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Subchronic steroid administration induces long lasting changes in neurochemical and behavioral response to cocaine in rats

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ABSTRACT

The abuse of anabolic androgenic steroids (AASs), such as nandrolone, is not only a problem in the world of sports but is associated with the polydrug use of non-athletes. Among other adverse effects, AAS abuse has been associated with long term or even persistent psychiatric problems. We have previously found that nandrolone decanoate treatment could produce prolonged changes in rats' brain reward circuits associated to drug dependence. The aim in this study was to evaluate whether AAS-induced neurochemical and behavioral changes are reversible.

The increases in extracellular dopamine (DA) and serotonin (5-HT) concentration, as well as stereotyped behavior and locomotor activity (LMA) evoked by cocaine were attenuated by pretreatment with nandrolone. The recovery period, which was needed for the DA system to return back to the basic level, was fairly long compared to the dosing period of the steroid. In the 5-HT system, the time that system needed to return back to the basal level, was even longer than in the DA system. The attenuation was still seen though there were no detectable traces of nandrolone in the blood samples.

Given that accumbal outflow of DA and 5-HT, as well as LMA and stereotyped behavior are all related to reward of stimulant drugs, this study suggests that nandrolone decanoate has significant, long-lasting but reversible effects on the rewarding properties of cocaine.

1. Introduction

Despite increased awareness of both the public and scientific communities of the profound neural changes that AASs are able to induce, experimental research on their neurobiological basis is limited. It has been hypothesized that by influencing neuronal activity and plasticity steroid hormones are important determinants of stimulant's effects on behavior [1–3]. Given that AASs modulate functions in the brain, it is likely that in addition to a persistent desire to enhance one's external features or sporting performance there are also neurochemical adaptations behind prolonged misuse of AASs.

There is evidence that dopaminergic as well as serotonergic activities in the brain are influenced by sex steroids. For instance, we have earlier found that AAS nandrolone decanoate, which resembles male gonadal hormones, interact with the brain by affecting the DA system, a key phenomenon in drug-dependence [4]. There are also studies where androgens are shown to have direct stimulating effects on central DA and 5-HT release [5,6]. Notably, when using microdialysis, we have also found that subchronic nandrolone administration reduces amphetamine- and MDMA-induced effects [7], as well as cocaine-induced [8] DA and 5-HT outflow in the rat nucleus accumbens.

Accumulating evidence suggests that abuse of supra-physiologic doses of AAS may have adverse effects on a number of organ systems, leading to both medical and psychiatric pathology. Importantly, some evidence suggests that some of these effects may persist long after the last AAS exposure. High concentrations of AAS, comparable to those likely to have been consumed by many AAS abusers, have been shown to produce e.g. apoptotic effects on various cell types, including neuronal cells [9] – raising the possibility of irreversible neuropsychiatric toxicity. Finally, AAS abuse appears to be associated with a range of potentially long-term psychiatric effects, including mood syndromes and progression to other forms of substance abuse in humans [10,11]. Surveys have revealed that young people who abuse AAS not only share personality related factors with individuals at risk for psychotropic substance abuse, but also tend to abuse alcohol and psychotropic drugs themselves [12–15]. There are also case studies where former users have reported depression and suicidal ideation, which were strongly related to the discontinuation of AAS abuse [16,17].

Our previous study supports the idea of these long-term effects of AAS on the central nervous system (CNS). We found that the changes seen in DA and 5-HT systems after nandrolone dosing, were still seen after a fairly long recovery period without nandrolone [8].

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These results suggest that AAS treatment would produce long-term changes in reward circuits in the brain. Following on from this, the overall goal of this study was to determine the duration of the AAS caused changes on rats' behavior and brain neurochemistry after psychostimulant exposure.

2. Experimental

2.1. Animals

Adult male Wistar rats, weighing 300–380 g, were supplied by Harlan Netherlands B.V. (The Netherlands) at least 1 week before the experiments. Animals were housed three per transparent cage (Techniplast Eurostandard type IV cage: 595 × 380 × 200 mm, floor area 1820 cm^2) in a temperature- and humidity-controlled room with a 12-h light cycle. The lights were on from 06.00 a.m. to 06.00 p.m., during which time all the experiments were conducted. Standard laboratory chow (RM1 IRR SQC, SDS, Witham, UK) and tap water were freely available. After surgery, the rats were housed individually. The State Provincial Office of Southern Finland Animal Experiment Board approved the animal experiments, and they were conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

2.2. Drugs and treatments

Nandrolone was administered at a dose of 20 mg/kg (calculated as free base) intramuscularly (i.m.) every other day over a 10-day period (total of 5 injections per animal). Nandrolone decanoate (Deca-Durabolin®) was a commercial preparation dispensed by NV Organon (Oss, The Netherlands). The matching vehicle for nandrolone decanoate, a mixture of arachinoid oil and benzylalcohol, was prepared by the University Pharmacy (Helsinki, Finland) for control purposes. The injections were given in the left and right hind leg alternately. There are considerable differences, regarding experimental design, between existing neurochemical studies of supratherapeutic AAS exposure in dose (1–45 mg/kg), dose interval (1–7 days), duration of administration (1 day–15 weeks) and route of administration (i.m. or s.c). We chose 20 mg/kg dose also to correspond, at least in some level, to one cycle of use during experienced AAS abuse, respectively, based on a 1 year follow-up study and a survey study of 500 AAS abusers [18,19]. Our preliminary experiment has showed that combined the half-life of nandrolone decanoate is 4.3 days, and the study of van der Vies demonstrated that nandrolone depot has a half-life of 5.4 days in rat [20].

Following the 10-day administration period, the animals were randomly assigned to five groups ($n = 6$): In group I the microdialysis experiments were conducted after 6 days from the last nandrolone injection, in group II the microdialysis experiments were conducted after 28 days, in group III after 35 days, in group IV after 49 days and in group V after 61 days from the last nandrolone injection. Different recovery periods were used to evaluate whether the effects of sub-chronic nandrolone treatment were reversible, and if they were, long this process took. The dose of nandrolone was chosen to correspond to dosages used in our previous studies without any observable adverse effects on animal welfare.

Cocaine HCl (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in saline (0.9% NaCl) to be injected at the dose of 20 mg/kg dose (calculated as free base). The drug was injected intraperitoneally (i.p.) in a volume of 1 ml/kg of body weight during the microdialysis experiment. The animals were weighed before each injection and the volume administered was adjusted accordingly. The cocaine dose chosen represents the minimum dose that produces stereotyped behavior in rats and sufficient increase in the extracellular DA in the NAc without any observable adverse effects on animal welfare (as determined by previous studies).

2.3. Microdialysis surgery and experiments

The rats were anesthetized using 5% halothane gas (Halothane Liquid BP; Rhodia Organique Fine Ltd., Bristol, UK) and placed in a stereotactic instrument. A guide cannula (MAB 9.14.IC; AgnTho's AB, Lidingö, Sweden) was implanted 2 mm above the NAc [A, +2.0; L, −0.9; D, −6.0] as calculated relative to the bregma and skull surface according to Paxinos and Watson [21] and secured with two small screws and dental cement (Stoelting Europe, Dublin, Ireland). During surgery halothane gas was administered at a concentration of 2.5%. The animals received subcutaneously (s.c.) 0.050 ml of buprenorphium preparation (Temgesic®, 0.3 mg/ml; Schering-Plough Europe, Brussels, Belgium) to alleviate the pain, and were allowed to recover from the surgery for at least 5 days.

One day before the experiment, the rats were allowed to habituate to the test cage, and a microdialysis probe (MAB 9.14.2, membrane length 2 mm; AgnTho's AB, Lidingö, Sweden) was inserted through the guide cannula into the NAc shell. The next day, the rats were placed in the test cage and the probes were connected to a CMA/100 microinjection pump and perfused with modified Ringer's solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$, pH 6) at a flow rate of 2 μl/min. In order to prevent degradation of monoamine transmitters, a 6.5-μl aliquot of an antioxidant solution (1.0 mM oxalic acid, 3.0 mM L-cysteine, 0.1 mM acetic acid) was added to each vial before collecting the dialysate samples.

The perfusate was discarded during the first 60 min, after which the samples were collected at 20-min intervals. An i.p. injection of cocaine was given after the collection of four basal samples (2 h 20 min from beginning of the perfusion). Animal behavior was video recorded from 20 min before the injection until 160 min after the injection. At the end of the experiment the animals were anesthetized with 5% halothane gas, and blood samples for drug measurements were drawn using cardiac puncture. After decapitation, their brains were dissected out and immersed in buffered 10% formalin solution to verify the correct placement of the probes. Only data from animals with accurate probe placements were included in statistical analyses.

2.4. Analytical procedures

2.4.1. Determination of DA, 5-HT and their metabolites in dialysate samples

In order to quantify DA, 5-HT and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), 20-μl aliquots of dialysate samples were injected into an ESA (ESA, Inc. Chelmsford, MA, USA) high performance liquid chromatography (HPLC) apparatus equipped with an Inertsil ODS-3V 5 μm (4.6 × 250 mm ID) reverse-phase column (GL-Sciences, Inc., Tokyo, Japan) and a coulometric ESA Coulochem III detector. The mobile phase was a mixture of a buffer containing 50 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 2.3 mM octanesulfonic acid, and acetonitrile (14% v/v in the final solution), with the pH adjusted to 3.0 with orthophosphoric acid (H_3PO_4). The mobile phase was filtered through a 47 mm hydrophilic polypropylene membrane filter with pore size of 0.22 μm (Gelman Sciences, Ann Arbor, MI, USA) and degassed under vacuum. The flow rate was 1.2 ml/min and the detector potentials of the two electrodes were −175 mV and +250 mV.

2.4.2. Determination of nandrolone and its metabolites in blood samples

The concentrations of nandrolone and its metabolites 19-noretiocholanolone and 19-norandrosterone were measured using gas

chromatography–mass spectrometry (GC–MS). Nandrolone and its metabolites were extracted from 1 ml of whole blood as follows:

One milliliter of pH 10 Na-tetraborate buffer ($Na_2B_4O_7{\cdot}10H_2O$; Merck, Darmstadt, Germany) and 5 ml of dichloromethane containing internal standard (IS) (17-α-methyltestosterone), was vortexed with 1 ml of the sample. After centrifugation the solvent layer was transferred into a clean test tube and 50 mg of sodium sulfate (Na_2SO_4; Merck, Darmstadt, Germany) was added to remove water. Clear liquid was transferred into a clean test tube and evaporated to dryness. The dry residue was derivatized with 50 μl of *N*-methyl-*n*-(trimethylsilyl)trifluoroacetamide (MSTFA; Sigma Aldrich Co Ltd., Dorset, UK) containing 3 mg/ml ammonium iodide (NH_4I) and 4 μl/ml 2-mercaptoethanol (C_2H_6OS) (both from Fluka Chemie GmbH Buchs, Switzerland). Samples were transferred into auto sampler vials and kept at 80 °C (Techne dri-block heater; Staffordshire, UK) for 30 min.

Then a 2-μl aliquot of the sample was injected into a GC–MS machine. The analysis was performed with an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890 N gas chromatograph, an Agilent 5973 mass selective detector (EI, positive ions, 70 eV) and an Agilent ChemStation data system. The system was operated in the splitless injector mode. Helium was used as the carrier gas. The GC column was a DB-35MS, with a length of 30 m and internal diameter of 0.32 mm and film thickness 0.25 μm (J&W Scientific, Inc., Folsom, CA, USA). The column temperature was initially 150 °C with a hold time of 1 min, and was increased 10 °C/min, up to at 325 °C. The inlet and MSD transfer line heater temperatures were maintained at 280 and 300 °C, respectively. MS detection was performed in the selected ion monitoring (SIM) mode. The lower limit of nandrolone quantitation was set at 0.5 μg/l.

Nandrolone (19-nortestosterone) and the metabolites 19-noretiocholanolone and 19-norandrosterone were purchased from Fluka Chemie GmbH (Buchs, Switzerland) and Cerilliant (Round Rock, TX, USA), respectively, while the IS 17-α-methyltestosterone was purchased from Fluka Chemie GmbH (Buchs, Switzerland).

2.4.3. Characterization of behavioral changes

Motor activity and the behavior of the animals were characterized from video tapes by an observer blind to drug conditions as described earlier [8]. Briefly, the locomotor activity (LMA) of the animals was estimated from the number of complete passes across the midline in the test cage at intervals of 20 min (corresponding to the sampling interval in the microdialysis experiments). In addition, for a more detailed behavioral analysis, the tapes were monitored visually for 1 min every 5th min. The rats were given a single behavioral score for each 1-min observation point and mean values were calculated for each 20-min sampling interval. Behavioral scores were: (0) passive motionlessness; (1) active motionlessness; (2) active motionlessness with occasional movements; (3) sniffing, grooming, occasional LMA; (4) LMA with burst of rearing, slight agitation; (5) stereotyped behavior; (6) intense stereotyped behavior; (7) ataxia.

2.5. Statistics

In the microdialysis experiments the mean of the four samples before the drug treatments was considered as basal release (100%), according to which relative changes after the injections were calculated. In the LMA test the absolute number of passes across the midline was counted, and for the more detailed behavioral analysis, scoring was conducted as described above. For statistical evaluations, both neurochemical and behavioral data were calculated as areas under the curves (AUCs) with the trapezoidal method. The microdialysis data were then subjected to a one-way analysis of variance (ANOVA) followed by Tukeýs test. The behavioral scores were analyzed with the Mann–Whitney *U* test or Kruskall–Wallis nonparametric ANOVA followed by the Mann–Whitney *U* test with Tukeýs adjustment for multiple comparisons. The results from measurements of nandrolone blood concentrations were analyzed with one-way ANOVA followed by Tukey's test. The results are presented as means ± SEM (standard error of the mean) and the level of statistical significance was set at $p \leqslant 0.05$.

3. Results

3.1. Blood drug concentrations

The concentration of nandrolone and its metabolites 19-noretiocholanolone and 19-norandrosterone were measured from samples collected 4 h after cocaine administration. The concentration of nandrolone was 12.73 ± 0.7 μg/l in the 6-day recovery group, and 4.15 ± 0.4 μg/l in the 28-day recovery group. Only the 28-day group differed statistically from the 6-day group ($p < 0.001$ ANOVA). In the other groups (35, 49 or 61 days), the concentrations of nandrolone were under the detectable limit. There were no detectable traces of the metabolites, 19-noretiocholanolone and 19-norandrosterone, in any the collected samples.

3.2. Microdialysis experiments

The absolute basal accumbal extracellular concentrations of DA, 5-HT and their metabolites did not differ significantly between the treatment groups (ANOVA), as seen in Table 1. Nandrolone pre-treatment alone had no effect on extracellular levels of DA, 5-HT or metabolites, as compared to vehicle pre-treatment. Administration of cocaine increased spontaneous release in the extracellular concentration of DA, 5-HT and decreased spontaneous release in the extracellular concentration of metabolite DOPAC in the NAc. Cocaine at a concentration of 20 mg/kg elevated DA ($p < 0.001$) and 5-HT ($p < 0.001$), when compared with saline in the NAc (AUC; one-way ANOVA, Fig. 1). Cocaine decreased the concentration of the DA metabolite DOPAC ($p = 0.004$, AUC; one-way ANOVA), while HVA and the 5-HT metabolite 5-HIAA remained unchanged (data not shown).

The temporal profiles of the effects of nandrolone pre-treatments and cocaine injections on extracellular DA and 5-HT levels are shown in Fig. 1. Nandrolone pre-treatment decreased the cocaine-induced elevation of extracellular DA-levels ($p < 0.001$ AUC ANOVA) and 5-HT levels ($p < 0.001$ AUC ANOVA) as compared to the vehicle oil.

The attenuating effect of nandrolone pre-treatment on cocaine-induced elevation of extracellular DA remained unaltered over the 28-day recovery period. The DA concentration collected from the NAc after the 20 mg/kg cocaine injection did not differ statistically between the groups with of the 6-day recovery period after nandrolone and the 28-day recovery period after nandrolone treat-

Table 1
The means (±SEM; $n = 11–12$) of the basal concentrations in the NAc dialysate in different treatment groups. Concentration fmol/40 μl.

Treatment	DA	DOPAC	HVA	5-HT	5-HIAA
Vehicle 6th	11.0 ± 1.2	18.3 ± 3.4	7.3 ± 1.2	6.4 ± 2.1	8.4 ± 2.0
Vehicle 28th	10.2 ± 1.4	17.7 ± 3.1	8.0 ± 1.5	5.6 ± 1.8	7.2 ± 1.8
Vehicle 35th	12.0 ± 3.4	14.0 ± 4.3	6.3 ± 0.9	7.9 ± 1.9	6.8 ± 2.1
Vehicle 49th	9.0 ± 1.5	16.2 ± 3.0	7.1 ± 1.1	7.1 ± 1.6	7.0 ± 2.0
Vehicle 61th	10.1 ± 1.3	16.9 ± 2.9	6.1 ± 1.9	6.2 ± 2.0	7.8 ± 1.8
Nandrolone 6th	9.3 ± 1.7	15.4 ± 4.1	7.8 ± 1.8	7.2 ± 1.8	7.9 ± 1.3
Nandrolone 28th	9.2 ± 1.5	16.3 ± 3.1	6.9 ± 1.2	7.3 ± 1.9	8.2 ± 1.6
Nandrolone 35th	8.8 ± 2.3	14.3 ± 3.9	6.9 ± 1.3	5.8 ± 2.2	8.6 ± 2.1
Nandrolone 49th	8.9 ± 1.9	16.6 ± 2.5	7.5 ± 0.8	7.7 ± 1.8	7.0 ± 2.0
Nandrolone 61th	10.2 ± 1.8	15.2 ± 3.2	7.1 ± 1.3	5.8 ± 1.9	9.0 ± 2.3

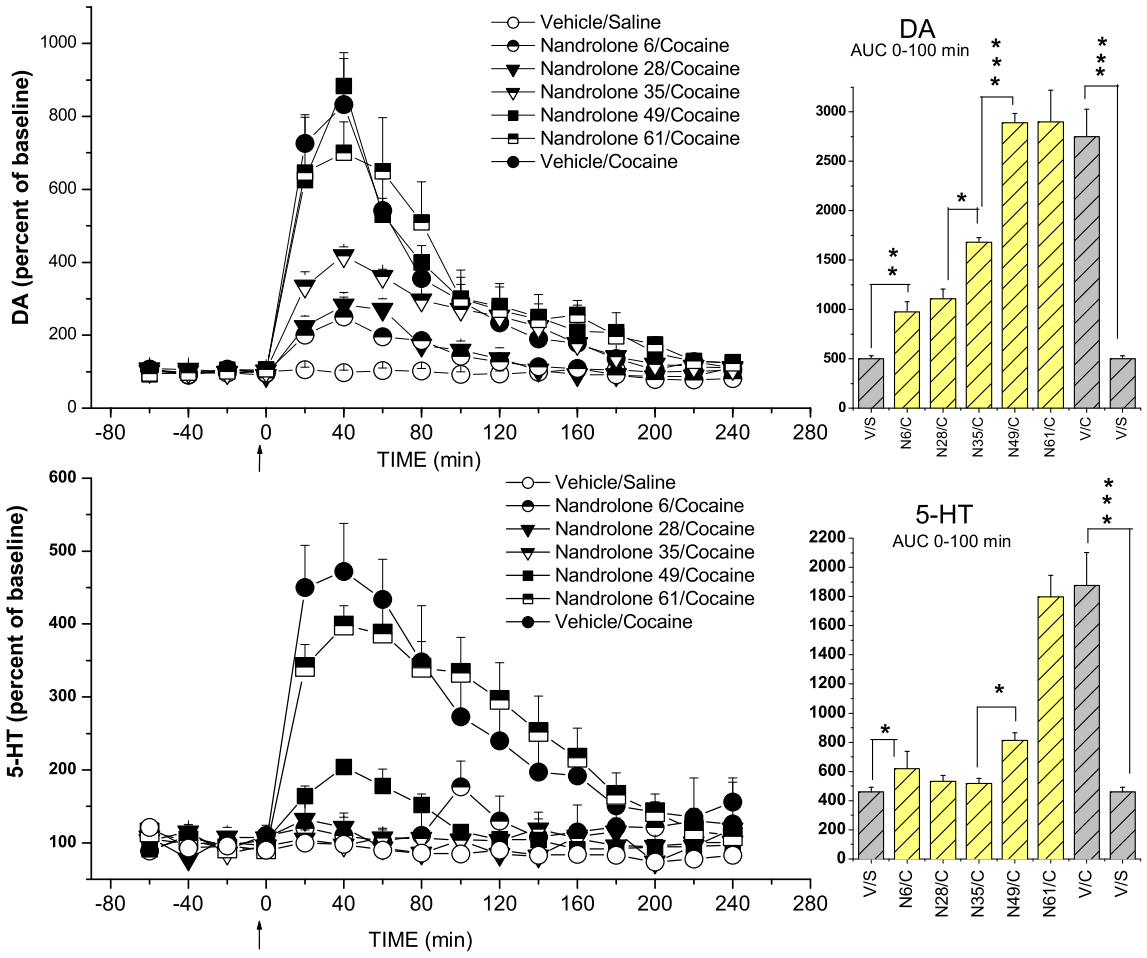

Fig. 1. The effects of nandrolone (5 × 20 mg/kg) and acute cocaine (20 mg/kg) injections on extracellular DA and 5-HT levels in the NAc. The times of the cocaine injections are indicated by arrows. Data expressed as percentages of basal release are given as means ± S.E.M. ($n = 6$). Histograms represent the area under the curve (AUC) after injection of the cocaine, and the minutes where AUC is counted are shown in the figure. $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$ Tukey's test. V/S = vehicle oil + saline, N6/C = nandrolone + cocaine, N28/C = nandrolone + cocaine after 28th day, N35/C = nandrolone + cocaine after 35th day, N49/C = nandrolone + cocaine after 49th day, N61/C = nandrolone + cocaine after 61th day, V/C = vehicle oil + cocaine.

ment (Fig. 2). The attenuation of DA elevation was partly restored after the 35-day recovery period. However, the nandrolone treated group differed still statistically from the vehicle oil treated (35-day recovery) group. After 49- and 61-day recoveries from nandrolone dosing, the cocaine-induced elevation of extracellular DA did not differ statistically from the vehicle oil treated group.

The 5-HT concentration collected from the NAc after the 20 mg/kg cocaine injection did not differ statistically between groups with the 6-, 28- and 35-day recovery period after nandrolone treatment, as seen in Fig. 2. The attenuation of 5-HT elevation was partly restored after the 49-day recovery period. However, the nandrolone treated group differed still statistically from vehicle oil treated (49-day recovery) group. The cocaine-induced elevation of extracellular 5-HT did not differ statistically from vehicle oil treated group after the 61-day recovery period.

There were no statistical differences between nandrolone and vehicle oil treated groups in DOPAC, HVA or 5-HIAA concentrations after cocaine (data not shown). As seen in Fig. 2, in vehicle oil groups, the length of the recovery period (6, 28, 35, 49 or 61 days) had no effect on cocaine's action *per se*.

3.3. Behavioral changes

Nandrolone pre-treatment *per se* or acute saline injections did not alter the behavior of the rats, while administration of cocaine induced profound effects on both LMA and stereotyped behavior. As shown in Fig. 3, administration of cocaine increased significantly the behavioral scores ($p = 0.001$; Mann–Whitney U test) and LMA ($p = 0.003$, t-test), as compared with saline. Cocaine-induced behavior is clearly distinguishable from the behavior of the saline-treated rats. While saline-treated animals were mostly sleeping or awake but motionless, the cocaine-treated animals exhibited behavioral patterns such as increased LMA with burst of rearing, slight agitation, and stereotyped behavior such as intensive sniffing, head or body weaving or head bobbing.

Nandrolone pre-exposure modified the ability of cocaine to increase LMA and stereotyped behavior ($p = 0.004$ LMA; $p = 0.002$ Behavior scores; ANOVA). Less stereotyped behavior was observed; the frequency and duration of rapid and repetitive purposeless behavior and intensive sniffing were reduced, head and body weaving were not so broad, and head bobbing was no longer observed. The intensity of these effects was related to length of the nandrolone recovery period. The effect of nandrolone on cocaine-induced LMA changes remained persistent over the 28-day recovery period. It seems that LMA is still decreased in the 35-day recovery group after nandrolone compared with the vehicle oil group, but there is no statistical difference. The reason for this is probably the high dispersion. Groups with recovery periods of 49 and 61 days after nandrolone treatments did not differ statistically from vehicle oil treated groups. The behavioral attenuation seen after cocaine injection in the nandrolone treated group was still seen after 28- and 35-day recovery periods. The 28-day group treated with nandrolone differed sta-

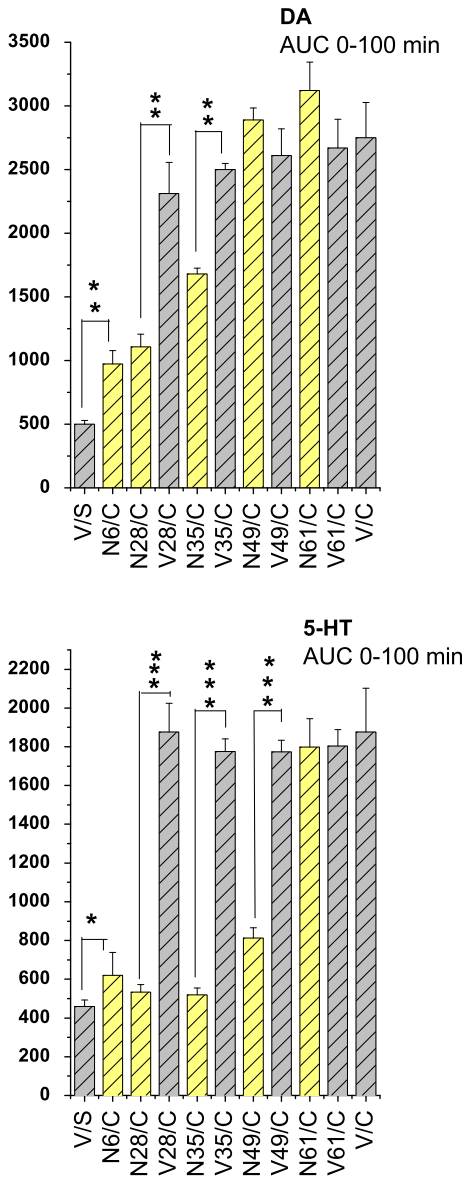

Fig. 2. The effects of nandrolone (5 × 20 mg/kg) or vehicle oil treatment and acute cocaine (20 mg/kg) injections after recovery periods on extracellular DA and 5-HT in the NAc. The times of the cocaine injections are indicated by arrows. Data expressed as percentages of basal release are given as means ± S.E.M. ($n = 6$). Histograms represent the area under the curve (AUC) after injection of the cocaine, and the minutes where AUC is counted are shown in the figure. $^{*}p \leqslant 0.05$, $^{***}p \leqslant 0.001$ Tukey's test. V/S = vehicle oil + saline, N6/C = nandrolone + cocaine, N28/C = nandrolone + cocaine after 28th day, V28/C = vehicle oil + cocaine after 28th day, N35/C = nandrolone + cocaine after 35th day, V35/C = vehicle oil + cocaine after 35th day, N49/C = nandrolone + cocaine after 49th day, V49/C = vehicle oil + cocaine after 49th day, V/C = vehicle oil + cocaine.

tistically from the vehicle oil treated 28-day group, and the nandrolone treated 35-day group also differed statistically from the vehicle oil treated 35-day group, as seen in Fig. 3. The same attenuation is still seen in the nandrolone 49-day recovery group, but the difference is no longer statistically significant when compared to the vehicle oil treated 49-day recovery group ($p = 0.060$). After 61 days from nandrolone dosing, there is no difference in behavior compared to vehicle oil groups.

4. Discussion

The main aim in the present study was to determine the length of the AASs induced effects on dopaminergic and serotonergic systems in brains. At the doses used here, the extracellular concentrations of DA after cocaine injection returned to the same levels as extracellular concentrations in the vehicle oil treated group 49 days from the last nandrolone dosing. This is a five times longer recovery period than the dosing period for doses and regimen of dosing used here, for the system to return back to the basic levels. The attenuation of cocaine's effects on the 5-HT system at the doses used here remained until 61 days before returning back to the basal levels. This is a six times longer recovery period than the dosing period, for the system to return back to the basic levels.

These results are in line with the concept that compounds that can cause dependence are hypothesized to produce changes in brain dopaminergic pathways that endure long after the person stops taking the drugs. It has also been shown that administration of nandrolone decanoate (14 × 15 mg/kg/in 2 weeks) increases the levels of the substance P N-terminal fragment SP1–SP1–7 in the NAc and that these higher concentration tended to remain elevated after 3 weeks of recovery [22]. Thus, a long-term effect of steroid administration is encountered in a tissue that is known to play an important role in the rewarding properties of abused drugs. The results seen here are also supported by the studies of Grimes et al., where they have suggested that AAS exposure has long-lasting effects on the 5-HT neural systems at least in adolescent AAS-treated Syrian hamsters [23,24]. To the author's knowledge, there are no studies of AAS long-lasting effects on the brains DA system. The 5-HT system returned more slowly back to the basal level than the DA system after nandrolone dosing suggesting that the same nandrolone dosing caused more attenuation on the 5-HT system than the DA system. Attenuation of the 5-HT system has been associated with increased aggression and dominance in both humans and animals [25–27], and aggression is one of the most commonly reported psychiatric side effects during use of AASs [28,29].

Acute administration of cocaine induced a rapid and long-lasting increase in locomotor activation and stereotypic behavior. Furthermore, nandrolone decanoate pre-treatment attenuated both the locomotor activity and stereotyped behavior induced by cocaine. These results are concordant with earlier studies in which testosterone has been shown to attenuate amphetamine induced locomotor activity and stereotyped behavior [30–32], and our own studies where nandrolone has been shown to blunt the stereotyped behavior and locomotor activity induced by amphetamine, MDMA and cocaine [8,33,7]. Correlation with microdialysis results is to be expected, because stimulant-induced hyperlocomotion is predominantly mediated by increased of DA in the NAc [34–36]. This is in line with previous studies where it has been shown that pre-treatment with nandrolone decanoate modulates, and induces, long lasting changes in the behavioral responses in rats. Johansson et al. [37] determined that nandrolone treatment enhanced steroid-induced defensive aggression 6–8 weeks after the end of the treatment period. Steensland et al. [38] showed that pre-treatment with nandrolone decanoate modulates the behavioral response and induces long lasting changes in the behavioral response to amphetamine.

The blood levels of nandrolone and its metabolites were measured in the present study. Our data shows that the dose 20 mg/kg (given five times during 10 days) induces 12.73 μg/l of nandrolone, measured 6 days after the last dose. After 28 days there was 4.15 μg/l nandrolone. Blood samples taken later did not contain any detectible residues of nandrolone or its metabolites, which suggests that changes are not caused by the presence of nandrolone or its metabolites. There are probably more permanent changes in the brain systems, but the mechanisms behind AAS-induced neurochemical and behavioral changes are not clear. We have previously found that pre-treatment with the androgen receptor antagonist flutamide robustly prevents, and the estrogen

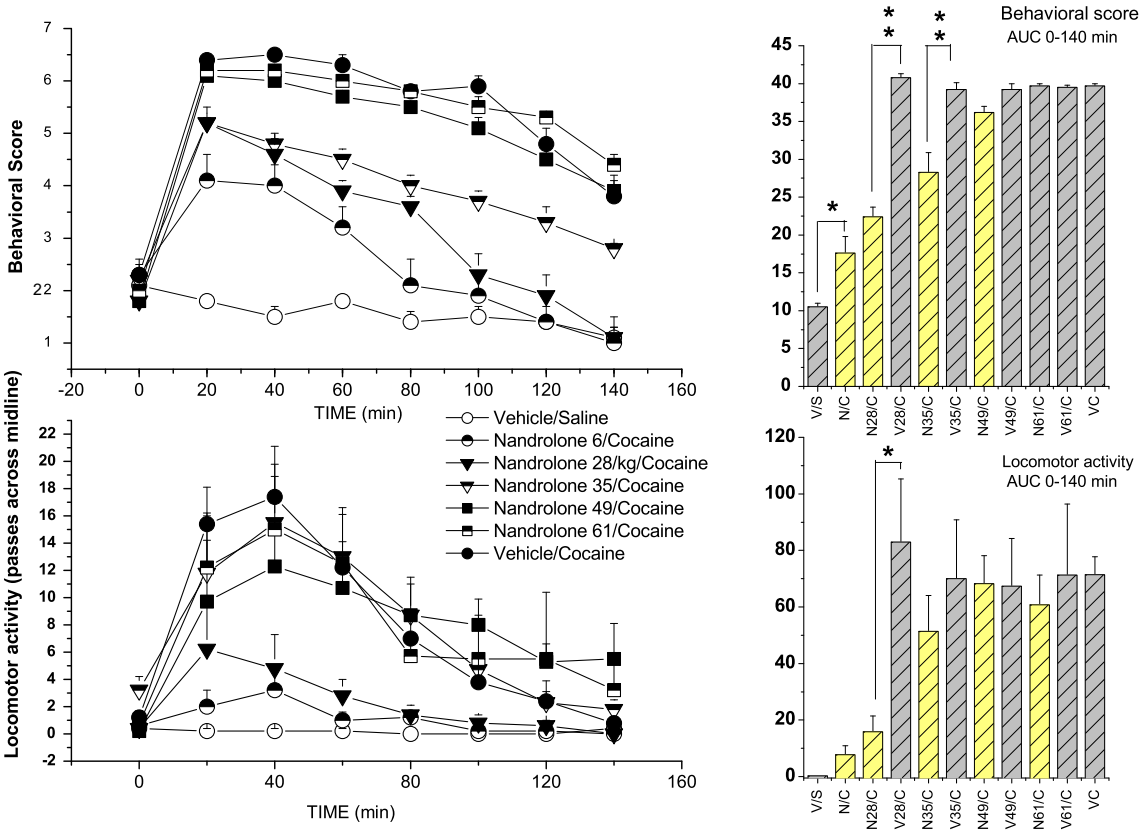

Fig. 3. The effects of nandrolone (5 × 20 mg/kg) and acute cocaine (20 mg/kg) injections on stereotyped behavior and locomotor activity. The rats were given a single behavioral score per each 1 min observation point and mean values were calculated per each 20 min sampling interval. Cocaine was administered at 0 min, indicated by arrows. Data expressed as absolute behavioral scores and passes across the midline ($n = 6$). Histograms represent AUC 0–140 min effect after injection of the cocaine. $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$, Tukey's test. V/S = vehicle oil + saline, N6/C = nandrolone + cocaine, N28/C = nandrolone + cocaine after 28th day, V28/C = vehicle oil + cocaine after 28th day, N35/C = nandrolone + cocaine after 35th day, V35/C = vehicle oil + cocaine after 35th day, N49/C = nandrolone + cocaine after 49th day, V49/C = vehicle oil + cocaine after 49th day, V/C = vehicle oil + cocaine.

receptor antagonist clomiphene partly reduces, nandrolone's attenuating effect on increased extracellular DA in the NAc after amphetamine dosing [33]. Anabolic steroids pass through the cell membrane and binds to the cytoplasmic androgen receptor (AR) which acts as transcription factors to modulate gene expression. Receptor activation could lead to alterations in neuronal excitability and signal transduction in brain regions implicated in addiction, which in turn could change the organism's neurochemical response to stimulant drugs in the brain. However AAS action on the central nervous system is complex. More recent studies in rats have demonstrated "non-classical" rapid cellular effects of androgens in brain regions that possess few classical, cytoplasmic receptors [39]. These steroid actions are thought to be mediated through membrane steroid receptors [40]. The simultaneous modification of both "classical" and "non-classical" target sites may account for the complex AAS abuse syndrome. Even with evidence as strong as the involvement of the steroid receptors, it has to be remembered that synthetic AASs and their metabolites also binds with higher doses, to glucocorticoid and progestin receptors [41]. They have also been shown to interact with GABA [42] and 5-HT receptors [43].

In can be concluded that AAS treatment causes long-lasting changes in monoaminergic systems. The recovery period, needed for the DA system to return back to the basic level, was fairly long compared to the dosing period of the steroid. In the 5-HT system, the time that system needed to return back to the basal level, was even longer than for the DA system. Since cocaine-induced responses were still attenuated after a long period without nandrolone, it seems that nandrolone induces long-lasting changes in the brains of rats. Further, nandrolone also decreased the behavioral effects induced by cocaine. Given that accumbal outflow of DA and 5-HT, as well as stereotyped behavior, are all considered to be related to gratification from stimulant drugs, this study suggests that AASs may induce changes in brain reward systems contributing to the maintenance of drug dependence.

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