories. When the profile of response of these behaviors were examined, the results were as follows:

The frequency of *attack* decreased in the high-attacking (F1, $22 = 6.57$, $p < 0.02$), increased in the low-attacking (F1, $22 = 16.96$, $p < 0.0001$), and did not significantly vary in the moderate-attacking group from screening to final test.

In total duration of *threat*, the significant increases in both moderate- and low-attacking groups in the final with respect to screening test (Fl, $23 = 7.80$, $p < 0.01$ and Fl, $22 = 43.90$, $p < 0.0001$, respectively) were not found in the high-attacking mice. The frequency of this behavior did not significantly vary in moderate-attacking, increased in low-attacking (Fl, $22 = 33.24$, $p < 0.0001$) and decreased in high-attacking subjects from screening to final test

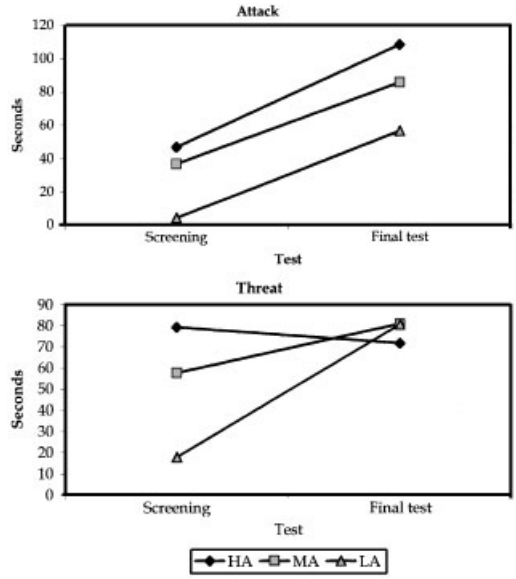

Fig. 3. Mean of the total duration (seconds) of attack and threat, in screening and final test, displayed by high, moderate and low attacking mice (HA, MA and LA).

(Fl, $22 = 32.45$, $p < 0.0001$). In latency of threat, the more aggressive the group, the lower the latency in both screening and final tests and the slighter the decreases shown (for all, $p < 0.001$).

In latency of *social investigation*, the high-attacking animals investigated the opponent later in comparison to the other two groups in the final test (F2, $69 = 4.20$, $p < 0.02$).

Finally, all groups showed *digging* behavior later in the final test with respect to the screening test (for all, $p < 0.0001$), but this effect was greater in treated groups than in controls.

Effects Due to the Social Experience

'Test' effect was significant in all the behaviors assessed (Table II, column of the 'Test' effects), although the direction of the changes was different in each behavioral category. Taking all the parameters in each behavior together, we can affirm that, for the whole sample, attack and threat increased, while non-social behavior and digging decreased in the final test with respect to the screening. The rest of behavioral categories showed different changes depending on the parameter considered. Total duration of exploration from a distance and body care increased in the final test with respect to the first one, but frequency of these behaviors decreased. Finally, total duration and frequency in social investigation increased but also the latency (for all, $p < 0.05$).

Testosterone and Corticosterone Levels

'Treatment' but not 'Group' was significant in testosterone levels (F2, $64 = 38.90$, $p < 0.0001$), with treated groups showing higher levels than control group ($p < 0.05$). No

significant differences were found in corticosterone levels. Mean values and Standard Errors of Mean (SEM) are presented in Table III.

Body and Organ Weights

'Group × Treatment' interaction was significant in final body weight ($F4, 56 = 2.52$, $p < 0.05$), although no significant effects of treatment were found in any aggressive group. In the 60 mg/kg dose group, the moderate-attacking mice weighed less than high- and low-attacking animals ($F2, 19 = 5.75$, $p < 0.01$) while no differences were observed in the other dose group.

Weights of kidney ($F2, 56 = 11.33$, $p < 0.0001$) and seminal vesicles ($F2, 55 = 4.59$, $p < 0.02$) of animals which received testosterone were higher than those of controls, but weight of testicles ($F2, 56 = 4.40$, $p < 0.02$) was lower in treated subjects in comparison with controls ($p < 0.05$).

No significant effects were found in weight of liver and preputial glands. However the color and structure of these glands in testosterone-treated mice revealed alterations.

For Mean values and SEM see Table III.

Hepatotoxicity

Testosterone-treated animals showed lower hepatic P450 cytochrome concentration in comparison with controls ($p < 0.05$) although no differences were found in the activity of the co-enzyme NADPH cytochrome c reductase and the P450 cytochrome dependent mono-oxygenase enzymes (see Table III).

Table III. Mean and Standard Error of Testosterone, Corticosterone, Body Weight, Several Body Organ Weights ((g/body weight)*1000) and the 450 Cytochrome Dependent System of Mice Which Received Testosterone Propionate or Sesame

	Sesame Oil	60 mg/kg of TP	120 mg/kg of TP
Testosterone (nmol/l)	10.63 ± 3.97	94.90 ± 12.95*	131.00 ± 12.23*
Corticosterone (ng/ml)	114.08 ± 7.99	96.86 ± 8.04	105.78 ± 7.92
Body Weight (g)	43.91 ± 0.82	43.42 ± 0.87	43.62 ± 0.93
Kidney	17.44 ± 0.39	20.61 ± 0.54*	20.93 ± 0.81*
Seminal Vesicles	8.43 ± 0.49	9.67 ± 0.47*	10.46 ± 0.51*
Testis	7.14 ± 0.18	6.44 ± 0.18*	6.27 ± 0.28*
Preputial Glands	3.56 ± 0.18	3.70 ± 0.22	3.91 ± 0.35
Liver	44.59 ± 1.05	46.99 ± 1.45	47.77 ± 1.28
Cytochrome P450 (nmol/mg prot)	0.40 ± 0.05	0.22 ± 0.05*	0.26 ± 0.03*
NADPH (nmol/min/mg prot)	57.90 ± 2.50	54.10 ± 7.40	63.40 ± 14.40
Anhiline hydroxilase (nmol/min/mg prot)	0.98 ± 0.09	0.88 ± 0.11	1.11 ± 0.14
Etoxicumarin desetilase (nmol/min/mg prot)	0.56 ± 0.12	0.59 ± 0.09	0.68 ± 0.08

*$p < 0.05$.

Correlations Between Behavior and Testosterone Levels

Correlations between testosterone and total duration, frequency and latency of behavioral categories were examined for total sample, aggressiveness groups, and treatment groups.

In the total sample, testosterone correlated positively with Social Investigation ($r = 0.32$, $p < 0.01$, $r = 0.29$, $p < 0.02$, for total duration and frequency, respectively) and with frequency of Body Care ($r = 0.25$, $p < 0.05$). In the high-attacking mice, testosterone correlated negatively with total duration of attack ($r = -0.44$, $p < 0.05$). In the low-attacking mice, testosterone was negatively associated with frequency of attack and threat ($r = -0.607$; $r = -0.582$, $p < 0.01$ for both) and, positively with latency of these same categories ($r = 0.455$; $r = 0.462$, $p < 0.05$, for attack and threat, respectively). Testosterone also correlated positively with Social Investigation ($r = 0.50$; $r = 0.46$, $p < 0.05$ for total duration and frequency, respectively). In moderate-attacking mice, no significant correlations with offensive behavior were found. There were positive correlations between testosterone and Social Investigation ($r = 0.51$, $p < 0.02$; $r = 0.52$, $p < 0.01$, for total duration and frequency, respectively) and negative ones with latency of Exploration from a distance ($r = -0.49$, $p < 0.02$).

In controls and those animals that received 60 mg/kg, testosterone correlated positively with latency of threat ($r = 0.44$; $r = 0.48$, $p < 0.05$, respectively) and attack ($r = 0.51$, $p < 0.01$; $r = 0.45$, $p < 0.05$, respectively). It also correlated with total duration of Social Investigation ($r = 0.56$, $p < 0.005$; $r = 0.49$, $p < 0.05$, respectively). Finally, in mice treated with the highest dose of the anabolic-androgenic steroid, testosterone correlated negatively with the total duration of Threat ($r = -0.54$, $p < 0.01$).

DISCUSSION

The most outstanding outcome of this investigation is that testosterone treatment has very specific behavioral effects depending on previous levels of aggression displayed by the experimental subjects as reported in other studies [Martínez-Sanchis et al., 1998a, 1998b]. The less aggressive animals (moderate- and low-attacking mice) were behaviorally unaffected by the treatment with respect to offensive categories. However, the most aggressive animals injected with testosterone spent less time attacking and more time exploring the environment than controls. Evolution from the first to the final test was also different, spending more time in exploratory behaviors in the latter.

It has been stated that in the Central Nervous System (CNS), testosterone interacts with neurotransmitters to modulate offensive behavior and this modulation is different in aggressive and non-aggressive animals. Ogawa et al. [1996] comparing two different strains of mice, concluded that the differences in the sensitivity to the effectiveness of testosterone replacement could be due to differences in steroid receptor concentration in the CNS. Additionally, Cologer-Clifford et al. [1999] and Simon et al. [1996, 1998] have stated strain differences in neural pathways through which testosterone can promote the display of aggression. The CF-1 strain has androgen and estrogen sensitive pathways through which testosterone can promote the display of aggression in the adult male brain, while other strains such as CFW are only estrogen sensitive.

It has been concluded that different levels of aggression among conspecific individuals are part of a wider behavioral pattern which includes different styles of coping with stressful events as is the case of intermale aggressive encounters [Benus et al., 1991]. This statement is confirmed by data related to a neuroendocrine axe related with stress. Sgoifo et al. [1996]

have concluded that the aggressive/active coping strategy is related to a high sympathetic-adrenomedullary activation which is not found in a passive strategy. This is supported by the results found by Serova et al. [1994], that is, the administration of two adrenergic receptor blockers has different effects on mice depending on their predisposition to be dominant or submissive. The drugs given separately had no effects on the dominant strain and induced a loss of dominance in the submissive one. On the other hand, the drugs given together decreased aggression only in those animals which were previously dominant.

The effects on attack displayed by high-attacking mice could be associated with the fact that, in mice, normal hormonal levels are necessary to display aggression although this behavior does not increase with concentrations above the normal range [Albert et al., 1990; Brain et al., 1971; Candland and Leshner, 1974; Leshner and Moyer, 1975; Leshner et al., 1973]. Additionally, in male hamsters, the short-day increases in aggressive behavior are inversely related to levels of serum testosterone [Jasnow et al., 2000]. On the other hand, in male talapoin monkeys, there were no differences in serum testosterone between animals of different rank [Martensz et al., 1987], and many species display aggression during autumn when they have low testosterone levels [Kendrick and Schlinger, 1996]. All testosterone-treated animals (high-, moderate-, and low-attacking mice) presented higher testosterone levels in a dose dependent way in comparison with their respective controls, as a consequence of the exogenous administration of testosterone. However, high levels of testosterone are not enough to change offensive behavior in itself. As is shown in the present experiment, individual configuration (basal levels of aggression) interacts with testosterone concentration to modify attack behavior. The diminution in the frequency of this behavior has been related, in animal models, to supraphysiological testosterone levels. It has been reported that low doses of the hormone given to eugonadal animals could be related to increases, as has been discussed elsewhere [Martínez-Sanchis et al., 1998b]. Thus, anabolic-androgenic steroid treatment induces biphasic effects: at low doses increased aggression while at high doses, decreased offensive behavior. These anti-aggressive effects could be related to a general effect on CNS. In fact, it has been stated that the psychological syndrome associated with anabolic-androgenic steroid abuse is a consequence of neurotoxic effects due to the use of high doses and a long-term administration. In this study, the treatment has probably caused a down-regulation of the receptors or changes in the neurotransmitters involved in the regulation of offensive aggression. In the present work, the increment in attack is buffered mostly in those high-attacking mice treated with testosterone. This effect is illustrated by the relationship between testosterone levels, aggressiveness, and treatment. In high-attacking mice, testosterone only correlated with attack (negatively).

Another remarkable finding was related to both groups of peripheral physiological effects: androgenic and anabolic. Androgenic action has as its main target organs those related to the production of endogenous androgens (testes) and those associated with the spermatogenesis (seminal vesicles). In the present work, testosterone-treated mice showed lighter testes and heavier seminal vesicles than controls. Although there were no differences in the weight of preputial glands among groups, their function could be affected in testosterone-treated animals as can be deduced by a change in color and structure, which is similar to that found by other authors [Agren et al., 1999]. Additionally, although no significant effects were found in weight of liver, an increment has been reported by several authors as a consequence of anabolic-androgenic steroid administration and is related to its hepatotoxicity potency [Friedl, 1993; Kochakian, 1990]. In relation to hepatotoxicity, our results showed a reduction in P450 cytochrome concentration in testosterone-treated mice, although this decrement did

not affect at all the system responsible for the metabolism of toxins and other substances. Boada et al. [1999] also found reductions in the levels of cytochrome P450 after chronic treatment with stanozolol, another anabolic-androgenic steroid. Bronson and Matherne [1997] found that a combination of four of these compounds shortened the life span of male mice suffering from tumors in the liver or kidney, lymphosarcomas and/or heart damage. Moreover, an increment in kidney weight was found in all testosterone-treated mice, which could be considered as reflecting the anabolic action of these substances [Kochakian, 1990].

In sum, these substances have had different impacts in peripheral physiological and behavioral parameters, the latter being modulated by previous basal levels of aggressiveness. Taking into account these effects, it would be interesting to explore more deeply the role of individual differences in the effects of anabolic-androgenic steroid abuse, which is widespread especially among the young population. Furthermore, severity of physiological effects of very high doses employed as an abuse substance has been demonstrated in this study.

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