Critical time-window for the actions of adrenal glucocorticoids in behavioural sensitisation to cocaine

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ABSTRACT

Glucocorticoids, secreted by the adrenals in response to stress, have profound effects on behavioural responsiveness to psychostimulant drugs. We have studied the critical time-window for the influence of corticosterone on behavioural sensitisation to cocaine in relation to i) the stage of behavioural sensitisation, and ii) the time of drug exposure.

Previously, we have identified a mouse strain (DBA/2) in which surgical removal of the adrenals (adrenalectomy: ‘ADX’) fully prevented locomotor sensitisation to cocaine. To investigate the role of corticosterone in expression of behavioural sensitisation, the glucocorticoid receptor (GR) antagonist mifepristone (RU38486) was administered to previously sensitised mice prior to a cocaine challenge. Furthermore, ADX mice were given corticosterone replacement at different intervals prior to each drug administration, to investigate the role of the glucocorticoid in initiation of behavioural sensitisation, and in relation to the time of drug exposure.

Administration of mifepristone to previously sensitised animals failed to block expression of cocaine-induced behavioural sensitisation. In ADX mice, intermittent replacement of corticosterone (1 mg/kg i.p., either 2 hours or 5 minutes prior to each cocaine administration), did not reverse the sensitisation deficit. By contrast, chronic corticosterone replacement (20% pellet) partially restored initiation of behavioural sensitisation. These data indicate that the presence of corticosterone facilitates the initiation rather than the expression of behavioural sensitisation to cocaine. However, because high corticosterone concentrations only partially reversed the sensitisation deficit of ADX mice, the adrenal glucocorticoid seems necessary, but not sufficient, for full behavioural sensitisation to cocaine in the DBA/2 strain.
INTRODUCTION

Adverse life experiences, and the neuroendocrine response they evoke, have a profound impact on vulnerability to the behavioural and reinforcing effects of psychostimulant drugs (reviewed in: 405,514). The contribution of the hypothalamic-pituitary-adrenal (HPA) axis and adrenal glucocorticoids (cortisol in humans, corticosterone in rodents) to psychostimulant sensitivity has been extensively studied 241,421. In laboratory rodents, glucocorticoids facilitate acquisition, maintenance and relapse of psychostimulant self-administration as well as drug-induced locomotion and behavioural sensitisation (reviewed in: 144,240,421,515). Furthermore, it has been shown that the propensity of animals to acquire psychostimulant self-administration positively correlates with their plasma corticosterone concentrations 244,516. The observation that glucocorticoids in the range of stress-induced levels are readily self-administered by laboratory rodents, suggests that these hormones themselves also possess reinforcing potential 512. In support of this, it has been shown that glucocorticoids can trigger synaptic adaptations in dopaminergic neurons similar to those induced by psychostimulants 582. There is convincing evidence that the actions of glucocorticoids on dopaminergic transmission and psychostimulant responsiveness are dependent on activation of the glucocorticoid receptor (GR) in the brain 153,168,308,579,582.

Despite the wealth of data showing that glucocorticoids mediate the effects of stress on psychostimulant sensitivity, the context- and time-dependency of their actions is poorly understood. Glucocorticoids can either facilitate or attenuate physiological or behavioural outcomes depending on timing, context and endpoint. For instance, the beneficial effects of glucocorticoids on memory consolidation and retrieval may only occur when stress is experienced closely linked in time to, and within the context of, the information to be learned (reviewed in: 318). Furthermore, glucocorticoids can exert their actions not only via nuclear receptor-mediated transcriptional regulation, but also via a non-genomic mechanism involving membrane-bound receptors and requiring a considerably shorter time span 55,103,176,338. Indeed, evidence is now accumulating that adrenal glucocorticoids regulate a wide range of behaviours via a rapid non-genomic mechanism (e.g. 339,450,451,584,589).

In the present study, we have investigated the critical time-window for the actions of corticosterone in sensitisation to the locomotor stimulant effects of cocaine. The DBA/2 inbred mouse strain was used, as it displays parallel sensitisation of cocaine-induced locomotion and corticosterone secretion, whereas surgical removal of the adrenals (adrenalectomy: ‘ADX’) prevents behavioural sensitisation 145. We have
investigated the time-window for the actions of corticosterone i) in relation to the stage of behavioural sensitisation (initiation vs. expression) and ii) in relation to the time of drug exposure. First, the glucocorticoid receptor (GR) antagonist mifepristone (RU38486) was administered to previously sensitised animals to investigate the contribution of corticosterone to expression of sensitisation. Second, ADX mice were given corticosterone replacement (5 minutes or 2 hours prior to each drug exposure or chronically), to study the role of the hormone during initiation of sensitisation and in relation to the time of drug exposure.

**METHODS**

**General methods**

*Animals*

Male DBA/2 Rj mice were obtained from Janvier (Le Genest Saint Isle, France) at the age of 8 weeks. Mice were housed in groups of four in perspex cages (35x19x14cm) with food and water available *ad libitum* at a 12 hour light-dark cycle (lights on: 7 am) in a temperature (21±1ºC) and humidity (55±5%) controlled room. Surgery was performed 2 weeks after arrival in the animal facility. Animals were briefly handled in the week before surgery and otherwise left undisturbed. Animal experiments were approved by the local Committee for Animal Health, Ethics and Research of Leiden University. Animal care was conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC).

*Surgery*

Animals were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where mice were allowed to recover from transportation for 2 hours. Inhalation anaesthesia consisted of a mixture of isoflurane (3 l/min), N₂O (0.8 l/min) and O₂ (0.4 l/min). Adrenalectomy was performed via the dorsal route as described previously. SHAM animals were treated similarly with the exception of the actual removal of the adrenals. Mice were kept individually housed for 24 hours following surgery after which they were housed two animals per cage of similar surgery. After surgery, all animals were given free access to 0.9% NaCl in addition to normal drinking water. The sensitisation paradigm was started following a recovery period of 1 week.
Drugs
Cocaine hydrochloride (BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) was dissolved in sterile saline and administered intraperitoneally (i.p.) in a dosage of 7.5 or 15.0 mg/kg. Mifepristone (RU38486, Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was dissolved to a concentration of 100 mg/ml in sterile saline containing 2.5 mg carboxymethylcellulose and 2 μl Tween-20 per ml. Animals received 50 μl of this solution applied on oats resulting in a dose of 200 mg/kg bodyweight. Corticosterone (corticosterone:HBC complex, Sigma-Aldrich) was dissolved in sterile saline and administered i.p. in a dosage of 1 mg/kg of the free base. Corticosterone pellets (100 mg, w x h: 9 x 2 mm) consisted of 20% corticosterone (ICN Biomedicals Inc, Aurora, Ohio, USA) in cholesterol vehicle (cholesterol 95% stabilised, Acros Organics, Geel, Belgium) and were implanted subcutaneously in the flank of the animal. Control groups received equal volumes of saline, 100% cholesterol pellets in the chronic corticosterone substitution study, and oats containing vehicle in the mifepristone study. From the start of the sensitisation paradigm, animals were weighed once every two days and the injection volumes were adjusted accordingly.

Sensitisation paradigm
One day prior to the first drug administration and thus the first behavioural test, animals were individually housed and kept single housed for the remainder of the experiment. The sensitisation paradigm consisted of a treatment phase (days 1-9), a withdrawal interval (days 10-14), a saline challenge (day 14), and a cocaine challenge (day 15). The treatment phase consisted of i.p. injections of 15.0 mg/kg cocaine (COC) or saline (SAL) on 9 consecutive days and locomotion was measured on days 1 (first administration) and 9 (last administration). On these days, animals received the treatment in the test setting as described in the following paragraph. On days 2-8 animals received the injections in the home cage. The treatment period was followed by a withdrawal interval of 5 days (no treatment). On the last day of the withdrawal interval (day 14), all animals received a saline challenge and on day 15, all animals received a 7.5 mg/kg cocaine challenge. In experiments 2-4, cocaine-treated mice were re-challenged with 7.5 mg/kg cocaine on day 21. For this day, only data of cocaine-treated mice are presented, as the saline-treated groups received cocaine for the second time and can therefore no longer be considered proper controls. All injections were given 2 to 5 hours after lights on.

Measurement of locomotor activity
All behavioural tests (days 1, 9, 14, 15 and 21) were performed in the room where animals were housed. Mice were placed in a test cage (same type and size
(35x19x14cm) as the home cage) containing a standardised amount of sawdust, covered with a perspex lid. Following a 2 hour habituation period, animals were injected and activity was monitored on video for 30 minutes. At the end of this period, a blood sample was taken from the tail vein for endocrine measurements and the animals were returned to their home cage.

Analysis of locomotor activity
Video images were digitised and analysed using Ethovision Videotracking, Motion Analysis & Behavior Recognition System version 1.96 (‘VTMAS’, Noldus Information Technology B.V., Wageningen, The Netherlands). The position of the animal was sampled 5 times per second. Of each recording (30 minutes) 27 minutes were analysed since the animals were subjected to blood sampling at 30 minutes after injection. Data are represented in total distance moved (cm) over the entire 27 minute treatment period. Locomotion was defined as movement with a minimal distance of 2 cm.

Corticosterone assay
Blood samples were taken from the tail vein by a small incision with a razorblade 30 minutes after treatment on the test days 1, 9, 14, 15 and 21. Blood was collected in small EDTA coated tubes (Microvette DB 200 K3E, Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 13000 rpm for 20 minutes at 4ºC and subsequently stored at -20ºC. Corticosterone concentrations were determined by in-duplo measurement using a radio-immuno-assay kit according to the protocol provided by the manufacturer (Corticosterone double antibody 125I RIA kit, MP Biomedicals, Asse-Relegem, Belgium). All samples were analysed in one assay to exclude inter-assay variability. ADX effectively clamped plasma corticosterone to basal concentrations and only animals with successful ADX were included in the study.

Experimental design

Experiment 1: Effect of mifepristone (RU38486) on expression of behavioural sensitisation to cocaine in intact mice
This experiment was designed to investigate whether corticosterone plays a critical role in expression of previously established behavioural sensitisation to cocaine. For this purpose, animals were administered the glucocorticoid receptor antagonist mifepristone (200 mg/kg, orally via oats) 2.5 hours prior to the cocaine challenge on day 15. Unoperated DBA/2 mice were used for this part of the study. In the week before start of the sensitisation paradigm, animals were familiarised with the smell
and taste of plain oats. A feeding cup was placed permanently in the home cage for oat administration. Initially, animals were divided over 2 experimental groups: SAL and COC (n=20, 34). Based on behavioural responses on the last day of the treatment period (day 9), animals were divided over vehicle and mifepristone (‘RU’) treatment groups such that a homogenous distribution of relatively low- and high-responders was created (SAL/VEH, SAL/RU, COC/VEH, COC/RU n=10-18). On day 15, the oats containing mifepristone or vehicle were given 2.5 hours prior to the cocaine challenge, thus 30 minutes prior to the onset of the habituation phase. When animals were transferred to the test cages, the home cages were checked to verify whether all oats had been eaten.

Experiments 2-4 were designed to investigate the role of corticosterone during initiation of behavioural sensitisation to cocaine and the time-window for the actions of the hormone in relation to drug exposure.

**Experiments 2 and 3: Effect of corticosterone substituted 5 minutes or 2 hours prior to cocaine on behavioural sensitisation in ADX mice**

This part of the study consisted of 6 experimental groups (n=8-12). Animals were either adrenalectomised (ADX) or SHAM operated. ADX animals were assigned to the corticosterone substitution or control groups (ADXcort, ADX). All groups were subdivided in saline (SAL) and cocaine (COC) groups, indicating the treatment given during the treatment phase of the sensitisation paradigm. Corticosterone (1 mg/kg, free base) or saline was injected i.p. 5 minutes (experiment 2) or 2 hours (experiment 3) prior to each treatment (days 1-9, 14 and 15). To investigate drug responsiveness in absence of corticosterone substitution, animals were re-challenged with 7.5 mg/kg cocaine one week after the initial cocaine challenge (day 21). On this day, all animals received saline 2 hours prior to drug treatment.

**Experiment 4: Effect of chronic corticosterone substitution on behavioural sensitisation in ADX mice**

Again, 6 treatment groups were used: SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) (n=8-12). Corticosterone (20%) and cholesterol pellets were implanted one day prior to the onset of the sensitisation paradigm. To this aim, animals were briefly anaesthetised with an isoflurane mixture (see surgical procedures) and the pellet was placed subcutaneously in the flank of the animal. Following surgery, animals were housed individually and the sensitisation paradigm was started the following day. Also in this study, animals were re-challenged on day 21 with 7.5 mg/kg cocaine.
Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). For the mifepristone study, analyses were performed per test day: one factor ANOVA for treatment (days 1, 9 and 14) and two factor ANOVA for treatment and mifepristone (day 15). For the corticosterone substitution studies, overall locomotor activity and corticosterone data were subjected to repeated measures ANOVA with three between subject factors (surgery, substitution and treatment) and one within subject factor (test day). Subsequent analyses were performed per test day: three factor ANOVA for surgery, substitution and treatment. When statistical significance was revealed, post hoc tests were performed (LSD, or for within subject comparison paired t-test). Differences were considered statistically significant when p<0.05.

RESULTS

Experiment 1: Effect of mifepristone (RU38486) on expression of behavioural sensitisation to cocaine in intact mice

The GR antagonist mifepristone (RU38486, ‘RU’) was administered orally to intact animals 2.5 hours prior to the 7.5 mg/kg cocaine challenge on day 15. Figure 1A depicts total distance moved for the treatment groups SAL and COC (days 1, 9 and 14) and SAL/VEH, SAL/RU, COC/VEH and COC/RU (day 15).

Cocaine stimulated locomotion in the treatment period (day 1: F[treatment]1,53=18.412, p<0.001, day 9: F[treatment]1,52=49.246, p<0.001, post hoc: p<0.001) and drug responses were enhanced on day 9 compared to day 1 (p<0.01, paired t-test). Furthermore, cocaine-treated mice displayed elevated locomotor responses to the saline challenge on day 14 (F[treatment]1,52=16.192, p<0.001, post hoc: p<0.001). Based on responsiveness on day 9, cocaine-treated mice were distributed over the RU and VEH groups such that relatively low- and high-responders were divided equally. The cocaine challenge on day 15 resulted in augmented locomotion in cocaine- compared to saline-treated mice (F[treatment]1,48=9.623, p<0.01, post hoc: p<0.05), while there was no effect of mifepristone on drug responses of either cocaine-treated or drug-naïve animals (F[RU]1,48=0.000, p=1.000).

Figure 1B depicts plasma corticosterone concentrations 30 minutes after drug treatment, thus 3 hours after RU or VEH administration. Plasma corticosterone concentrations were elevated in RU compared to VEH-treated groups (F[RU]1,53=126.566,
p<0.001, post hoc: p<0.001), with the most pronounced elevation in the COC/RU group (F[treatment]_{1,51}=126.566, p=0.210, F[treatment x RU]_{1,51}=9.672, p<0.01, post hoc: p<0.01 vs. SAL/RU).

The results of experiment 1 did not support a critical role for glucocorticoid signalling via GR in expression of locomotor sensitisation in DBA/2 mice. We therefore hypothesised that corticosterone may contribute to induction of behavioural sensitisation. Experiments 2-4 were designed to investigate whether the deficit of
ADX mice in locomotor sensitisation to cocaine can be reversed by replacement of corticosterone from the start of the sensitisation paradigm, at different time intervals in relation to drug exposure.

**Plasma corticosterone concentrations**

Table 1 shows the effects of ADX and the different hormone replacement regimens on plasma corticosterone concentrations 30 minutes after treatment on days 1, 9, 14, and 15.

For SHAM and ADX mice, data were replicated across experiments and therefore pooled. Main effects were found for treatment, surgery, day and the interaction between these factors ($F_{[\text{treatment}],89}=19.897$, $p<0.001$, $F_{[\text{surgery}],89}=314.423$).

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 9</th>
<th>Day 14</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM SAL</td>
<td>108.9 ± 12.3</td>
<td>140.4 ± 17.3 †</td>
<td>138.8 ± 13.6</td>
<td>216.2 ± 15.7</td>
</tr>
<tr>
<td>SHAM COC</td>
<td>220.5 ± 13.4 *</td>
<td>364.0 ± 16.4 †</td>
<td>167.7 ± 18.0</td>
<td>261.1 ± 17.6 *</td>
</tr>
<tr>
<td>ADX SAL</td>
<td>18.6 ± 2.9 f</td>
<td>30.0 ± 4.6 f</td>
<td>35.7 ± 5.3 f</td>
<td>40.4 ± 6.6 f</td>
</tr>
<tr>
<td>ADX COC</td>
<td>20.8 ± 3.0 f</td>
<td>30.9 ± 4.2 f</td>
<td>35.6 ± 4.0 f</td>
<td>38.7 ± 4.8 f</td>
</tr>
<tr>
<td>CORT 5 min SAL</td>
<td>422.9 ± 50.3 f a</td>
<td>530.1 ± 31.6 f a</td>
<td>577.2 ± 17.1 f a</td>
<td>418.5 ± 64.0 f a</td>
</tr>
<tr>
<td>CORT 5 min COC</td>
<td>557.7 ± 31.3 f a</td>
<td>633.0 ± 43.2 f a</td>
<td>521.6 ± 67.5 f a</td>
<td>469.5 ± 63.8 f a</td>
</tr>
<tr>
<td>CORT 2 hrs SAL</td>
<td>71.7 ± 13.4 f a</td>
<td>72.2 ± 6.8 f</td>
<td>95.3 ± 10.8 a</td>
<td>102.0 ± 15.0 f a</td>
</tr>
<tr>
<td>CORT 2 hrs COC</td>
<td>867.0 ± 13.9 f a</td>
<td>84.3 ± 10.3 f</td>
<td>56.8 ± 11.0 f</td>
<td>65.0 ± 9.9 f</td>
</tr>
<tr>
<td>CORT pellet SAL</td>
<td>474.1 ± 22.8 f a</td>
<td>129.5 ± 6.2 f a</td>
<td>118.6 ± 6.5 f a</td>
<td>135.6 ± 5.9 f a</td>
</tr>
<tr>
<td>CORT pellet COC</td>
<td>527.5 ± 19.5 f a</td>
<td>130.8 ± 4.9 f a</td>
<td>110.2 ± 4.6 f a</td>
<td>120.5 ± 5.5 f a</td>
</tr>
</tbody>
</table>

Plasma corticosterone concentrations 30 minutes after treatment on days 1, 9, 14 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and given corticosterone (CORT) replacement: 5 minutes or 2 hours prior to drug administration (1 mg/kg, i.p.) or chronically (20% pellet). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average plasma corticosterone concentration (ng/ml) ± SEM, $n=7-29$ animals/group. * $p<0.05$ vs. SAL (LSD), † $p<0.05$ vs. SHAM (LSD), Ω $p<0.05$ vs. ADX (LSD), ‡ $p<0.05$ vs. day 1 (paired t-test).
Corticosterone concentrations were attenuated in ADX compared to SHAM mice on all test days (p<0.001). In agreement with previous observations (chapter 2, 145), cocaine-induced corticosterone secretion in SHAM mice was enhanced on day 9 compared to day 1 (p<0.001, paired t-test) and a similar effect was observed for the response to saline treatment, though less pronounced (p<0.05, paired t-test). In addition, cocaine-treated SHAM mice showed greater corticosterone secretion in response to the drug challenge on day 15, when compared to saline-treated mice (p<0.05).

Corticosterone substitution 5 minutes prior to drug treatment resulted in a pronounced elevation of the hormone to concentrations even higher than in the SHAM groups (F[substitution]1,103=805.511, p<0.001, post hoc: p<0.001 vs. SHAM and ADX on all days). By contrast, when corticosterone was administered 2 hours prior to drug administration, hormone concentrations were lower than of SHAM mice, though not fully normalised to ADX levels 30 minutes after drug treatment (F[substitution]1,102=9.839, p<0.01). On day 21, corticosterone substitutions were omitted and hormone concentrations were similar to those of ADX mice (data not shown).

In animals with corticosterone pellets (F[substitution]1,105=171.434, p<0.001), hormone concentrations were elevated above SHAM levels on day 1 (p<0.001) and thereafter declined to on average 125 ng/ml on days 9, 14 and 15, being higher than in the ADX groups (at least p<0.01), but in the case of cocaine treatment, lower than in the SHAM groups (at least p<0.05). A further decline was observed from day 15 to 21, when corticosterone concentrations in the substituted groups dropped below 100 ng/ml (ADXcort/SAL: 92 ± 8, ADXcort/COC: 81 ± 6). These data indicate that the subcutaneous corticosterone pellet generated plasma corticosterone concentrations that were very high on the first day of the treatment phase and thereafter declined to concentrations in the range of stress-induced levels that persisted until the first cocaine challenge on day 15.

**Experiment 2: Effects of corticosterone substituted 5 minutes prior to cocaine on behavioural sensitisation in ADX mice**

Corticosterone (1 mg/kg, free base) or saline was administered 5 minutes prior to drug treatment on days 1-9, 14 and 15, but not 21. Main effects were found for treatment, day and the interaction between day, surgery and treatment (F[treatment]1,43=37.622, p<0.001, F[day]4,172=12.413, p<0.001, F[day x surgery x treatment]4,172=3.444, p<0.05). Responses to saline treatment were not affected by either surgery or substitution in any of the tests.
Figure 2 depicts total distance moved for the treatment groups SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) on days 1, 9 and 15. On the first day of the treatment period, there was no effect of surgery or substitution on cocaine-induced locomotion ($F_{[\text{treatment}]}=4.532, p<0.05$, $F_{[\text{surgery}]}=0.292, p=0.591$, $F_{[\text{substitution}]}=0.235, p=0.630$). Consistent with previous observations, drug responses of SHAM ($p<0.05$, paired t-test), but not of ADX mice ($p=0.735$, paired t-test), were enhanced on day 9 compared to day 1. The ADXcort group also displayed increased cocaine responsiveness ($p<0.01$, paired t-test), although this effect appeared to be mainly attributable to the slightly lower drug response.
on day 1, rather than to true sensitisation. In support of this, cocaine responses of ADX mice were lower than of SHAM mice on day 9 (p<0.01), irrespective of substitution (F[treatment]_{1,54}=39.504, p<0.001, F[surgery]_{1,54}=4.339, p<0.05, F[substitution]_{1,4}=0.040, p=0.843, F[surgery x treatment]_{1,54}=4.886, p<0.05). Furthermore, in contrast to SHAM/COC mice (p<0.001), neither ADX/COC nor ADXcort/COC mice displayed augmented responsiveness to the 7.5 mg/kg cocaine challenge on day 15 (F[treatment]_{1,53}=29.765, p<0.001, F[surgery]_{1,53}=1.786, p=0.188, F[substitution]_{1,53}=1.571, p=0.216, F[surgery x treatment]_{1,53}=12.160, p<0.01). Moreover, locomotor responses of cocaine-treated ADX and ADXcort mice were lower than of SHAM mice on day 15 (p<0.01) and on day 21 (ADXcort: p<0.05, ADX: p=0.068), when animals were re-challenged with 7.5 mg/kg cocaine without prior corticosterone substitution (day 21: SHAM: 5603 ± 1038, ADX: 3771 ± 619, ADXcort: 3017 ± 650, F[treatment]_{1,51}=10.536, p<0.01, F[surgery]_{1,51}=0.195, p=0.661, F[substitution]_{1,51}=1.965, p=0.168, F[surgery x treatment]_{1,51}=8.059, p<0.01).

**Experiment 3: Effects of corticosterone substituted 2 hours prior to cocaine on behavioural sensitisation in ADX mice**

Corticosterone (1 mg/kg, free base) or saline was administered 2 hours prior to drug treatment on days 1-9, 14 and 15, but not 21. Locomotion was influenced by surgery, treatment and day and interactions between these factors (F[surgery]_{1,41}=5.566, p<0.05, F[treatment]_{1,41}=16.192, p<0.001, F[day]_{4,164}=29.030, p<0.001, F[day x surgery]_{4,164}=4.500, p<0.01, F[day x treatment]_{4,164}=2.504, p<0.05). Responses to saline treatment were not affected by either surgery or substitution in any of the tests.

Locomotor responses of the SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) groups on days 1, 9 and 15 are depicted in figure 3. On the first day of the treatment period, there was no effect of surgery or substitution on cocaine-induced locomotion (F[treatment]_{1,57}=9.719, p<0.01, F[surgery]_{1,57}=1.529, p=0.222, F[substitution]_{1,57}=0.232, p=0.632). Similar to experiment 2, only the SHAM group displayed enhanced drug responsiveness on day 9 compared to day 1 (p<0.05, paired t-test). Furthermore, cocaine responses of ADX mice were lower than of SHAM mice on day 9, irrespective of substitution, although this just failed to reach significance for the ADX group (F[treatment]_{1,50}=26.694, p<0.001, F[surgery]_{1,50}=2.157, p=0.149, F[substitution]_{1,50}=0.566, p=0.456, post hoc: ADX: p=0.055, ADXcort: p<0.01 vs. SHAM). Also in this study, only SHAM/COC mice (p<0.05) displayed enhanced responsiveness to the 7.5 mg/kg cocaine challenge on day 15 (F[treatment]_{1,49}=7.188, p<0.05, F[surgery]_{1,49}=1.572, p=0.216,
Furthermore, locomotor responses of cocaine-treated ADX (p=0.066) and ADXcort (p<0.05) mice were lower than of SHAM mice on day 15 and this was even more pronounced on day 21 (p<0.05 for both groups), when animals were re-challenged with 7.5 mg/kg cocaine without prior corticosterone substitution (SHAM: 9859 ± 1481, ADX: 4290 ± 1182, ADXcort: 5960 ± 1338, F[treatment]₁,₄₇=3.920, p=0.054, F[surgery]₁,₄₇=9.646, p<0.01, F[substitution]₁,₄₇=0.483, p=0.491).

Figure 3: The effect of corticosterone substituted 2 hours prior to drug administration on behavioural sensitisation in ADX mice. Locomotion in response to treatment on days 1, 9 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and given corticosterone replacement 2 hours prior to drug administration (ADXcort). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average total distance moved (TDM, cm) ± SEM, n=7-12 animals/group. * p<0.05 vs. SAL (LSD), #1 p<0.01, #2 p<0.05 vs. SHAM (LSD), † p <0.05 vs. day 1 (paired t-test).
Experiment 4: Effect of chronic corticosterone substitution on behavioural sensitisation in ADX mice

Corticosterone (20% corticosterone in cholesterol) or vehicle (100% cholesterol) pellets were implanted subcutaneously (s.c.) one day prior to the first drug administration. Main effects were found for surgery, treatment, day and the interaction between these factors ($F_{\text{surgery}}=10.640, p<0.01$, $F_{\text{treatment}}=37.136, p<0.001$, $F_{\text{day}}=35.683, p<0.001$, $F_{\text{day x surgery x treatment}}=2.800, p<0.05$). Responses to saline treatment were not affected by either surgery or substitution in any of the tests.

![Graph](Image)

**Figure 4:** The effect of chronic corticosterone substitution on behavioural sensitisation in ADX mice.

Locomotion in response to treatment on days 1, 9 and 15. Animals were SHAM operated, adrenalectomised (ADX), or ADX and implanted with a 20% corticosterone pellet (ADXcort). Mice were treated during 9 consecutive days with cocaine (COC) 15.0 mg/kg or saline (SAL), followed by a 5 day withdrawal interval. All animals received a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. Data are represented in average total distance moved (TDM, cm) ± SEM, n=6-10 animals/group. * $p<0.01$ vs. SAL, #1 $p<0.001$, #2 $p<0.01$, #3 $p<0.05$ vs. SHAM (LSD), $\Omega p<0.05$ vs. ADX (LSD), †1 $p<0.01$, †2 $p<0.05$ vs. day 1 (paired t-test).
Figure 4 depicts locomotor responses of SHAM (SAL/COC), ADX (SAL/COC) and ADXcort (SAL/COC) groups on days 1, 9 and 15. On the first day of the treatment period, corticosterone substitution reduced cocaine responses (F[treatment]_{1,53}=9.398, p<0.01, F[substitution]_{1,53}=4.659, p<0.05, F[surgery]_{1,53}=1.099, p=0.300, post hoc: p<0.05 vs. SHAM/COC and ADX/COC). In this experiment, not only SHAM, but also ADXcort mice, displayed enhanced cocaine-induced locomotion on day 9 compared to day 1 (p<0.05, paired t-test). The apparent sensitisation in the ADXcort group was not solely due to the reduced drug response on day 1, as cocaine responses of these mice were higher than of ADX mice on day 9 (p<0.05), although not fully normalised to SHAM levels (p<0.001) (F[treatment]_{1,49}=46.014, p<0.001, F[surgery]_{1,49}=18.168, p<0.001, F[substitution]_{1,49}=2.481, p=0.122, F[surgery x treatment]_{1,49}=16.909, p<0.001). However, only SHAM/COC animals (p<0.01) displayed enhanced drug responsiveness to the 7.5 mg/kg cocaine challenge on day 15 (F[treatment]_{1,50}=5.106, p<0.05, F[substitution]_{1,50}=8.135, p<0.01, F[substitution]_{1,50}=0.979, p=0.328, F[surgery x treatment]_{1,50}=4.645, p<0.05). Furthermore, locomotor responses of cocaine-treated ADX and ADXcort mice were lower than of SHAM mice on day 15 (p<0.05). On day 21 however, when animals were re-challenged with 7.5 mg/kg cocaine, only cocaine responses of ADX mice were significantly lower than of SHAM mice (SHAM: 7101 ± 1102, ADX: 4651 ± 761, ADXcort: 5311 ± 572, F[treatment]_{1,49}=3.558, p=0.066, F[surgery]_{1,49}=1.506, p=0.226, F[substitution]_{1,49}=1.333, p=0.255, post hoc: ADX: p<0.05, ADXcort: p=0.112).

**DISCUSSION**

The present study shows that adrenal glucocorticoids play a role during initiation rather than expression of cocaine-induced behavioural sensitisation in DBA/2 mice. The time window for the actions of corticosterone during initiation of sensitisation was investigated in ADX mice. It was demonstrated that whereas intermittent corticosterone replacement, either 5 minutes or 2 hours prior to each drug administration, was ineffective in reversing the sensitisation deficit of ADX mice, initiation of behavioural sensitisation was partially restored by chronic corticosterone substitution.

The observation that ADX prevents both initiation and expression of locomotor sensitisation to cocaine in DBA/2 mice, suggests that adrenal stress hormones contribute to the developmental stage of behavioural sensitisation (the present study, 145).
This hypothesis was strengthened by the present finding that the GR antagonist mifepristone (RU38486) administered 2.5 hours prior to the cocaine challenge (day 15) was ineffective in reversing expression of previously established behavioural sensitisation of intact mice (figure 1A). In agreement with this, it has been demonstrated that ADX performed in already sensitised animals does not prevent expression of the behavioural hyperresponsiveness. The present finding does however contradict two reports in which the motivation to self-administer cocaine, and amphetamine-induced behavioural sensitisation, were blocked by pretreatment with mifepristone in animals that had previously acquired these behaviours. This discrepancy could result from the use of different species and genetic backgrounds. In support of this, Deroche-Gamonet et al. show that mifepristone only reduces the motivation to obtain cocaine in a subset of outbred rats that display an exceptionally high drug response, and we have demonstrated previously that the effects of ADX on behavioural sensitisation to cocaine are highly strain-dependent.

The dramatic increase in plasma corticosterone observed in mifepristone-pretreated mice (as measured 30 minutes after drug treatment, figure 1B), indicates that the antagonist was effective in preventing negative feedback on the HPA-axis and thus in blocking GR. From these data it can however not be concluded whether sufficient concentrations of mifepristone reached the brain, but this is likely to have occurred since other studies using lower oral doses have shown effects of the antagonist on brain function and behaviour.

The observation that the 20% corticosterone pellet facilitated initiation of sensitisation in ADX mice, is in agreement with reports showing that chronic administration of the adrenal glucocorticoid in concentrations similar to those induced by stress, enhances behavioural responsiveness to single and repeated psychostimulant exposure. However, mice implanted with the 20% pellets did not show behavioural hyperresponsiveness to the cocaine challenge on day 15. It is conceivable that a certain degree of behavioural sensitisation did develop in mice receiving chronic corticosterone, and we hypothesise that a longer withdrawal duration may be required for this behaviour to become expressed. Indeed, psychostimulant sensitisation is characterised by a time-dependent cascade of cellular changes that differs between short- and long-term withdrawal periods. The observation that locomotor responses of ADXcort and SHAM mice were no longer different by the time of the second cocaine challenge (day 21), supports this hypothesis. This finding was replicated in a subsequent experiment in which drug responses of ADXcort mice were even equal to those of SHAM mice on day 21 (see chapter 5). Further
Several explanations can be proposed for the inefficacy of corticosterone in fully reversing the effects of ADX on behavioural sensitisation to cocaine. First, it is conceivable that the hormone was not replaced in sufficient amounts. This is however not likely, since the dose of 1 mg/kg used in the intermittent replacement studies resulted in extraphysiological peak plasma concentrations of 1200 ng/ml (5 minutes after i.p. administration, data not shown). Furthermore, corticosterone in the same dose range has been shown to sensitisise the locomotor response to amphetamine in rats. However, it has also been demonstrated that administration of 10 mg/kg, but not 5 mg/kg, corticosterone prior to cocaine enhances behavioural sensitisation to the drug in intact rats, indicating that even in the extra-physiological dose range, there may be dose-dependency for the effects of corticosterone. In the case of the 20% corticosterone pellet, mice were exposed to high glucocorticoid levels especially during the initiation of behavioural sensitisation, to which the hormone was hypothesised to contribute. Furthermore, concentrations in the range of stress-induced levels were still present at the time of the first cocaine challenge. Previously, a 20% corticosterone pellet has been demonstrated to fully restore amphetamine-induced locomotion in ADX mice.

Second, sensitisation of HPA-axis responsiveness to cocaine may be a requirement for behavioural sensitisation in this strain. Indeed, DBA/2 mice display parallel sensitisation of locomotion and corticosterone secretion during the treatment phase and in response to the cocaine challenge on day 15 (the present study). However, it has been shown previously that sensitisation of corticosterone secretion may not be a prerequisite for long-term expression of behavioural sensitisation. Further studies are therefore required to investigate the potential contribution of endocrine sensitisation to behavioural responsiveness in this mouse strain.

Third, it may be necessary to restore the circadian and/or ultradian rhythmicity in corticosterone secretion. In contrast to rats, mice maintain basal levels of corticosterone that are considered sufficient for occupation of the high-affinity mineralocorticoid receptor after ADX (the present study). However, ADX mice do lack the rise in plasma corticosterone that precedes the nocturnal period. Although diurnal concentrations of corticosterone are sufficient for the locomotor response to a single cocaine exposure in rats, a full circadian cycle might be required for behavioural sensitisation to repeated drug exposure.

Assuming that sufficient amounts of corticosterone were replaced, the observation that only chronic high concentrations of the hormone partially restored initiation of
sensitisation, suggests that glucocorticoids may not be the only adrenal factors that contribute to behavioural sensitisation in the DBA/2 strain. This conclusion is supported by several observations. It is well accepted that stress-induced behavioural sensitisation to psychostimulants depends on corticosterone secretion \(^{164,166}\). However, studies using ADX, genetic- or pharmacological inactivation of the GR have generated conflicting results regarding the necessity of adrenal glucocorticoids in psychostimulant sensitisation \(^{21,168,527,552,609}\). Furthermore, two recent reports have indicated that corticosterone may be necessary, but not sufficient for the effects of stress on escalation of cocaine self-administration \(^417\) and on the neurochemical and rewarding effects of morphine \(^{161}\). Mantsch et al. elegantly showed that restoration of corticosterone levels in the range of those induced by footshock stress is necessary to reinstate stress-induced escalation of cocaine self-administration in ADX animals. However, corticosterone alone was not sufficient to reproduce the effects of the stressor in animals that were not exposed to footshock \(^{417}\). The requirement of the stressor suggests that stress-induced factors other than corticosterone play an additional role. In view of the present data with ADX, it is tempting to speculate a role for the adrenal catecholamine epinephrine. Indeed, the HPA-axis and the sympathetic nervous system have been demonstrated to interact in regulation of behaviour, most notably in memory processing \(^{53,571}\). Further research is required to investigate the potential role of other adrenal factors such as epinephrine, in addition to that of corticosterone, in behavioural sensitisation to cocaine.

The present study showed that, in a mouse strain in which behavioural sensitisation to cocaine is critically dependent on adrenal hormones, the glucocorticoid corticosterone facilitated the initiation and retention of the behavioural sensitisation to repeated drug exposure, provided it was continuously circulating. To full blown recovery of the impaired behavioural sensitisation to psychostimulants in ADX mice other adrenal factors, such as epinephrine, may contribute.

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