Vulnerability to cocaine: role of stress hormones

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Adrenalectomy prevents behavioural sensitisation of mice to cocaine in a genotype-dependent manner

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ABSTRACT

The objective of the present study was to investigate the contribution of adrenal stress hormones to strain differences in cocaine sensitivity.

For this purpose, we have studied sensitisation to the locomotor stimulant effect of cocaine and, in parallel, cocaine-induced corticosterone secretion in two inbred mouse strains: C57BL/6 and DBA/2. Adrenalectomy ('ADX': surgical removal of the adrenal glands) was performed in a subset of animals to investigate the contribution of the adrenals. ADX and SHAM operated mice were subjected to repeated injections of cocaine (15.0 mg/kg) or saline for 9 consecutive days, followed by a 5 day withdrawal interval and a saline challenge on day 14. All animals were challenged with 7.5 mg/kg cocaine on day 15.

We report that repeated cocaine exposure induced locomotor sensitisation in both strains, while endocrine sensitisation was only observed in the DBA/2 strain. By contrast, cocaine attenuated corticosterone responses in C57BL/6 mice throughout the sensitisation paradigm. We have therefore identified one strain, the DBA/2 strain, that displays parallel sensitisation of cocaine-induced locomotion and -corticosterone secretion. Most interestingly, ADX prevented locomotor sensitisation only in DBA/2 mice, suggesting that behavioural sensitisation depends on the integrity of adrenal function and on secretion of adrenal glucocorticoids in this strain.

The present results demonstrate that adrenal stress hormones facilitate behavioural sensitisation to cocaine in a genotype-dependent manner and suggest that glucocorticoids contribute to strain differences in psychostimulant sensitivity.

INTRODUCTION

Behavioural responses to psychostimulant drugs are characterised by a large degree of individual variability, both in humans and laboratory animals [191,479,509]. Psychostimulants activate the mesocorticolimbic dopamine system and individual vulnerability to their effects may reflect a given predisposition to dopaminergic psychosis, such as observed in drug addiction, schizophrenia and psychotic depression. Knowledge of factors that enhance vulnerability to psychostimulants will therefore greatly increase our insight in the neurobiology of dopaminergic psychopathologies.

The existence of marked strain differences in responsiveness to drugs such as amphetamine and cocaine has demonstrated that genetic traits contribute to variations in psychostimulant vulnerability. Two inbred mouse strains that have been used frequently to study the psychopharmacology of dopamine are the C57BL/6 and DBA/2 strains. These strains display profound differences in the anatomy and functioning of the mesocorticolimbic dopamine system and in behavioural responsiveness to dopaminergic agonists and addictive drugs (reviewed in: [531]). Compared to DBA/2 mice, C57BL/6 mice are more sensitive to amphetamine-induced locomotion and reward and display higher drug-induced dopamine release in the nucleus accumbens [65,71,700,701,760]. Paradoxically, while C57BL/6 mice are also more vulnerable to the rewarding effects of cocaine, they appear less sensitive to cocaine-induced locomotion [483,665]. Robust differences between the two strains have also been reported for behavioural sensitisation to repeatedly administered psychostimulants, although the magnitude and direction thereof appears to be highly dependent on the design of the sensitisation paradigm [20,65,483,559].

Interestingly, the strain differences in dopaminergic transmission and sensitivity to the rewarding properties of psychostimulants are not stable, but can change under the influence of environmental challenges, pointing towards a role for the neuroendocrine stress system in psychostimulant vulnerability [70,71,533,704]. Indeed, a wealth of data suggests that stress modulates behavioural and neurochemical responses to psychostimulants and other addictive drugs [241,358,514,515,618].

Stressful stimuli, either physical or mental, induce concomitant activation of the hypothalamic-pituitary-adrenal axis (HPA-axis) and the sympathetic nervous system resulting in release of glucocorticoid hormones and epinephrine from the adrenal glands [146]. Glucocorticoids in particular, have been shown to modulate transmission in the mesocorticolimbic dopamine system and to facilitate behavioural responses to psychostimulants such as locomotor activity, behavioural sensitisation,

self-administration and relapse (reviewed in: [421]). Furthermore, corticosterone in the range of stress-induced levels has reinforcing potential and stress can, like drugs of abuse, increase strength of excitatory synapses on midbrain dopaminergic neurons [512,582]. Strong evidence indicates that the glucocorticoid-dopamine interactions are dependent on activation of the glucocorticoid receptor, that is widely distributed throughout the brain and is expressed by the majority of the midbrain dopaminergic neurons [144,153,168,279,308,579,582].

Taken together, these data suggest that variations in HPA-axis responsiveness to stress may contribute to individual differences in psychostimulant vulnerability, as was elegantly addressed by Piazza *et al.* [516]. In this respect, laboratory mouse or rat strains with differential stress responsivity provide a valuable tool to study the interaction between the neuroendocrine stress system and the mesocorticolimbic dopamine circuit. With respect to the C57BL/6 and DBA/2 inbred strains, few studies have addressed differences in HPA-axis activation and findings are contradictory. In one study, C57BL/6 mice displayed higher peak corticosterone levels in response to novelty which is in line with our findings (S. Dalm, personal communication), but contradictory to two reports using other stressors and experimental designs [66,321,622]. In addition, there may be differences between these strains in psychostimulant-induced HPA-axis activation, but this has to our knowledge not been reported yet. Differences in basal, stress- or psychostimulant-induced glucocorticoid release may however play a prominent role in the observed strain differences in psychostimulant sensitivity.

The present study was designed to test the hypothesis that adrenal stress hormones contribute to strain differences in cocaine sensitivity. The C57BL/6 and DBA/2 mouse strains were used as model for genetic differences in dopamine and HPA-axis function. We have measured behavioural sensitisation to the locomotor stimulant effect of cocaine and, in parallel, corticosterone responses to single and repeated cocaine exposure. In order to show involvement of the adrenal, we have tested whether strain differences persist when the adrenal is surgically removed (adrenalectomy: 'ADX') prior to the onset of drug treatment.

METHODS

Animals

Male C57BL/6 Rj (C57BL/6) and DBA/2 Rj (DBA/2) mice were obtained from Janvier (Le Genest Saint Isle, France) and received in the animal facility at the age of 8

weeks. Mice were housed in groups of four of the same strain 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).

Experimental design

The study consisted of 8 experimental groups. Per mouse strain (C57BL/6 and DBA/2) animals were either SHAM operated (SHAM) or adrenalectomised (ADX). Each surgical group was subdivided into a cocaine (COC) and a saline (SAL) group, indicating the treatment given during the treatment period of the sensitisation paradigm. Each experimental group consisted of 9-12 animals.

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), N_2O (0.8 l/min) and O_2 (0.4 l/min). During surgery mice were placed on a heat pad (37ºC). The skin on the back was shaved and disinfected and an incision of approximately 1 cm was made above and parallel to the spinal cord. Through a small opening in the muscle layer left and right of the spinal cord the adrenals were removed from the surrounding fat tissue. The skin was closed using a simple running suture. 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 and strain. 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, stored in aliquots at -20˚C and defrosted on the day of administration. Cocaine (room temperature) was administered intraperitoneally

(i.p.) in a volume of 200µl/25 grams bodyweight and a dosage of 7.5 or 15.0 mg/kg. Control groups received an equal volume of saline. 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 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 period (day 14), all animals received a saline challenge and on day 15, all animals received a challenge of 7.5 mg/kg cocaine. All injections were given 2 to 5 hours after lights on.

Measurement of locomotor activity

Behavioural tests were performed on days 1, 9, 14 and 15 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. The cage was 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 over the entire 27 minute

treatment period and per 3 minute time blocks (cm). Locomotion was defined as movement with a minimal distance of 2 cm.

Corticosterone and adrenocorticotrophic hormone (ACTH)

Blood samples were taken from the tail vein by a small incision with a razorblade 30 minutes after injection at the test days 1, 9, 14 and 15. Blood was collected in small EDTA coated tubes (Microvette DB 200 K3E, Sarstedt, Nümbrecht, Germany). Mice were euthanised in the morning of day 16, 24 hours after the cocaine challenge, and trunk blood was collected following decapitation in large EDTA coated tubes (Tube 10 ml, 95x16.8 mm, K3E, Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 13000 rpm for 20 minutes at 4ºC and subsequently stored at -20ºC. Corticosterone and ACTH concentrations were determined by in-duplo measurement using radio-immuno-assay (RIA) kits from MP Biomedicals according to the protocol provided by the manufacturer (Corticosterone double antibody ^{125}I RIA kit and ACTH double antibody ^{125}I 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 in both strains, and only animals with successful ADX were included in the study.

Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). Locomotor activity, corticosterone and bodyweight data were subjected to repeated measures ANOVA with two between subject factors (surgery and treatment) and one within subject factor (test day). Subsequent analyses were performed per test day: two factor ANOVA for surgery and treatment. Locomotion represented per 3 minute time blocks was subjected to repeated measures ANOVA with two between subject factors (surgery and treatment) and one within subject factor (time block). ACTH data were analysed by two factor ANOVA for surgery and treatment. Correlations between locomotor activity data and corresponding corticosterone concentrations were analysed using Pearson's test (two-tailed). When statistical significance was revealed, post hoc tests were performed (Tukey HSD, or for within-subject comparison paired t-test). Differences were considered statistically significant when $p<0.05$.

RESULTS

Locomotor activity

The effects of ADX on locomotion of C57BL/6 and DBA/2 mice were studied during the different phases of the sensitisation paradigm. Figures 1A (C57BL/6) and 1B (DBA/2) depict total distance moved in the four behavioural tests (days 1, 9, 14 and 15) for the treatment groups SHAM/SAL, SHAM/COC, ADX/SAL and ADX/COC.

C57BL/6

The effects of ADX on cocaine-induced locomotion and sensitisation of C57BL/6 mice are shown in figure 1A. Surgery did not influence the response of C57BL/6 mice in any treatment group or at any time point ($F[surgery]_{1,35}=0.374$, $p=0.545$). Locomotion was significantly affected by treatment ($F_{1,35}=58.745$, $p<0.001$), day ($F_{3,105}=20.466$, $p<0.001$) and the interaction between both ($F_{3,105}=17.921$, $p<0.001$).

On days 1 and 9 of the treatment period, locomotion was increased three-fold by cocaine (15.0 mg/kg) in comparison with saline, irrespective of surgery (day 1: $F[treatment]_{1,39}=40.438$, $p<0.001$, $F[surgery]_{1,39}=0.508$, $p=0.418$, day 9: $F[treatment]_{1,40}=101.574$, $p<0.001$, $F[surgery]_{1,40}=0.569$, $p=0.455$, post hoc: $p\leq0.001$ compared to saline-treated for both surgical groups on both days). Remarkably, cocaine responses were not enhanced on the last compared to the first day of the treatment period. Similarly, saline responses on days 1 and 9 were comparable.

On day 14, *all* animals received a *saline* challenge in the test environment. Responses were higher in the cocaine-treated groups irrespective of surgery ($F[treatment]_{1,39}=9.290$, $p<0.01$, $F[surgery]_{1,39}=0.001$, $p=0.974$), although statistical significance was not reached when comparing individual groups. The treatment effect was however confirmed when locomotion was plotted per 3 minute time blocks ($F[treatment]_{1,36}=9.312$, $p<0.01$, $F[surgery]_{1,36}=0.001$, $p=0.975$, data not shown). These data indicate that cocaine treatment induced a distinct, yet small, conditioned hyperresponsiveness to the experimental conditions in both surgical groups.

On day 15, *all* animals received a challenge dose of 7.5 mg/kg *cocaine*. Responses are depicted in figure 1A (total distance moved) and figure 2A (distance moved per 3 minute time blocks). Subsequent statistics refer to total distance moved in figure 1A. Cocaine-treated mice displayed augmented locomotion in response to the cocaine challenge when compared to saline-treated mice, irrespective of surgery ($F[treatment]_{1,39}=18.773$, $p<0.001$, $F[surgery]_{1,39}=0.256$, $p=0.616$, post hoc:

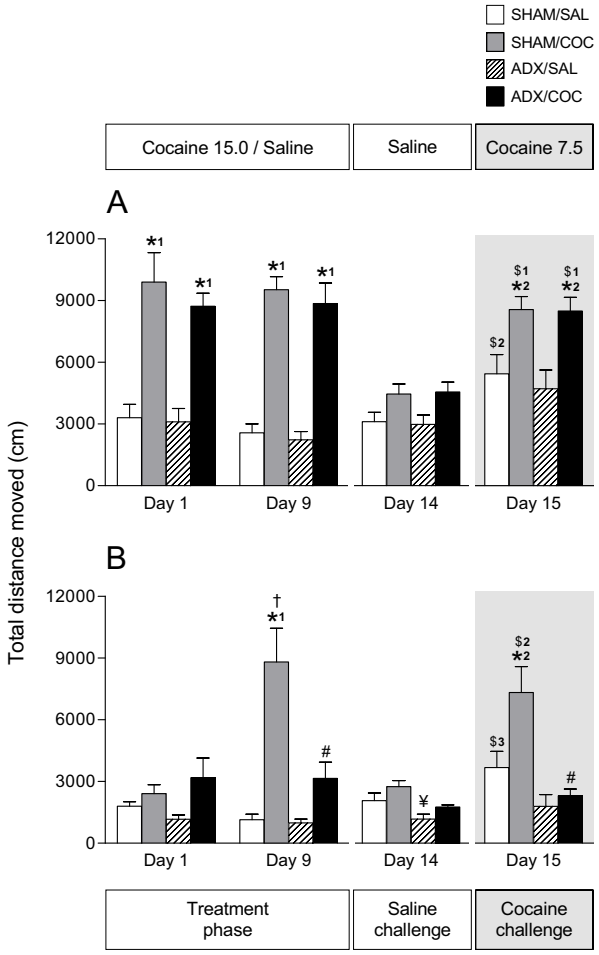

Figure 1: *Initiation and expression of sensitisation.*
Locomotion of C57BL/6 (A) and DBA/2 (B) mice in response to treatment on days 1, 9, 14 and 15 of the sensitisation paradigm. Adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal interval, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as mean total distance moved over the entire 27 minute treatment period (cm) ± SEM, n= 9-12 animals/group. *1 $p<0.001$, *2 $p<0.05$ vs. SAL (Tukey HSD), # $p<0.01$ vs. SHAM (Tukey HSD), ¥ $p<0.01$ vs. SHAM/COC (Tukey HSD), † $p<0.01$ vs. day 1 (paired t-test), $1 $p<0.001$, $2 $p<0.01$, $3 $p<0.05$ vs. saline challenge on day 14 (paired t-test).

$p<0.05$ for both SHAM and ADX). In addition, the cocaine-treated groups displayed twofold higher locomotion in response to the cocaine challenge when compared to the saline challenge (day 14), indicating that conditioned responsiveness cannot

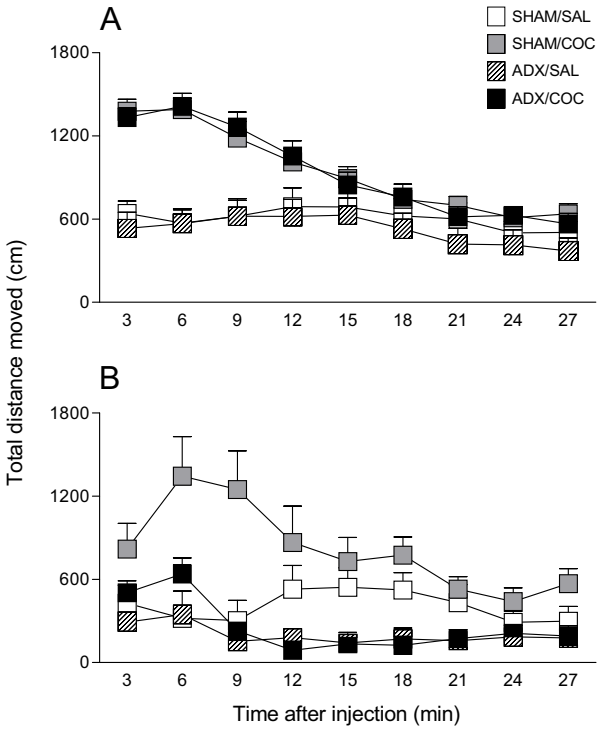

Figure 2: *Expression of sensitisation.*
Locomotion of C57BL/6 (A) and DBA/2 (B) mice in response to the 7.5 mg/kg cocaine challenge on day 15. Previously, adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal interval and a saline challenge (day 14). Data are represented as mean distance moved per 3 minute time blocks (cm) ± SEM, n= 9-12 animals/group. C57BL/6 (A): $F[surgery]_{1,36}=0.258$, $p=0.615$, $F[treatment]_{1,36}=18.757$, $p<0.001$, $F[time\ block]_{8,288}=54.845$, $p<0.001$, $F[time\ block \times treatment]_{8,288}=30.951$, $p<0.001$. DBA/2 (B): $F[surgery]_{1,38}=15.054$, $p<0.001$, $F[treatment]_{1,38}=5.449$, $p<0.05$, $F[time\ block]_{8,304}=5.098$, $p<0.001$, $F[time\ block \times surgery]_{8,304}=2.223$, $p<0.05$, $F[time\ block \times treatment]_{8,304}=4.220$, $p<0.001$.

have accounted for the full magnitude of the sensitised response ($p<0.001$, paired t-test for both surgical groups). The dose of 7.5 mg/kg was sufficient to enhance locomotion of drug-naïve SHAM mice above their saline response on day 14 and a similar effect was observed for ADX mice, although this just failed to reach statistical significance (SHAM/SAL: $p<0.01$, ADX/SAL: $p=0.06$, paired t-test).

Figure 3A depicts individual cocaine responses of SHAM operated C57BL/6 mice on days 1, 9 and 15. While responses to the first drug exposure were characterised by a considerable degree of inter-individual variability, variation became much less

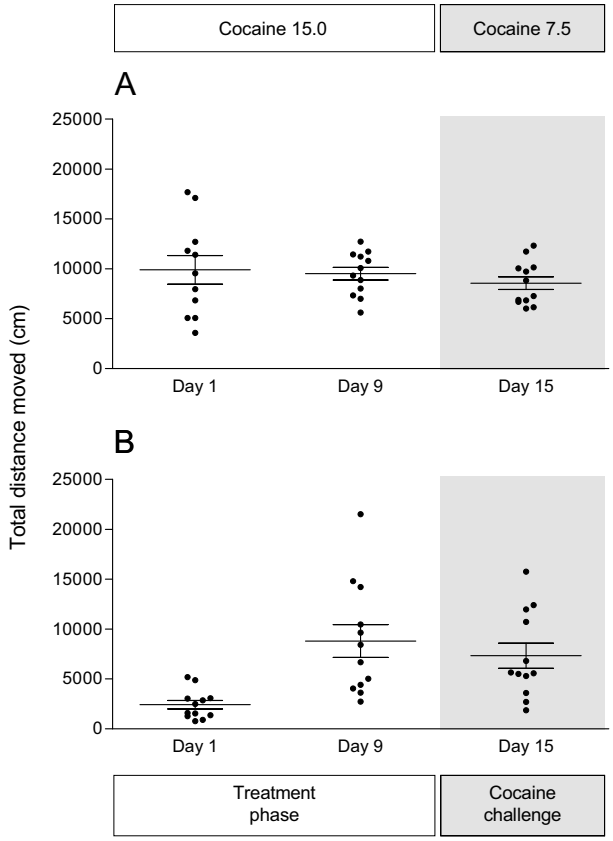

Figure 3: *Cocaine responses of individual animals.*
Locomotion of SHAM operated C57BL/6 (A) and DBA/2 (B) mice in response to cocaine on days 1, 9 (15.0 mg/kg) and 15 (7.5 mg/kg) of the sensitisation paradigm. Data are represented as mean total distance moved over the entire 27 minute treatment period (cm) ± SEM, n= 11-12. The black dots represent the data points for the individual animals.

during the course of the sensitisation paradigm and standard errors were very small (days 9 and 15). The behavioural responses or the degree of sensitisation thereof were not correlated with corticosterone concentrations on any day (Pearson's correlation coefficients: day 1: r=-0.059, p=0.864, day 9: r=0.023, p=0.942, day 15: r=0.496, p=0.101, day 9/day 1: r=-0.003, p=0.994, day 15/day 1: r=-0.241, p=0.476).

In summary, these results indicate that while C57BL/6 mice responded strongly to the first administration of cocaine (15.0 mg/kg), this locomotor response was not further enhanced by 8 drug administrations in this test setting. Despite the lack of increased responsiveness during the treatment phase, sensitisation was revealed when animals were challenged with 7.5 mg/kg cocaine following a 5 day withdrawal period. In addition, a small conditioned hyperactivity was observed in the

cocaine-treated C57BL/6 mice when challenged with saline. Remarkably, ADX did not affect any of the behavioural responses of the C57BL/6 mice measured in this study.

DBA/2

The effects of ADX on cocaine-induced locomotion and sensitisation of DBA/2 mice are shown in figure 1B. In this mouse strain, not only treatment ($F_{1,38}$=16.562, p<0.001) and test day ($F_{3,114}$=9.369, p<0.001) but also surgery ($F_{1,38}$=9.979, p<0.01) significantly affected locomotion. In addition, a surgery x treatment x day interaction was found ($F_{3,114}$=7.051, p<0.001).

Cocaine (15.0 mg/kg) increased locomotion of DBA/2 mice when compared to saline on both days 1 and 9 of the treatment period (day 1: $F[treatment]_{1,44}$=5.178, p<0.05, day 9: $F[treatment]_{1,41}$=22.684, p<0.001). On day 1, drug responses were very small, there were no surgery effects and post hoc analysis did not reveal significant differences between the treatment groups ($F[surgery]_{1,44}$=0.012, p=0.915). On day 9 however, SHAM animals were strongly activated by cocaine and locomotor responses were 8-fold higher compared to those of saline controls (p<0.001) and threefold enhanced compared to drug responses on day 1 (p<0.01, paired t-test). In ADX mice, on the contrary, cocaine did not increase activity above saline responses on day 9 (p=0.494) and cocaine responsiveness was not augmented when compared to day 1 (p=0.701, paired t-test). Furthermore, cocaine-induced locomotion on day 9 was significantly lower in ADX compared to SHAM mice ($F[surgery]_{1,41}$=7.910, p<0.01, $F[surgery \times treatment]_{1,41}$=7.072, p<0.05, post hoc: p<0.01).

On day 14, *all* animals received a *saline* challenge in the test environment. Behavioural responses were significantly affected by treatment and surgery ($F[treatment]_{1,41}$=4.988, p<0.05, $F[surgery]_{1,41}$=11.092, p<0.01), but both effects were mainly attributable to the less relevant group comparison between the SHAM/COC and ADX/SAL groups (p<0.01). No other significant differences were found between the treatment groups by means of post hoc analysis.

On day 15, following a 5 day withdrawal period, *all* animals were challenged with 7.5 mg/kg *cocaine*. Responses are depicted in figure 1B (total distance moved) and figure 2B (distance moved per 3 minute time blocks). Subsequent statistics refer to total distance moved in figure 2. In SHAM (p<0.05), but not in ADX animals (p=0.981), cocaine treatment resulted in augmented locomotor responsiveness to the cocaine challenge when compared to saline treatment ($F[treatment]_{1,41}$=5.459, p<0.05, $F[surgery]_{1,41}$=15.040, p<0.001, $F[surgery \times treatment]_{1,41}$=3.144, p=0.084). In addition, the locomotor responses of cocaine-treated ADX mice were significantly lower compared to those of cocaine-treated SHAM mice (p<0.01). Interestingly, the cocaine responses of the drug-naïve SHAM mice exceeded their saline responses

on day 14 ($p<0.05$, paired t-test) while this was not the case for the drug-naïve ADX mice ($p=0.385$, paired t-test). These data suggest that ADX may also reduce sensitivity of drug-naïve mice to the activating effects of low doses of cocaine, although statistical significance was not reached when comparing cocaine responses of the SHAM/SAL and ADX/SAL groups by post hoc analysis.

The DBA/2 strain was characterised by a relatively high degree of individual variability in cocaine responses after repeated drug exposure (days 9 and 15), reflecting the existence of considerable inter-individual differences in behavioural sensitisation (figure 3B). The variability within the DBA/2 strain was confirmed in subsequent experiments (data not shown). The behavioural responses or the degree of sensitisation thereof in the SHAM/COC group were not correlated with corticosterone concentrations on any day (Pearson's correlation coefficients: day 1: $r=0.024$, $p=0.939$, day 9: $r=-0.121$, $p=0.695$, day 15: $r=0.230$, $p=0.450$, day 9/day 1: $r=-0.097$, $p=0.764$, day 15/day 1: $r=0.318$, $p=0.314$).

In summary, these results indicate that while DBA/2 mice appeared relatively insensitive to the first administration of cocaine (15.0 mg/kg), sensitisation to the locomotor stimulant effects did develop in SHAM mice during the course of 9 drug treatments. Furthermore, sensitisation was expressed in SHAM animals in response to a challenge of 7.5 mg/kg cocaine after a 5 day withdrawal period. However, ADX abolished the ability of DBA/2 mice to develop and express sensitisation to the locomotor stimulant effects of cocaine.

Corticosterone

Plasma corticosterone concentrations were measured 30 minutes after treatment in the four behavioural tests on days 1, 9, 14 and 15 (figures 4A: C57BL/6 and 4B: DBA/2) and following decapitation in the morning of day 16 (figures 5A: C57BL/6 and 5B: DBA/2, left panels).

C57BL/6

The effects of ADX and drug treatment on plasma corticosterone concentrations of C57BL/6 mice are shown in figure 4A. In this strain, main effects were found for surgery ($F_{1,34}=224.014$, $p<0.001$), day ($F_{4,136}=63.347$, $p<0.001$) and the interaction between surgery, treatment and day ($F_{4,136}=2.438$, $p=0.05$). On all test days, corticosterone concentrations were significantly lower in the ADX groups (day 1: $F[\text{surgery}]_{1,40}=121.402$, $p<0.001$, day 9: $F[\text{surgery}]_{1,40}=126.309$, $p<0.001$, day 14: $F[\text{surgery}]_{1,38}=220.125$, $p<0.001$, day 15: $F[\text{surgery}]_{1,39}=115.274$, $p<0.001$, post hoc: $p<0.001$ compared to SHAM groups on all days). Endocrine responses of the SHAM animals are described in the following paragraphs.

On day 1, cocaine attenuated corticosterone secretion observed in response to saline treatment ($F[treatment]_{1,40}=4.890$, $p<0.05$, post hoc: $p<0.05$). A similar trend

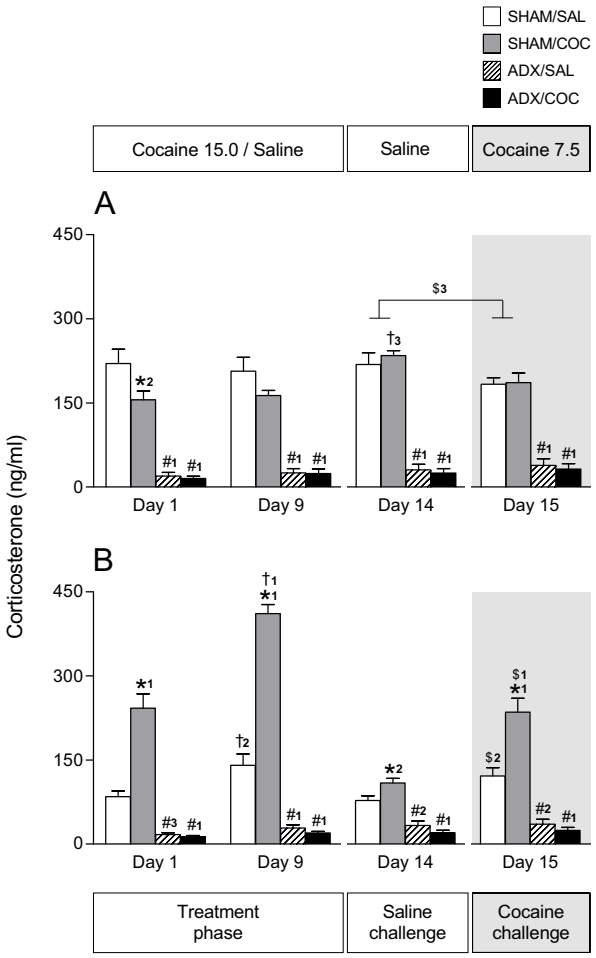

Figure 4: *Corticosterone concentrations.*
Plasma corticosterone concentrations of C57BL/6 (A) and DBA/2 (B) mice 30 minutes after treatment on days 1, 9, 14 and 15 of the sensitisation paradigm. Adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal period, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as mean plasma corticosterone concentration (ng/ml) ± SEM, n= 9-12 animals/group. *1 $p<0.001$, *2 $p<0.05$ vs. SAL (Tukey HSD), #1 $p<0.001$, #2 $p<0.01$, #3 $p<0.05$ vs. SHAM (Tukey HSD), †1 $p<0.001$, †2 $p<0.05$ vs. day 1 (paired t-test), †3 $p<0.001$ vs. days 1 and 9 (paired t-test), $1 $p<0.001$, $2 $p<0.05$ vs. saline challenge on day 14 (paired t-test), $3 $p<0.05$ vs. saline challenge on day 14, SHAM groups pooled (paired t-test).

was still observed on day 9 ($F[treatment]_{1,40}$=2.417, p=0.129, post hoc: p=0.136). Within the SAL and COC treatment groups, corticosterone responses were comparable on days 1 and 9, suggesting that neither sensitisation nor desensitisation occurred in response to either treatment.

When *all* animals were administered *saline* on day 14, the difference between the two SHAM groups disappeared due to a relative increase in the corticosterone response of the cocaine-treated mice. In this group, corticosterone concentrations were significantly enhanced compared to days 1 and 9 when animals were treated with cocaine (p<0.001, paired t-test for both days) and no longer different from the saline-treated group ($F[treatment]_{1,38}$=0.151, p=0.700). In addition, corticosterone responses of both SHAM groups were similar to those of saline-treated mice on days 1 and 9, indicating that responses to saline were stable throughout the paradigm.

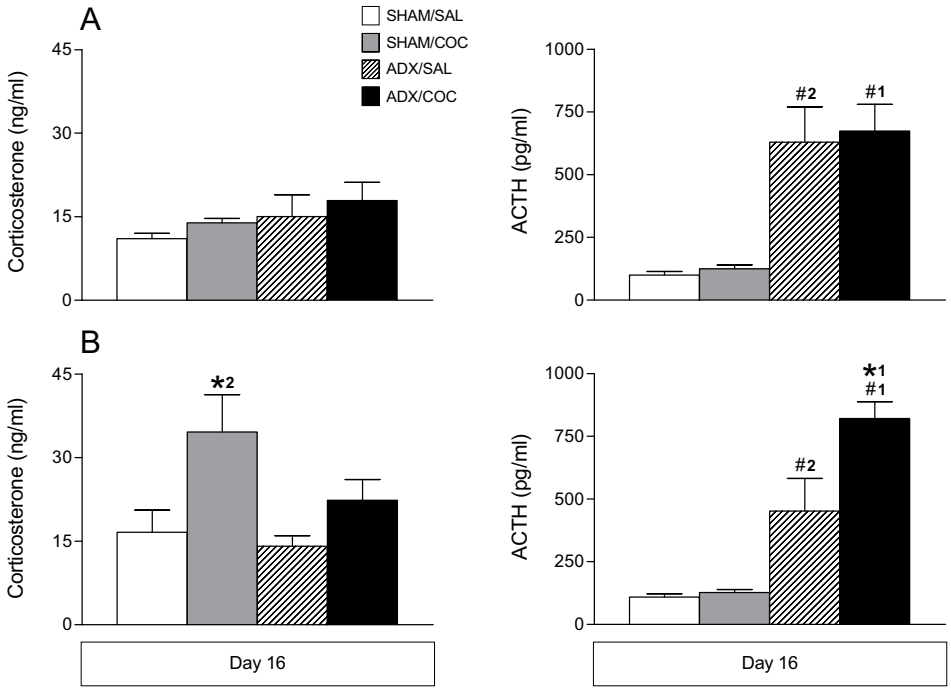

Figure 5: *Morning corticosterone and ACTH after sensitisation.*
Morning plasma corticosterone and ACTH concentrations of C57BL/6 (A) and DBA/2 (B) mice following decapitation on day 16. Previously, adrenalectomised (ADX) or SHAM operated animals received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal period, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as mean plasma corticosterone (ng/ml) and ACTH (pg/ml) concentration ± SEM, n= 9-12 animals/group. #1 p<0.001, #2 p<0.01 vs. SHAM (Tukey HSD), *1 p<0.01, *2 p<0.05 vs. SAL (Tukey HSD).

The *cocaine* (7.5 mg/kg) challenge on day 15 again attenuated the corticosterone response in the two SHAM groups when compared to the saline challenge on day 14 ($p<0.05$, paired t-test). The effect of 7.5 mg/kg was less pronounced than that of 15.0 mg/kg, indicating that cocaine may dose-dependently attenuate HPA-axis activation in this strain.

Morning plasma corticosterone at the time of decapitation on day 16 when animals were taken directly from the home cage (figure 5A, left panel), was not affected by surgery or previous treatment ($F[surgery]_{1,38}=2.654$, $p=0.112$, $F[treatment]_{1,38}=1.362$, $p=0.251$).

In summary, ADX was effective in clamping plasma corticosterone concentrations to stable low levels. Compared to saline administration, cocaine treatment (15.0 mg/kg and to a lesser extent 7.5 mg/kg) attenuated corticosterone responses in SHAM operated animals, an effect that was observed throughout the sensitisation paradigm. No sensitisation or desensitisation was detected in response to either saline or cocaine treatment in the C57BL/6 strain.

DBA/2

The effects of surgery and drug treatment on corticosterone responses of DBA/2 mice are shown in figure 4B. Main effects were found for surgery ($F_{1,34}=224.104$, $p<0.001$), treatment ($F_{1,34}=51.631$, $p<0.001$), day ($F_{4,136}=78.390$, $p<0.001$) and the interaction between these factors ($F_{4,136}=25.179$, $p<0.001$). Also in the DBA/2 strain, corticosterone was significantly attenuated in the ADX groups on each test day (day 1: $F[surgery]_{1,43}=100.558$, $p<0.001$, day 9: $F[surgery]_{1,40}=315.116$, $p<0.001$, day 14: $F[surgery]_{1,39}=76.399$, $p<0.001$ and day 15: $F[surgery]_{1,40}=75.619$, $p<0.001$, post hoc comparison with SHAM groups: at least $p<0.05$ on all test days). The endocrine responses of the SHAM animals are described in the following paragraphs.

In contrast to C57BL/6 mice, DBA/2 mice responded to cocaine treatment (15.0 mg/kg) with an elevation of corticosterone when compared to saline treatment on both days 1 and 9 of the treatment period (day 1: $F[treatment]_{1,43}=27.059$, $p<0.001$, $F[surgery \times treatment]_{1,43}=29.911$, $p<0.001$, day 9: $F[treatment]_{1,40}=85.531$, $p<0.001$, $F[surgery \times treatment]_{1,40}=97.050$, $p<0.001$, post hoc: $p<0.001$ compared to saline on both days). In addition, cocaine-induced corticosterone secretion was markedly enhanced on day 9 compared to day 1 ($p<0.001$, paired t-test), an effect that was also observed for the response to saline treatment, although less pronounced ($p<0.05$, paired t-test).

When *all* animals received *saline* on day 14, cocaine-treated SHAM mice responded with a greater elevation in corticosterone compared to saline-treated mice ($F[treatment]_{1,39}=1.464$, $p=0.234$, $F[surgery \times treatment]_{1,39}=8.390$, $p<0.01$, post hoc: $p<0.05$).

On day 15, when *all* animals were challenged with 7.5 mg/kg *cocaine*, the corticosterone response of the cocaine-treated SHAM group was augmented compared to that of the saline-treated group ($F[treatment]_{1,40}=9.026$, $p<0.01$, $F[surgery \times treatment]_{1,40}=13.452$, $p<0.01$, post hoc: $p<0.001$). Furthermore, the response to 7.5 mg/kg cocaine was not different from that on day 1 when animals were treated with 15.0 mg/kg. In both SHAM groups, the cocaine challenge enhanced corticosterone concentrations above those in response to the saline challenge on day 14 (at least $p<0.05$, paired t-test).

Interestingly, at the time of decapitation on day 16 when animals were taken directly from the home cage (figure 5B, left panel), morning corticosterone concentrations of the cocaine-treated SHAM mice were higher compared to those of saline-treated mice ($F[treatment]_{1,41}=7.452$, $p<0.05$, $F[surgery]_{1,41}=2.359$, $p=0.133$, post hoc: $p<0.05$).

In summary, ADX was effective in clamping plasma corticosterone concentrations to stable low levels. Cocaine increased corticosterone secretion in SHAM operated DBA/2 mice and repeated drug treatment resulted in sensitisation of HPA-axis activation that persisted until the cocaine challenge. Furthermore, cocaine-treated SHAM mice displayed higher corticosterone secretion in response to the saline challenge and elevated basal morning corticosterone on day 16.

ACTH

Basal plasma ACTH concentrations, measured following decapitation in the morning of day 16, are depicted in the right panels of figure 5A (C57BL/6) and 5B (DBA/2). In both strains, ACTH levels were significantly elevated in ADX mice (C57BL/6: $F[surgery]_{1,37}=40.779$, $p<0.001$, DBA/2: $F[surgery]_{1,39}=58.105$, $p<0.001$, post hoc: at least $p<0.01$ compared to SHAM for both strains). In the DBA/2 strain, the ACTH elevation was dependent on treatment, being higher in cocaine-treated ADX mice ($F[treatment]_{1,39}=8.150$, $p<0.01$, $F[surgery \times treatment]_{1,39}=6.645$, $p<0.05$, post hoc: $p<0.01$ compared to ADX/SAL), whereas this was not the case in the C57BL/6 strain ($F[treatment]_{1,37}=0.165$, $p=0.688$). In both strains, ACTH levels of SHAM operated mice were around 100 pg/ml, irrespective of treatment.

Body weight

Table 1 depicts bodyweights on the day prior to surgery and test days 1, 9 and 15 (7, 15 and 21 days after surgery respectively). Average pre-surgical bodyweight of each strain was set at 100%. In the C57BL/6 strain, bodyweight gradually increased and exceeded pre-surgical weight on all test days with no effects of

Table 1: *Bodyweight relative to pre-surgical weight (%).*

			Pre-surgical	Day 1	Day 9	Day 15
C57BL/6	**SHAM**	**SAL**	100.5 ± 0.7	104.3 ± 1.0 *1	107.6 ± 1.1 *1 #2	109.4 ± 1.2 *1 #2 †2
		COC	100.2 ± 1.2	104.6 ± 1.1 *1	107.0 ± 1.1 *1 #2	108.6 ± 1.3 *1 #1
	ADX	**SAL**	100.5 ± 1.7	103.8 ± 1.6 *3	108.9 ± 2.0 *1 #2	107.2 ± 1.8 *1 #3
		COC	98.6 ± 0.6	104.3 ± 1.3 *1	108.8 ± 1.5 *1 #2	106.5 ± 1.6 *2 #3 †2
DBA/2	**SHAM**	**SAL**	101.9 ± 1.5	98.7 ± 1.5 *2	102.2 ± 2.3 #2	102.6 ± 2.3 #3
		COC	99.2 ± 1.7	97.5 ± 1.9	98.8 ± 1.5	101.2 ± 1.7 #2 †1
	ADX	**SAL**	100.5 ± 1.4	97.2 ± 1.1	103.5 ± 2.0 #2	101.7 ± 2.0 #3
		COC	98.5 ± 1.5	95.5 ± 1.6 *3	96.2 ± 2.0	98.5 ± 2.3 †2

Relative bodyweights of C57BL/6 and DBA/2 mice, prior to surgery and on test days 1, 9 and 15 (7, 15 and 21 days after surgery respectively). For both strains, average pre-surgical bodyweight was set at 100%. Animals were adrenalectomised (ADX) or SHAM operated and received daily administrations of 15.0 mg/kg cocaine (COC) or saline (SAL) (days 1-9), followed by a 5 day withdrawal period, a saline challenge (day 14) and a 7.5 mg/kg cocaine challenge (day 15). Data are represented as percentage of mean pre-surgical bodyweight (%) ± SEM, n= 9-12 animals/group. *1 $p<0.001$, *2 $p<0.01$, *3 $p<0.05$ vs. pre-surgical (paired t-test), #1 $p<0.001$, #2 $p<0.01$, #3 $p<0.05$ vs. day 1 (paired t-test), †1 $p<0.01$, †2 $p<0.05$ vs. day 9 (paired t-test).

surgery or treatment ($F[day]_{3,108}=125.281$, $p<0.001$, $F[surgery]_{1,36}=0.150$, $p=0.701$, $F[treatment]_{1,36}=0.137$, $p=0.713$, post hoc: at least $p<0.05$ compared to pre-surgical on all test days, paired t-test). In the DBA/2 strain, bodyweight was reduced on day 1 compared to pre-surgical weight and recovery occurred faster in the saline-treated groups ($F[day]_{3,114}=12.099$, $p<0.001$, $F[day \times treatment]_{3,114}=3.252$, $p<0.05$). In contrast to the C57BL/6 strain, final bodyweight did not exceed pre-surgical weight. Also in this strain, there was no main effect of surgery or treatment on any day ($F[surgery]_{1,38}=0.648$, $p=0.426$, $F[treatment]_{1,38}=3.098$, $p=0.086$).

DISCUSSION

The present data show similarities, but also profound differences, between the C57BL/6 and DBA/2 strains in behavioural and endocrine sensitisation to cocaine. We have identified one strain, the DBA/2 strain, in which repeated cocaine exposure induces sensitisation of both drug-induced locomotion and -corticosterone secretion. Furthermore, only in this strain, behavioural sensitisation was prevented

by ADX, suggesting that adrenal hormones facilitate sensitisation to the locomotor stimulant effects of cocaine and do so in a genotype-dependent manner.

In both strains, cocaine-treated mice expressed behavioural sensitisation in response to a challenge dose of cocaine (7.5 mg/kg) after a 5 day withdrawal period. Occurrence of sensitisation in both strains was considered an important requirement, as the sensitisation paradigm was subsequently employed to investigate the contribution of adrenal stress hormones. Experimental parameters that may influence susceptibility of the two strains to psychostimulant sensitisation include i) repeated drug administration within or beyond the test context [65], ii) the duration of the withdrawal interval [20,559], and iii) habituation to the test setting [20].

It has been argued that C57BL/6 mice are more susceptible to context-dependent sensitisation, while DBA/2 mice are more likely to develop context-independent sensitisation [20,65,559]. The observed hyperactivity of cocaine-treated C57BL/6 mice following saline administration in the test setting supports the notion that the C57BL/6 strain is susceptible to the influence of contextual information. Therefore, a 'mixed' design was used in which animals received cocaine both in the test cage (days 1, 9 and 15) and in the home cage (days 2-8) on several occasions. This might allow the development of either type of sensitisation resulting in augmented cocaine responsiveness in both strains. In addition, a withdrawal interval was chosen comparable to that employed in studies demonstrating expression of amphetamine sensitisation in both strains [65,559]. Furthermore, animals were habituated to the test setting 2 hours prior to drug administration, in order to minimise the contribution of strain differences in the behavioural and endocrine response to novelty [66]. Finally, mice were single housed to avoid variable social influences that could play a role during drug exposure in the home cage.

Pronounced strain differences were observed for cocaine responsiveness in the treatment phase. In contrast to DBA/2 mice, C57BL/6 mice did not display increased drug-responsiveness on the last (day 9) compared to the first day. In view of the augmented response of cocaine-treated C57BL/6 mice to the cocaine challenge, this observation is not likely to reflect a complete lack of sensitisation. As discussed in the previous paragraph, a longer time interval may need to elapse before sensitisation becomes expressed in the C57BL/6 strain. It is generally accepted that expression of behavioural sensitisation intensifies with prolonged withdrawal and that associated neuroadaptations change over time [497,520]. In addition, it is conceivable that C57BL/6 mice reached a ceiling response already at the first drug administration, while behavioural sensitisation could only be unmasked by the lower challenge dose of 7.5 mg/kg cocaine. Indeed, an acute administration of cocaine (15.0 mg/kg) resulted in pronounced hyperactivity in the C57BL/6 strain

while being virtually ineffective in the DBA/2 strain. This finding is coherent with data obtained on amphetamine [65,700,760], but discrepant to two reports on cocaine [483,665]. The discrepancy may result from aforementioned differences in experimental methods.

Striking strain differences were also observed for HPA-axis responsiveness of SHAM operated animals throughout the sensitisation paradigm. In the DBA/2 strain, repeated cocaine treatment resulted in a persistent sensitisation of drug-induced corticosterone secretion, that was still evident in response to a cocaine challenge after a withdrawal period. By contrast, in the C57BL/6 strain, cocaine attenuated rather than enhanced corticosterone secretion in the context of a stress-induced response (e.g. induced by i.p. injection in the test setting) and there was no difference between the first and last drug exposure of the treatment phase.

The observed HPA-axis sensitisation in DBA/2 mice is in line with previous reports showing that repeated amphetamine treatment can induce hypersecretion of corticosterone to a subsequent drug challenge, even after as little as one exposure [29,694]. Furthermore, cocaine-treated DBA/2 mice displayed an augmented corticosterone response to a saline challenge, that may either reflect conditioned activation of the HPA-axis in response to drug-associated stimuli specifically [169], or enhanced sensitivity to challenging situations in general. In support of the latter, it has been shown repeatedly that the effects of psychostimulants and stress on behavioural and endocrine sensitisation are interchangeable (e.g. [13,29,493]). Furthermore, a small degree of sensitisation was also observed during saline treatment, indicating that the HPA-axis of DBA/2 mice can sensitise to repeated treatment procedures. It is conceivable that cocaine induced more pronounced and persistent endocrine sensitisation due to its higher potency in activating the HPA-axis. Interestingly, morning corticosterone concentrations of cocaine-treated mice were elevated one day after the drug challenge. This finding supports the notion of a deregulation of HPA-axis activity, although the contribution of either drug anticipation or withdrawal symptoms to this phenomenon cannot be excluded. Further studies are necessary to clarify whether and to what extent the observed HPA-axis sensitisation of DBA/2 mice is specific for cocaine or cocaine-associated stimuli.

In agreement with previous studies, our findings indicate that sensitisation takes place at the level of the pituitary or its regulatory areas, since elevated basal ACTH levels were revealed in cocaine- compared to saline-treated DBA/2 mice in which the negative glucocorticoid feedback was relieved by ADX [29,610]. In addition, the elevated morning corticosterone concentrations observed in SHAM operated DBA/2 mice on day 16 did not correspond with elevated ACTH levels. We interpret this observation as hypersensitivity of the adrenal to ACTH. From these data it cannot

be concluded at what time point during the sensitisation paradigm the apparent adrenal hypperresponsiveness develops and how it contributes to the enhanced corticosterone responsiveness.

The observation that, in C57BL/6 mice, cocaine attenuated rather than enhanced corticosterone responses in comparison with saline, contradicts many reports showing enhanced HPA-axis activation in response to psychostimulants, including the present results for the DBA/2 strain. In an additional study (data not shown), we have confirmed that, in C57BL/6 mice, cocaine attenuates corticosterone secretion in the context of a stress-induced response over the entire time span of the endocrine response. Strain differences in functioning or wiring of the HPA-axis itself and of the neurotransmitter systems that regulate the cocaine-induced corticotrophin releasing hormone and corticosterone release may underlie the opposite effects of cocaine on stress-induced corticosterone secretion in the two strains [52,66,285,321,622]. Alternatively, the difference in corticosterone responsiveness may reflect different strategies for coping with drug-induced arousal [454]. In addition, cocaine may alter either the perception of stressful events (e.g. test- and treatment procedures), or the kinetics of HPA-axis activation in response to such events, in a strain-dependent manner.

We have made the remarkable observation that ADX prevents behavioural sensitisation to cocaine in a genotype-dependent manner. ADX prevented development and expression of sensitisation to the locomotor stimulant effects of cocaine in the DBA/2 strain, while being ineffective in the C57BL/6 strain. Strain differences have also been reported for these two inbred strains regarding the impact of stress on dopaminergic transmission, dopamine receptor expression, amphetamine-induced conditioned place preference, -locomotion, and -sensitisation, and stereotyped behaviour [20,67,69-71,533,704]. With respect to psychostimulant-induced behaviour, the DBA/2 strain is consistently found to be stress-responsive, while C57BL/6 mice appear resistant to environmental manipulations [20,67,71]. In the present report, we show a similar strain-dependency for the effects of ADX. ADX did not influence behavioural responsiveness to cocaine in the C57BL/6 strain, in which cocaine attenuated corticosterone responses, while fully preventing behavioural sensitisation in the DBA/2 strain, that displayed cocaine-induced corticosterone secretion and sensitisation thereof.

These data suggest that adrenal glucocorticoids, and possibly sensitisation of their release, contribute to behavioural sensitisation of DBA/2 mice to cocaine. Many studies have shown that glucocorticoid hormones have a facilitatory role in behavioural responses to psychostimulant drugs, such as locomotor activity, self-administration and relapse (reviewed in: [421]). Furthermore, strong evidence exists

that activation of the glucocorticoid receptor is critically involved in the glucocorticoid effects on drug responses [168,308]. In mice, basal levels of corticosterone that are considered sufficient for occupation of the high-affinity mineralocorticoid receptor remain after ADX (the present study, [300]), also pointing towards a role for the relatively low-affinity glucocorticoid receptor in behavioural sensitisation of the DBA/2 strain to cocaine. There were however no strain differences in residual corticosterone following ADX, excluding this as explanation for the strain-dependent effects of ADX on behaviour.

The finding that locomotor sensitisation to cocaine depends on the integrity of adrenal function only in the DBA/2 strain, suggests that DBA/2 mice are more sensitive to the impact of adrenal stress hormones on cocaine sensitivity than C57BL/6 mice. In this respect, it is interesting to note that the DBA/2 strain was also characterised by a higher degree of individual variability in behavioural responses to repeatedly administered cocaine than the C57BL/6 strain, a phenomenon that has been reported previously [483]. As inbred strains are considered to be genetically homogenous, individual differences are assumed to arise from epigenetic changes induced by variations in life-experiences, either in the past or in the present, further strengthening the notion that the neuroendocrine stress system may play a regulatory role in drug sensitivity of DBA/2 mice. However, the behavioural cocaine responses or the degree of sensitisation thereof among the SHAM operated DBA/2 mice did not correlate with corticosterone levels at any day. Moreover, there was very little variation in corticosterone responsiveness and sensitisation. Therefore, behavioural sensitisation of DBA/2 mice to cocaine may depend on glucocorticoids, and possibly on sensitisation of their release, but these hormones do not account for the full extent of individual variability. Further research is therefore required to verify the involvement of corticosterone and to investigate whether other adrenal factors such as epinephrine also contribute to cocaine sensitivity of DBA/2 mice.

It is of great interest to unravel the mechanisms via which adrenal stress hormones facilitate cocaine responsiveness in a genotype-dependent manner. Compared to C57BL/6 mice, DBA/2 mice display lower amphetamine-induced dopamine release in the nucleus accumbens as a consequence of higher drug-induced dopamine release in the prefrontal cortex [700,701]. It would be interesting to investigate whether a similar mechanism controls the strain differences in cocaine sensitivity and whether adrenal hormones modulate the balance between drug-induced dopamine release in the prefrontal cortex and the nucleus accumbens. Interestingly, it has recently been shown in DBA/2 mice that food restriction can increase action potential-dependent dopamine release in the nucleus accumbens, the component of dopamine release that is controlled by the prefrontal cortex and most likely mediates

behavioural sensitisation [705]. Alternatively, the strain difference in dopamine D2 autoreceptor-postsynaptic receptor ratio between the cell body and terminal regions of the mesocorticolimbic dopamine system, being higher in DBA/2 mice, may have contributed to the differences in cocaine sensitivity. Moreover, it has been demonstrated that dopamine D1 and D2 receptor expression can be regulated by stressful experiences in a genotype-dependent manner [69].

The present finding, that adrenal stress hormones play an essential role in behavioural sensitisation to cocaine only in certain strains of animals, may provide an explanation for some of the discrepancies in literature regarding the contribution of adrenal hormones to psychostimulant sensitisation. Strong evidence for the role of adrenal glucocorticoids in behavioural sensitisation has been presented most notably by Piazza and co-workers (e.g. [168,510]), although conflicting data have also been reported [21,609]. In the light of the current data, these different outcomes can be accounted for by different genetic, and perhaps environmental, backgrounds. In addition, some of the conflicting data obtained with the C57BL/6 and DBA/2 strains might be explained by environmental and experimental factors that influence adrenal stress hormones.

In summary, the present results demonstrate that the C57BL/6 and DBA/2 inbred mouse strains not only differ profoundly in behavioural, but also in endocrine responsiveness to cocaine. In both strains, intermittent cocaine administration resulted in locomotor hyperresponsiveness to the cocaine challenge, while only the DBA/2 strain displayed endocrine sensitisation. Remarkably, ADX prevented behavioural sensitisation only in the DBA/2 strain, suggesting that in this strain, glucocorticoids facilitate behavioural sensitisation to cocaine. All together, these results suggest that adrenal stress hormones modulate behavioural sensitisation to cocaine in a genotype-dependent manner and that genotypic differences in cocaine sensitivity may arise not only from differences in reward related signalling but also from differential HPA-axis responsiveness.

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