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Chronic nandrolone administration induces dysfunction of the reward pathway in rats

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ABSTRACT

Data in animal models and surveys in humans have revealed psychiatric complications of long-term anabolic androgenic steroid abuse. However, the neurobiochemical mechanisms behind the observed behavioral changes are poorly understood.

The aim of the present study was to investigate the effects of nandrolone decanoate on emotional behavior and neurochemical brain alterations in gonadally intact male rats.

The behavioral reactivity to the elevated plus maze and the social interaction test was used to assess anxiety-related symptoms, and the sucrose preference test was used to evaluate anhedonia. Dopaminergic, serotonergic and noradrenergic transmissions were also evaluated in selected brain areas.

The chronic administration of nandrolone, at 5 mg kg^{-1} injected daily for 4 weeks, induced the loss of sweet taste preference, a sign of anhedonia and dysfunction of the reward pathway. The behavioral outcomes were accompanied by reductions in the dopamine, serotonin and noradrenaline contents in the nucleus accumbens. Alterations in the time spent in the open arms and in the social interaction test were not found, suggesting that nandrolone did not induce an anxiogenic profile. No differences were revealed between the experimental groups in the amygdala in terms of the neurotransmitters measured.

Our data suggest that nandrolone-treated rats have a depressive, but not anxiogenic-like, profile, accompanied by brain region-dependent changes in dopaminergic, serotonergic and noradrenergic neurotransmission. As anabolic androgenic steroid dependence is plausibly the major form of worldwide substance dependence that remains largely unexplored, it should be highlighted that our data could contribute to a better understanding of the altered rewards induced by nandrolone treatment and to the development of appropriate treatments.

1. Introduction

Although originally developed for clinical purposes, anabolic androgenic steroids (AAS) are currently used not only among high-profile and non-professional athletes as performance enhancing drugs but also among the general population, especially adolescents, to improve physical appearance and increase self-esteem [1,2]. Thus, the adverse effects of AAS rapidly became of significant concern from a public health perspective, outside of sports. In this regard, several studies have reported numerous peripheral side effects [3].

Moreover, central nervous system processing and behavioral output have been reported to be altered. Both data in animal models and large population surveys in humans have shown that AAS misuse could induce numerous adverse psychiatric effects, including the lack of impulse control, labile mood with quick swings and depression [see [4] for review], although AAS-induced alterations have been shown to be dependent upon the type of AAS administered. However, constellations of personality traits have been related to several biological parameters.

Until now, the neurobiochemical mechanisms behind the observed behavioral changes have been poorly understood, although the wide range of altered behaviors observed in animals subjected to AAS administration suggests a widespread involvement of different signaling systems in the brain.

In this regard, recent animal studies have found that signaling mediated by both classical neurotransmitters and neuromodulatory peptides in the brain is significantly altered and that these alterations could be related to different AAS-induced behavioral

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changes [5,6]. Brain opioid peptides have been demonstrated to be involved in the addictive behavior after AAS misuse [see [7] for review]. Matrisciano and co-workers recently showed that repeated injections of AAS, at doses considered equivalent to those abused by humans, induced behavioral changes and alterations in neurotrophin levels suggestive of a depressed state in rats [8].

Three systems considered of particular interest are the neurotransmitters dopamine (DA), 5-hydroxytryptamine (5-HT) and noradrenaline (NA). They have an evident role in shaping mood and behavior. In particular, sub-chronic treatment with nandrolone alters DA metabolism in brain regions involved in the development of drug dependence, such as the nucleus accumbens (NAc) [9]. Moreover, dysfunction in 5-HT neurotransmission has been associated with disinhibitory behavior [6,10], and early AAS exposure showed anxiogenic effects during withdrawal partly modulated by 5-HT signaling [11]. Elevated levels of hypothalamic noradrenergic and serotonergic amines were also found [12]. Our previous study supported the idea of numerous neurochemical alterations in the brain after the chronic administration of AAS. We found alterations in the DA, 5-HT and NA contents in several brain areas of stanozolol-treated rats [13].

Thus, the aim of the present study was to characterize the neurobehavioral and neurochemical profiles induced by nandrolone decanoate. As reported by the World Anti-Doping Agency, after stanozolol, nandrolone is the second most used prohibited drug in all sports [14].

Few data exist on the impact of nandrolone treatment on anxiety and depressive behavior, with even less on the aminergic systems as neurochemical correlates for behavioral alterations in selected brain areas. Therefore, here we investigated the effects of chronic nandrolone on the behavioral reactivity to the elevated plus maze (EPM) [15] and social interaction tests [16], two “gold standard tests” for anxiety-related behaviors. Additional testing of anhedonia, a cardinal symptom of depression [17], was performed using the sucrose preference test.

Moreover, we investigated the neurochemical alterations induced by chronic nandrolone treatment, studying key biological systems relevant for anxiety- and depression-induced alterations of neural plasticity, such as the DA, 5-HT and NA contents in two brain regions predominantly involved in regulating emotional responses, the NAc and amygdala (Amy).

2. Materials and methods

2.1. Animals

Male Wistar rats (Harlan, S. Pietro al Natisone, Udine, Italy) weighing 225–250 g were used. They were housed at constant room temperature (22 ± 1 °C) and relative humidity (55 ± 5%) under a 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM). During normal housing conditions, food and water were freely available. The procedures involving animals and their care were conducted in conformity with the institutional guidelines of the Italian Ministry of Health (D.L. 116/92), the Declaration of Helsinki, the Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2004), the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Adequate measures were taken to minimize the number of animals used and their pain or discomfort.

2.2. Animal treatment

Different groups of gonadally intact, experimentally naive male rats were randomly assigned according to the experimental protocols. For each experimental group, rats received a subcutaneous (s.c.) injection of nandrolone decanoate (5 mg kg^{-1}, Sigma, Milan, Italy) or vehicle (polyethylene glycol, PEG, 1 ml kg^{-1}, Carlo Erba Reagents, Milan, Italy) once daily, 5 days per week for 4 weeks. The dosage was chosen on the basis of the previously described model [18]. The animals were weighed daily; at the end of the treatment, they were sacrificed, and the testis weight was assessed. Each experimental procedure was conducted before 2:00 PM, 24 h after the last injection of nandrolone decanoate, and different sets of animals were used for each behavioral test ($n = 10$/group) and for the neurochemical analysis ($n = 8$/group).

2.3. Sucrose preference test

The sucrose preference test was performed in the animal’s individual cages, where non-food-deprived rats were given a 48-h two-bottle exposure, one containing a 2% sucrose solution and the other containing water according to the protocol previously described by Monteggia et al [19].

To prevent a potential locational drinking preference, the position of the bottles was changed after 24 h. At the end of the test, the bottles were removed and weighed to determine the amount of sucrose solution or water consumed. The preference for the sucrose solution was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed.

2.4. Social interaction test

The social interaction procedure was adapted from File et al. [16]. The test was performed in a circular open arena (made of dark plastic; diameter 60 cm; height 31 cm), unfamiliar to the animals and placed in a highly lit room. To increase social interaction, the rats were single-housed for 5 days before the test [20]. On the day of testing, all rats were weighed, and pairs were assigned on the basis of weight and treatment (nandrolone-treated rat was tested with a vehicle-treated partner on the basis of weight, ensuring that they did not differ by more than 10 g). The animals were marked on their back and placed head to head simultaneously in the arena, and their behavior was recorded for 10 min by a camera mounted vertically above the test arena. During quantification, the observer, who was blinded to the experimental conditions, scored the total time that each rat spent performing the following behaviors: *sniffing* (sniffing several body parts of the other rat, including the anogenital region), *following* (moving towards and following behind the other rat around the arena), *climbing* (climbing over and under the conspecific’s back), *aggressive behavior* (including kicking and boxing) and *exploratory behavior* (rearing and wall rearing).

2.5. Elevated plus maze test

The experimental procedures were performed according to Pellow et al. [15]. Briefly, the Plexiglas apparatus consisted of two opposite open arms (50 × 10 cm) without side walls and two closed arms (50 × 10 × 40 cm) extending horizontally at right angles from a central area (10 × 10 cm). The maze was situated in a separate brightly lit room illuminated with four, 32-W fluorescent overhead lights that produced consistent illumination within the room. The apparatus had similar levels of illumination on both the open and closed arms. The maze was elevated to a height of 50 cm in the lit room. At the beginning of the experiment, the rat was placed in the central platform facing an open arm and allowed to explore the maze for 5 min. The following parameters were analyzed: number of entries into the open and closed arms and the time spent in the open arms. An arm entry was counted when the hindpaws were placed on the open arm. Principal component analysis of the conventional plus-maze [21] has shown that the percentage of time spent in the open arms and the number of entries into the open arms are the best measures of anxiety (these parameters are increased by anxiolytic treatments and decreased by anxiogenic treatments). The number of closed arm entries is the best measure of locomotor activity.

2.6. Post-mortem tissue analyses

The rats were killed by decapitation 24 h after the last nandrolone administration, and the brains were immediately removed. The brains were placed dorsal side up in an ice-chilled rat brain matrix (World Precision Instruments, Inc., Aston, Stevenage, UK) with slits spaced at 1 mm. Using an ice-chilled razor blade, the target regions (all subregions of the NAc and Amy) were dissected according to the atlas of Paxinos and Watson [22]. The tissues were frozen and stored at −80 °C until analysis [23]. At the time of analysis, the samples were homogenized in 10 volumes (w V^{-1}) of 0.1 M perchloric acid. The homogenates were stored on ice for 30 min and then centrifuged at 10,000*g* for 10 min at 4 °C. The supernatants were then filtered and diluted before high performance liquid chromatography (HPLC) analysis.

2.7. HPLC analysis

The DA, 5-HT and NA concentrations were determined by HPLC coupled with an electrochemical detector (Intro, Antec Leyden, the Netherlands). The separation was performed by LC18 reversed phase cartridge column (ODS Hypersil, 150 mm × 3 mm, 5 μm; Thermo Scientific, Milan, Italy). Detection was accomplished by a Unijet cell (BASi, Kenilworth, U.K.) with a 6 mm diameter glassy-carbon electrode at a working potential of 0.65 V vs. Ag/AgCl. The substrate concentration was expressed as fmol per milligram of wet tissue, as previously described [23].

2.8. Statistical analysis

All statistical analyses were performed using Graph Pad® 5.0 for Windows (GraphPad Software, San Diego, CA). The behavioral data were analyzed by two-way analysis of variance (two-way Anova, treatment × behavior) followed by a Bonferroni's multiple comparison test or unpaired *t*-test, as required. The body weight differences were analyzed by two-way Anova repeated measures (RM) (treatment × weeks of treatment) followed by a Bonferroni's multiple comparison test. The neurotransmitter concentrations were analyzed using the unpaired *t*-test. The results are expressed as the means ± SEM. The differences were considered statistically significant when the *P* value was less than 0.05.

3. Results

3.1. Body and testis weight

The body weight of each animal was reported weekly, and the testis weight was evaluated after 4 weeks of treatment. No difference was found between the two experimental groups in terms of body weight (Table 1; two-way Anova RM: $F_{\mathrm{tx}(1,54)} = 0.07$, n.s), whereas a significant reduction in the testis weight was observed in the nandrolone-treated rats (Table 1; unpaired *t*-test: $P < 0.01$).

Table 1
Body and testis weight.

Parameters	Groups	
	Vehicle	Nandrolone
Body weight (g)		
Week 1	252.6 ± 3.0	251.9 ± 4.1
Week 2	280.3 ± 3.2	278.6 ± 3.7
Week 3	362.0 ± 7.5	359.8 ± 6.7
Week 4	386.3 ± 6.5	384.9 ± 7.3
Testis weight (g)	4.9 ± 0.1	4.4 ± 0.1*

3.2. Behavioral results

3.2.1. Sucrose preference test

We sought to detect anhedonia in the nandrolone-treated rats using the sucrose preference test. Sucrose preference was monitored during two days of sucrose habituation, and a significant decrease was observed on day 2 in the treated animals compared with the controls, as shown in Fig. 1 (unpaired *t*-test: $P < 0.01$). In addition, the total intake of liquid did not differ between the control and nandrolone-exposed animals throughout the experiment (data not shown).

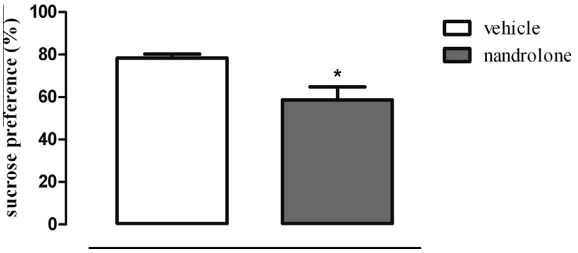

Fig. 1. Sucrose preference test in male Wistar rats after a 4-week administration of nandrolone decanoate (5 mg kg^{-1} daily, s.c., filled bar) or vehicle (PEG, 1 ml kg^{-1}, s.c., empty bar). The data are expressed as the mean ± SEM of the percentage of sucrose consumption ($n = 10$/group; unpaired *t*-test $^{*}P < 0.01$ vs. vehicle-treated group).

3.3. Elevated plus maze test

As shown in Fig. 2, we did not detect any changes in anxiety-like behavior between the nandrolone- and vehicle-treated groups as there were no significant differences in the time spent in the open arms (Fig. 2A) or in the number of entries into the open arms (Fig. 2B; unpaired *t*-test: n.s).

3.4. Social interaction test

The effects of chronic nandrolone administration on anxiety-like behavior were further explored in the social interaction test. The data from this test were examined with two-way Anova, using *behavior* as the within-subjects variable and *treatment* as the between-subjects variable. The defined social interactions included the following: sniffing, following and climbing the partner as characteristics of non-aggressive behavior; rearing and wall rearing as exploratory behavior; and boxing and kicking as aggressive behavior. The results showed that there were no significant differences in the non-aggressive (Fig. 3A; $F_{\mathrm{tx}}[1,72] = 0.1963$, n.s), exploratory (Fig. 3B; $F_{\mathrm{tx}}[1,36] = 0.4548$, n.s) and aggressive (Fig. 3C; $F_{\mathrm{tx}}[1,54] = 0.04355$, n.s) between the two treatment groups.

3.5. Neurochemical results

3.5.1. Effects of nandrolone in NAc

As shown in Fig. 4A, the DA levels were significantly decreased after the nandrolone treatment (unpaired *t*-test, $P < 0.05$). Similarly, the tissue contents of the other studied neurotransmitters were influenced by the chronic administration of the anabolic steroid. In fact, we found considerable reductions in the 5-HT (Fig. 4B;

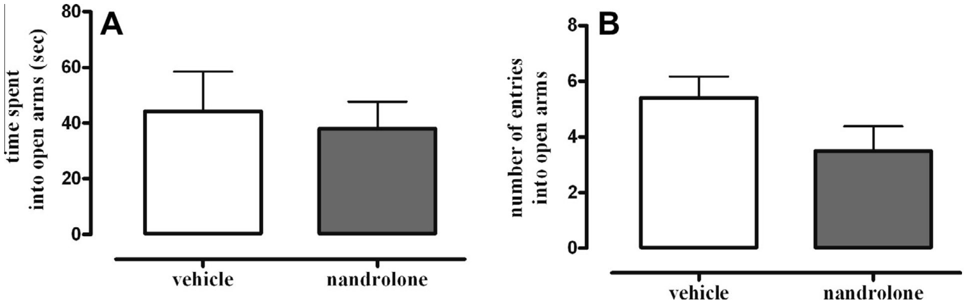

Fig. 2. Elevated plus maze test in male Wistar rats after a 4-week administration of nandrolone decanoate (5 mg kg^{-1} daily, s.c., filled bar) or vehicle (PEG, 1 ml kg^{-1}, s.c., empty bar). The data are expressed as the mean ± SEM of the time spent in (panel A) and number of entries into the open arms (panel B). ($n = 10$/group; unpaired *t*-test, n.s vs. vehicle-treated group).

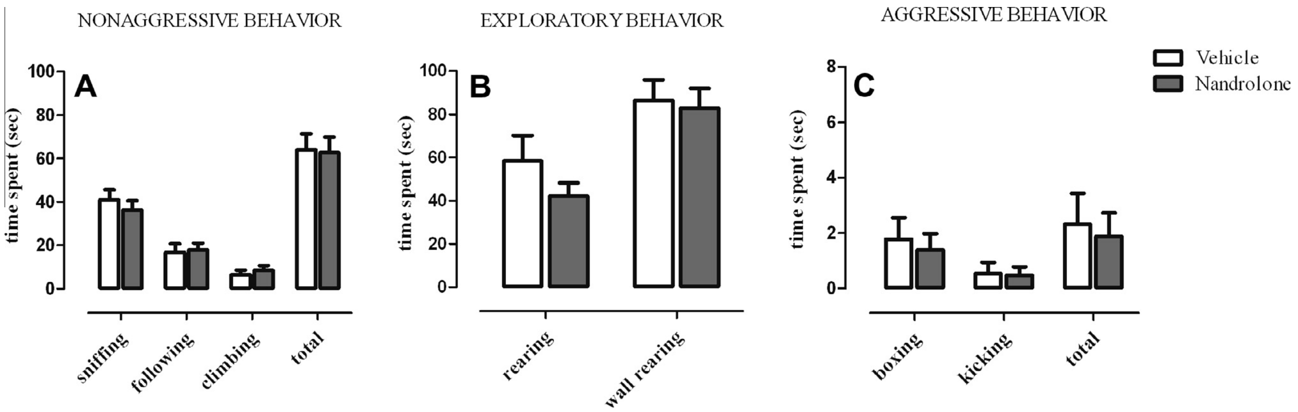

Fig. 3. The social interaction test in male Wistar rats after a 4-week administration of nandrolone decanoate (5 mg kg^{-1} daily, s.c., filled bar) or vehicle (PEG, 1 ml kg^{-1}, s.c., empty bar). The data are expressed as the mean ± SEM of the time spent performing non-aggressive (panel A), exploratory (panel B) and aggressive behaviors (panel C). ($n = 10$/group; Two-way Anova followed by Bonferroni's test, n.s. vs. vehicle-treated group).

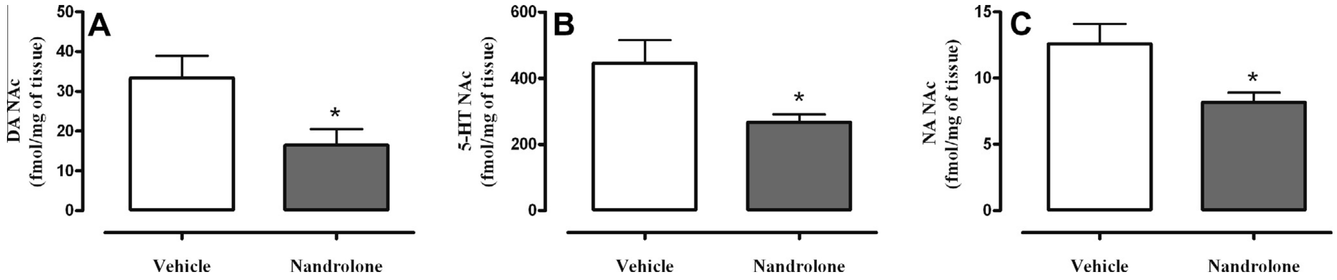

Fig. 4. Tissue content of DA (panel A), 5-HT (panel B) and NA (panel C) in the NAc of male Wistar rats after a 4-week administration of nandrolone decanoate (5 mg kg^{-1} daily, s.c., filled bar) or vehicle (PEG, 1 ml kg^{-1}, s.c., empty bar). The data are expressed as the mean ± SEM ($n = 8$/group; unpaired *t*-test, $P < 0.05$ vs. vehicle-treated group).

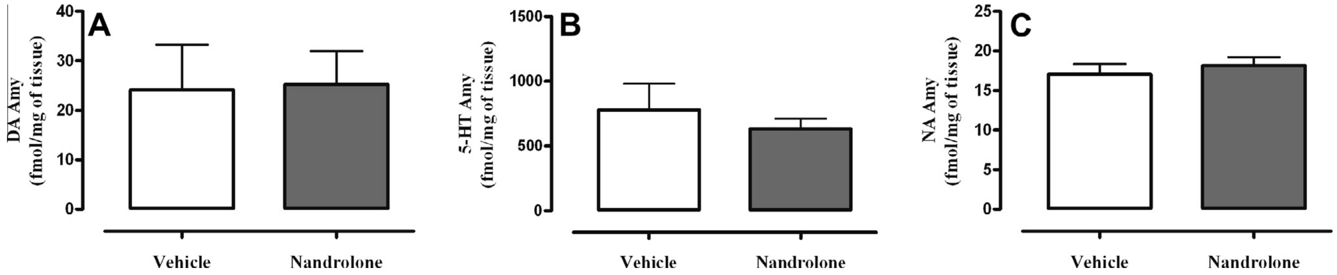

Fig. 5. Tissue content of DA (panel A), 5-HT (panel B) and NA (panel C) in the Amy of male Wistar rats after a 4-week administration of nandrolone decanoate (5 mg kg^{-1} daily, s.c., filled bar) or vehicle (PEG, 1 ml kg^{-1}, s.c., empty bar). The data are expressed as the mean ± SEM ($n = 8$/group; unpaired *t*-test, n.s. vs. vehicle-treated group).

unpaired *t*-test: $P < 0.05$) and NA amounts in nandrolone-treated rats (Fig. 4C; unpaired *t*-test: $P < 0.05$).

3.6. Effects of nandrolone in Amy

The statistical analysis showed that the DA (Fig. 5A; unpaired *t*-test: n.s.), 5-HT (Fig. 5B; unpaired *t*-test: n.s.) and NA concentrations (Fig. 5C; unpaired *t*-test: n.s.) in the Amy were not influenced by chronic nandrolone administration.

4. Discussion

The present study examined the emotional behavior and possible associated neurochemical brain alterations in gonadally intact male rats at the end of a chronic nandrolone decanoate treatment period. In particular, we first evaluated, from a behavioral point of view, whether the emotional behavior of the animals was differentially affected in tests for anxiety- and depressive-like symptoms. Our results suggested that nandrolone-treated animals showed a depressive- but not anxiogenic-like phenotype. These outcomes were accompanied by alterations in the dopaminergic, serotonergic and noradrenergic neurotransmissions in the NAc. No effects of the nandrolone treatment were found in the aminergic contents in the Amy.

The recommended therapeutic use of nandrolone decanoate is approximately 0.4–0.7 mg kg^{-1} body weight in human subjects. Athletes likely consume 10–100 times the recommended therapeutic dose used for medical purposes [24]. In humans, the psychiatric side effects of such chronic AAS misuse have been documented in both case reports and observational studies [25,26]. Symptoms of the chronic use of supra-therapeutic doses of AAS include mania, irritability, anger, abnormal levels of aggression, hostility, depression, and even suicide. Interestingly, some studies have shown that AAS elicited electroencephalographic changes similar to those observed with amphetamine abuse [27]. In the present study, we used 5 mg kg^{-1} daily, a dose considered equivalent to that usually taken by AAS abusers [28]. To evaluate the peripheral effects of nandrolone at the dosing regimen used, we monitored the weight of the animals and their testis weight. In accordance with earlier findings [29], we found comparable body weight gains in both experimental groups throughout the treatment period. However, a significant decrease in the testis weight was observed in the treated rats, which could be considered proof of the biological activity of nandrolone at the dose used.

In our study, the chronic administration of nandrolone induced a depression-related sign, such as anhedonia, which is defined as the inability to experience pleasure from activities formerly found to be enjoyable. Although this behavioral trait has been studied in different neuropsychiatric disorders, anhedonia is considered a core feature of human depressive disorder, according to DSM-IV criteria for major depression and the definition of the melancholic subtype [30]. The evaluation of anhedonia in rodents takes advantage of their innate preference for sweets and can be evaluated using the sucrose preference test. In this test, when given free access to both tap water and a sucrose solution, a healthy subject will strongly prefer the latter, whereas animals with experimental depression will consume equal amounts of water and sucrose, a validated sign of anhedonia [19]. Notably, in the present study, the decrease in the sucrose intake in the nandrolone-treated animals was not associated with changes in fluid consumption or increased locomotion, as indicated by the number of entries in the closed arms in the EPM (data not shown), further suggesting that the effect was not due to changes in the physiological processes interfering with ingestion or motor activity.

Our results are in line with previous studies reporting that nandrolone-treated rats behaved similar to spontaneously depressed rats, the Flinders Sensitive Line. This animal model showed an increased duration of immobility in the forced swim test, which was reversed by the antidepressant clomipramine [8,31]. The mechanism by which the chronic administration of nandrolone might induce depression with melancholic features, such as anhedonia, is not yet clear; however, our data raise the hypothesis that the alteration of aminergic neurotransmission in specific brain areas might be implicated. This hypothesis is based on the assumption that normal levels of brain DA are important for normal motivation. Specifically, the accumulation of considerable neuropharmacological data provides now strong evidence linking the mesolimbic DA system with reward and motivational processes [32].The cerebral DA terminal area that has received most attention with respect to reward function is NAc. Indeed, selective lesions of this neurotransmitter system were shown to interfere with cocaine self-administration [33]. Since then, numerous studies from the drug addiction research field have characterized the NAc as one of the most important brain regions involved in drug and natural rewards [34]. In this context, the development of anhedonia could be attributed to dysfunctions in the reward pathway [35]. Indeed, DA release in the NAc may underlie the approach responses and guidance towards positive incentives, such as motivation. During exposure to novel food, DA release from the NAc is shown to be modulated by several characteristics of the stimulus and by the motivational state [36]. Thus, to directly assess whether nandrolone inhibits dopaminergic neurotransmission as the above data suggest, we measured the DA levels in the NAc. We found that the DA content was selectively reduced. These results are supported by recent data that demonstrated that DA neurons in the limbic circuitry modulate neural encoding and the expression of depression-related behavior, such as anhedonia [37]. Our results are especially relevant in light of the recent underestimated emerging evidence of AAS dependence in the general population, especially among adolescent users. The important role of DA in the brain reward system does not imply that it should be considered an independent factor nor that this neurotransmitter is the final pathway to achieve the rewarding state. 5-HT also regulates reward processing and anhedonic behaviors. For example, 5-HT has a recognized effect on the modulation of DA and opioid activities [38] and, therefore, could exert a regulatory function in the reward process. Furthermore, chronic treatment with selective 5-HT reuptake inhibitors increased sucrose preference in mice [39]. Based on the above results, we also measured 5-HT levels in the NAc, and as expected, we found that nandrolone selectively reduced the 5-HT content in this area. Our findings are in line with previous studies. Indeed, alterations of DA metabolism and DA receptor densities in the rat nucleus accumbens shell have already been shown [9,40]. Moreover, nandrolone pre-exposure is able to modulate the acute neurochemical and behavioral effects of amphetamine in rats [41] and to attenuate increases in the extracellular DA and 5-HT concentrations and the stereotypical behavior evoked by cocaine [6]. To the best of our knowledge, no data exist on the impact of nandrolone treatment on the noradrenergic system as neurochemical correlates for behavioral alterations in selected brain areas, such as the NAc.

Beyond the serotonergic system, another important neurobiological feature of depressive disorders is a dysregulation of noradrenergic neurotransmission [42]. In our study, we found that nandrolone treatment also significantly reduced the NA content in the NAc. We also evaluated the effects of chronic nandrolone treatment on anxiety-like behavior. In the field of anxiety, the EPM has become one of the most popular animal tests [4]. The reluctance of rats to explore the open arms of the maze has been suggested to be caused by the fear of open spaces [4]. Anxiolytic compounds increase, whereas anxiogenic compounds decrease, the percentage of time spent in the open arms [4,43]. Under our experimental conditions, a possible anxiogenic effect of nandrolone is doubtful as no alterations were observed in this test. In addition, the effects of nandrolone on anxiety-like behavior were further evaluated in the social interaction test. In the context of environment-dependent social behavior, this test has been shown to have a high degree of ethological validity. Usually, social anxiety-like behaviors are negatively correlated with the time spent engaging in active social interaction [44]. This phenomenon has been empirically described and validated with anxiolytic and anxiogenic drugs [44,45]. Also in line with the above results, we found no effects of nandrolone administration on sociability.

Numerous studies have highlighted the critical role of the Amy in behavioral phenomena that involve anxiety-like emotional process. Critically, the Amy is widely accepted to be important for the recognition of negative emotions and for linking environmental stimuli with aversive events, and anxiety is continuously regulated by balanced antagonistic pathways within the Amy [for review see [46]. Aminergic neurotransmission has been shown to represent a key biological system relevant for anxiety-induced symptoms in the Amy [47]. Thus, we measured the biogenic amine content in this area, and as expected from the behavioral analysis, we did not find alterations in the NA levels or in the DA and 5-HT tissue contents.

In conclusion, the findings described herein suggest that the chronic administration of nandrolone decanoate at a dosing regimen equivalent to that usually taken by AAS abusers induces dysfunction of the reward pathway. As AAS dependence is considered a valid diagnostic entity and a growing public health issue, it should be highlighted that our data could provide a fruitful basis to better clarify not only the mechanisms underlying the effects of nandrolone abuse but also an improved understanding of the psychiatric complications of long-term AAS exposure.

Conflict of interest

The authors declare no conflict of interest.

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