Vulnerability to cocaine: role of stress hormones

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Strain differences in the effects of adrenalectomy on the midbrain dopamine system: implication for behavioural sensitisation to cocaine

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ABSTRACT

Adrenalectomy (ADX) abolishes behavioural sensitisation to cocaine in DBA/2, but not C57BL/6 inbred mice. The present study therefore tests the hypothesis that this ADX effect on behavioural sensitisation in the susceptible DBA/2 strain involves changes in midbrain dopamine systems that do not occur in the resistant C57BL/6 strain.

For that purpose, we have measured tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA and D1- and D2-like receptor binding in C57BL/6 and DBA/2 mice that were i) unoperated, ii) ADX or SHAM operated, or iii) ADX or SHAM operated and subjected to a cocaine sensitisation regimen (15.0 mg/kg cocaine on 9 consecutive days, followed by a 7.5 mg/kg challenge after a 5 day withdrawal). ADX prevented behavioural sensitisation to cocaine in the DBA/2, but not the C57BL/6 strain. Mice were sacrificed under basal conditions, in the latter case 24 hours after the cocaine challenge.

ADX did not affect markers of the dopamine system in drug naïve mice. By contrast, strain-dependent neuroadaptations were found in the midbrain dopamine system of mice subjected to the sensitisation regimen. In the DBA/2 strain, the sensitisation-resistant ADX mice were characterised by reduced D2 binding in the nucleus accumbens core and rostral caudate putamen (CP). Furthermore, ADX prevented the increase in TH and DAT mRNA expression in the substantia nigra (SN), and the decrease in D2 binding in the dorsomedial subdivision of the caudal CP associated with sensitisation in SHAM mice. In the C57BL/6 strain ADX only marginally affected dopaminergic adaptations.

These data suggest that adrenal hormones modulate behavioural sensitisation to cocaine in a genotype-dependent fashion possibly through adaptations in pre- and post-synaptic components of the midbrain dopamine system. During cocaine sensitisation, the DBA/2, but not the C57BL/6 strain, was susceptible to ADX in the dopamine system with respect to somatic TH and DAT and terminal D2 receptor expression.

INTRODUCTION

There are marked individual differences in behavioural responsiveness to psychostimulant drugs that depend on the interplay between heritable traits and environmental factors [191]. The midbrain dopamine system, which plays a critical role in the behavioural and reinforcing properties of psychostimulant drugs [202], provides an important substrate for gene x environment interactions underlying dopaminergic psychopathologies. Studies using inbred mouse strains have indicated that there is prominent genetic control over anatomy and function of the midbrain dopamine system and related behavioural output. Furthermore, environmental factors and most notably stress, facilitate the neurochemical, behavioural and reinforcing effects of psychostimulant drugs [241,510,635] and there is ample evidence that this depends on the interaction between the midbrain dopamine system and adrenal glucocorticoid hormones, that are secreted upon activation of the hypothalamic-pituitary-adrenal (HPA) axis (for reviews see: [421,515]).

Inbred mouse strains provide a valuable tool to study gene x environment interactions in the workings of the midbrain dopamine system. The C57BL/6 and DBA/2 mouse strains have been widely exploited as a model for genetic differences in dopaminergic transmission and associated behavioural output (reviewed in: [531]). In addition, the two strains exhibit differences in the behavioural and endocrine adaptation to stress [66,67,321,622]. With respect to behavioural responsiveness to psychostimulants, the DBA/2 strain is susceptible to environmental manipulations, whereas C57BL/6 mice appear resistant to stress [20,67,71]. The difference in susceptibility to environmental influences may in part be explained by strain-dependent adaptations of the midbrain dopamine system to stress, involving genotype-specific changes in dopamine D1 and D2 receptor density and -function, and in dopamine release and -metabolism in distinct brain regions [69,70,704].

In a previous study, we have investigated in more detail the contribution of adrenal stress hormones to cocaine sensitivity of the C57BL/6 and DBA/2 strains [145]. C57BL/6 mice were found to be more responsive to the locomotor stimulant effects of an initial cocaine exposure, whereas surgical removal of the adrenals (adrenalectomy: 'ADX') did not influence the acute drug response in either strain. By contrast, both strains exhibited psychomotor sensitisation after repeated drug exposure, while this was prevented by ADX in the DBA/2, but not the C57BL/6 strain. These findings indicate that adrenal stress hormones play an essential role in behavioural sensitisation to cocaine, but only in the DBA/2 strain.

The present study was designed to investigate whether a neural correlate for the strain differences in behavioural responsiveness to cocaine and the contribution of adrenal stress hormones can be found in the midbrain dopamine system. The hypothesis is tested that DBA/2 and C57BL/6 strains are susceptible and resistant, respectively, to the effects of ADX on midbrain dopamine transmission. For this purpose, we have measured dopaminergic markers in the mesocorticolimbic and nigrostriatal dopamine systems of C57BL/6 and DBA/2 mice that were i) unoperated, ii) ADX or SHAM operated, or iii) ADX or SHAM operated and subjected to a cocaine sensitisation regimen. We have determined tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression and D1- and D2-like receptor binding using the radiolabelled ligands SCH23390 and iodosulpride respectively. Although these ligands cannot dissociate between the D1 (D1 and D5) and D2 (D2, D3 and D4) receptor subtypes, they allow for discrimination between the two dopamine receptor families.

METHODS

General 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. Experiments started two weeks after arrival in the animal facility. Prior to this, animals were briefly handled 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

In experiments 2 and 3, animals were adrenalectomised (ADX) or SHAM operated. Mice were individually housed 1 day prior to surgery. The cages were transported to the operating room on the morning of the surgery where animals 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). Adrenalectomy was performed via the dorsal route as described previously [145]. SHAM animals were

treated similarly with the exception of the actual removal of the adrenals. ADX effectively clamped plasma corticosterone to basal concentrations and only animals with successful ADX were included in the study.

Tissue preparation
In all experiments, animals were sacrificed in the morning (3-5 hours after lights on) under basal conditions. Trunk blood was collected for endocrine measurements and brains were rapidly dissected, snap frozen in isopentane (cooled in ethanol placed on pulverised dry ice) and stored at -80 ºC until further use.

Sections of 16 µm were cut on a cryostat (Leica CM3050). Based on the brain atlas of Franklin and Paxinos [220] (figure 1), sections were taken at the level of the nucleus accumbens (NAc: core and shell subregions, bregma +1.44), rostral caudate putamen (rCP, bregma +1.44), caudal CP (cCP: lateral (Lat), dorsomedial (DM) and ventromedial (VM) subsections, bregma +0.14), ventral tegmental area (VTA,

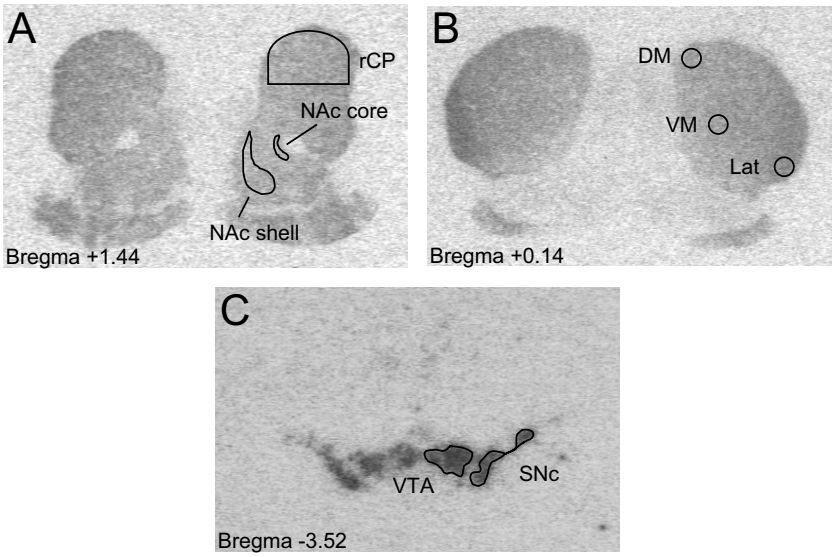

Figure 1: *Coronal sections of the mouse brain, indicating the areas sampled for optical density measurement.*
Autoradiographs at the level of A) the nucleus accumbens (NAc) and rostral caudate putamen (rCP), and B) caudal caudate putamen after binding of ^{125}I-iodosulpride, and C) the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) after hybridisation with the TH mRNA specific oligonucleotide probe. The line-shapes indicate the areas sampled for optical density measurement. DM: dorsomedial, VM: ventromedial, Lat: lateral.

bregma -3.52) and substantia nigra pars compacta (SNc, bregma -3.52). Sections were thaw-mounted on 0.001% poly-L-lysine (Sigma-Aldrich) coated slides and stored at -80 ºC until further use.

In situ hybridisation

Tyrosine hydroxylase (TH) and dopamine transporter (DAT)

In situ hybridisation was performed to investigate the mRNA expression of TH and DAT in the cell body regions of the midbrain dopamine systems. The oligonucleotides (table 1) were 3′ end-labelled with α [^{33}P]deoxyadenosine triphosphate (Perkin Elmer, Groningen, The Netherlands, 3000 Ci/mmol, 10 mCi/ml) using terminal deoxynucleotidyl transferase (GE Healthcare Europe Gmbh, Diegem, Belgium) according to the manufacturer's protocol.

Table 1: *The TH and DAT mRNA-specific oligonucleotides.*

TH	**Match**	5′-ATTTAGCTAATGGCACTCAGTGCTTGGGTCAGGGTGTGCAGCTCA-3′
	Mismatch	5′-ATTTAG**A**TAATGG**A**ACTCAG**G**GCTTGG**T**TCAGGGT**T**TGCAG**A**TCA-3′
DAT	**Match**	5′-CTTGTCCTCCGTGGCTCAGAACAGACCCTGCGTGTGTGTAATA-3′
	Mismatch	5′-CTTGT**A**TCCGTG**T**CTCAGA**C**CAGACC**A**TGCGTG**G**GTGTAA**G**A-3′

Sequences of the 45 and 43-mer oligonucleotide probes complementary to respectively tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA. To control for specificity, oligonucleotides were used that were identical, except for 6 mismatches (transversions, underlined) evenly spaced by 5-7 nucleotides.

For prehybridisation purposes, sections were fixed for 30 min. in 4% para-formaldehyde in PBS (pH 7.4), washed twice in PBS, acetylated in 0.1 M triethanolamine (pH 8.0) with 0.25% acetic anhydride for 10 min., rinsed for 10 min. in 2 x SSC (150 mM sodium chloride, 15 mM sodium citrate), dehydrated in an ethanol series, delipidated in chloroform for 1 min., air dried and stored at room temperature. To each slide, hybridisation mix (100 µl, containing 1 x 10^6 dpm. of labelled oligonucleotide) was applied. The hybridisation mix consisted of 50% formamide, 10% dextran sulfate, 4 x SSC, 25 mM sodium phosphate (pH 7.0), 1 mM Na pyrophosphate, 20 mM DTT, 5 x Denhardt's, 100 µg/ml poly-A and 100 µg/ml sheared herring sperm DNA. Sections were coverslipped and hybridised overnight in a moist chamber at 42 °C. The next morning, after removal of the coverslips, sections were rinsed in 1 x SSC at room temperature, washed twice for 30 min. in 1 x SSC at 50 °C, washed for 5 min. in 1 x SSC at room temperature, dehydrated in an ethanol series, air dried and

apposed to Kodak Biomax MR film (Kodak, Rochester, NY). After development of the films, sections were counter-stained with 0.5% cresyl violet.

Radioligand binding

D1 receptors

D1 receptor binding sites were analysed by in vitro autoradiography using the ^{3}H-labelled D1-like receptor antagonist SCH23390. Sections were pre-incubated in a 50 mM Tris buffer (containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.5) for 60 min. at room temperature. Subsequently, sections were incubated with 1 ml of the Tris buffer containing 2 nM ^{3}H-SCH23390 (GE Healthcare Europe Gmbh, Diegem, Belgium, 66 Ci/mmol, 1 mCi/ml) for 60 min. at room temperature. As SCH23390 has been reported to bind to serotonergic sites, 1 µM mianserin hydrochloride (Sigma-Aldrich Chemie B.V., Zwijndrecht, The Netherlands) was added to the incubation buffer. Non-specific binding was determined in the presence of 40 µM R(+)-SCH23390 hydrochloride (Sigma-Aldrich). After incubation, sections were drained, dipped in distilled water (4 ºC), washed two times in the Tris buffer (4 ºC) for 10 min., dipped in distilled water (4 ºC) and air-dried. Sections were apposed to a Kodak Biomax MS film (Kodak, Rochester, NY).

D2 receptors

Binding sites of the D2 receptor family were analysed by in vitro autoradiography using the ^{125}I-labelled D2-like antagonist iodosulpride. Sections were pre-incubated in a 50 mM Tris buffer (containing 5.7 mM ascorbic acid, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.0) for 60 min. at room temperature. Subsequently, sections were incubated with 0.4 ml of the Tris buffer containing 0.1 nM ^{125}I-iodosulpride (GE Healthcare Europe Gmbh, Diegem, Belgium, 2000 Ci/mmol, 100 µCi/ml) for 30 min. at room temperature. Non-specific binding was determined in the presence of 2 µM haloperidol (Sigma-Aldrich). After incubation, sections were drained, dipped in distilled water (4 ºC), washed two times in the Tris buffer (4 ºC) for 3 min., dipped in distilled water (4 ºC) and air-dried. Sections were apposed to a Kodak Biomax MS film (Kodak, Rochester, NY).

Analysis of mRNA expression and radioligand binding

Autoradiographs were digitised and relative expression levels or binding densities (grey values on film) were determined by computer-assisted optical densitometry using Analysis (analySIS 3.1, Soft Imaging System GmbH) and Scion Image software (a PC-version of the Macintosh program NIH Image: http://rsb.info.nih.gov/nih-image), available from Scion Corporation: http://www.scioncorp.com/). Standard

curves of ^{14}C (*in situ* hybridisation and D2 binding) and ^{3}H (D1 binding) were included to ensure that grey values were within the linear range between 0 and 255. Average optical density per region (in arbitrary units) was corrected for optical density in an adjacent region where no mRNA expression / receptor binding was observed (deep mesencephalic nucleus for VTA and SNc, corpus callosum for NAc, rCP and cCP).

Experimental design

Experiment 1: *The basal dopamine system*
Drug naïve C57BL/6 and DBA/2 mice were sacrificed under basal conditions two weeks after arrival in the animal facilities.

Experiment 2: *The effects of ADX on the dopamine system of drug naïve mice*
Mice of both strains were adrenalectomised (ADX) or SHAM operated and sacrificed after a post-surgery interval of 3 weeks, which is an identical protocol as used in experiment 3.

Experiment 3: *The effects of ADX and cocaine sensitisation on the dopamine system*
C57BL/6 and DBA/2 mice were ADX or SHAM operated and, after a recovery period of one week, subjected to a cocaine sensitisation regimen as described previously [145]. In brief, animals received i.p. injections of 15.0 mg/kg cocaine (COC, BUFA Pharmaceuticals B.V., Uitgeest, The Netherlands) or saline (SAL) on 9 consecutive days, followed by a withdrawal interval of 5 days, a saline challenge on day 14 and a 7.5 mg/kg cocaine challenge on day 15. On days 1, 9, 14 and 15, locomotor responses were recorded on video and analysed using Ethovision Videotracking, Motion Analysis & Behavior Recognition System version 1.96 ('VTMAS', Noldus Information Technology B.V., Wageningen, The Netherlands). Previously we discovered that ADX prevented initiation and expression of behavioural sensitisation to cocaine in the DBA/2, but not the C57BL/6 strain [145]. Mice were sacrificed in the morning of day 16, 24 hours after the cocaine challenge and 3 weeks after surgery.

Statistics

Statistical analysis was performed using SPSS for Windows software (release 7.5, SPSS Benelux B.V., Gorinchem, The Netherlands). In experiments 1 and 2, expression and binding data were subjected to ANOVA with strain (experiment 1) or strain and surgery (experiment 2) as factor(s). In experiment 3, analyses were performed per strain (two-factor ANOVA for surgery and treatment). When statistical

significance was revealed, post hoc tests were performed (Fisher's LSD). Differences were considered statistically significant when $p<0.05$.

RESULTS

The basal dopamine system

In drug naïve mice, either unoperated (experiment 1) or subjected to SHAM surgery (experiment 2), replicable strain differences were found for the dopaminergic markers, which are summarised in the following paragraphs.

SNc and VTA

Tables 2A and 3A depict the dopaminergic markers in somatic regions of unoperated or SHAM operated mice respectively. D1 receptor binding was undetectable and is therefore not shown. TH mRNA expression was higher in DBA/2 than in C57BL/6 mice, both in the SNc and the VTA. Similarly, DBA/2 mice displayed higher DAT mRNA expression in the SNc and a comparable, but non-significant, tendency was observed in the VTA. In agreement with previous reports, D2 receptor binding was higher in DBA/2 compared to C57BL/6 mice in the two dopaminergic cell groups.

Both TH and DAT mRNA are present within the dopaminergic neurons. Similarly, D2-like receptors in the cell body regions can function as autoreceptors and are localised on the dopaminergic neurons themselves. As all three markers were found to be higher in DBA/2 compared to C57BL/6 mice, we hypothesised that DBA/2 mice possess greater numbers of dopaminergic neurons. To test this, in experiment 1, the number of TH-positive cells was quantified in *in situ* hybridisation slides that were dipped in photographic emulsion and counterstained with Hoechst to visualise cell nuclei. Indeed, the number of TH-positive cells was higher in DBA/2 compared to C57BL/6 mice in the SNc (21%) and the VTA (32%) (table 2A).

NAc and CP

Strain differences were also observed in D1 and D2 receptor binding in the projection areas of the midbrain dopamine system (tables 2B and 3B), with a specific pattern for each receptor. DBA/2 mice displayed higher D1 binding in the NAc when compared to C57BL/6 mice, which was more pronounced in the shell compared to the core subdivision and a similar trend was observed in the rostral CP, which reached significance in experiment 2. Because this finding contradicts a previous report [69] we have in addition measured D1 receptor mRNA expression with a specific riboprobe and found this to correspond with receptor binding (DBA/2

Table 2: *The basal dopamine system.*

A

		C57BL/6	DBA/2	F
TH	**SNc**	47.8 ± 1.5	53.7 ± 1.5 *	$F_{1,19}$=8.149, p<0.05
	VTA	60.2 ± 3.1	72.3 ± 2.3 **	$F_{1,19}$=9.849, p<0.01
DAT	**SNc**	52.7 ± 1.4	58.7 ± 1.8 *	$F_{1,19}$=6.714, p<0.05
	VTA	50.5 ± 2.0	56.4 ± 2.3	$F_{1,18}$=3.811, p=0.068
D2	**SNc**	43.9 ± 0.7	48.2 ± 0.6 ***	$F_{1,18}$=19.672, p<0.001
	VTA	42.6 ± 0.3	48.0 ± 0.9 ***	$F_{1,19}$=30.891, p<0.001
TH+	**SNc**	66.2 ± 2.6	80.1 ± 5.8 *	$F_{1,19}$=4.755, p<0.05
	VTA	113.6 ± 8.6	149.9 ± 9.8 *	$F_{1,19}$=7.770, p<0.05

B

		C57BL/6	DBA/2	F
D1	**NAc core**	15.9 ± 0.7	18.2 ± 0.8 *	$F_{1,19}$=4.783, p<0.05
	NAc shell	13.3 ± 0.8	17.8 ± 0.6 ***	$F_{1,17}$=22.031, p<0.001
	rCP	22.4 ± 1.1	23.8 ± 0.9	$F_{1,19}$=0.945, p=0.344
	cCP Lat	20.5 ± 1.3	18.1 ± 1.0	$F_{1,19}$=2.086, p=0.166
	cCP DM	21.2 ± 1.3	20.8 ± 1.2	$F_{1,19}$=0.068, p=0.797
	cCP VM	17.7 ± 1.2	18.1 ± 1.0	$F_{1,19}$=0.081, p=0.780
D2	**NAc core**	30.7 ± 1.3 *	25.3 ± 2.0	$F_{1,18}$=5.020, p<0.05
	NAc shell	29.7 ± 1.5	27.7 ± 1.3	$F_{1,17}$=1.016, p=0.329
	rCP	42.8 ± 0.9 **	36.9 ± 1.1	$F_{1,15}$=16.969, p<0.01
	cCP Lat	49.3 ± 1.4	56.8 ± 1.8 **	$F_{1,17}$=10.524, p<0.01
	cCP DM	40.3 ± 0.9 *	35.9 ± 1.5	$F_{1,18}$=6.871, p<0.05
	cCP VM	34.8 ± 1.2	34.8 ± 1.2	$F_{1,18}$=0.001, p=0.976

Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression, TH+ cell numbers and D1 and D2 receptor binding in the somatic (A) and dendritic (B) dopaminergic subfields of unoperated, drug naïve C57BL/6 and DBA/2 mice. Data represent optical density on film (arbitrary units) ± SEM. n=8-10 animals/group. *** p<0.001, ** p<0.01, * p<0.05 vs. the other strain (Fisher's LSD). SNc: substantia nigra pars compacta, VTA: ventral tegmental area, NAc: nucleus accumbens, (r/c) CP (Lat,DM,VM): (rostral/caudal) caudate putamen (lateral, dorsomedial, ventromedial regions).

Table 3: *The effects of ADX on the basal dopamine system.*

A

		C57BL/6		DBA/2	
		ADX	SHAM	SHAM	ADX
TH	SNc	55.4 ± 2.6	49.7 ± 2.0	57.9 ± 2.1 *	56.5 ± 2.1
	VTA	49.5 ± 2.5	47.1 ± 1.6	52.8 ± 1.4 *	57.2 ± 2.3 *
DAT	SNc	31.8 ± 1.2	33.9 ± 0.8	40.0 ± 1.1 **	36.8 ± 1.9 *
	VTA	32.2 ± 1.5	31.1 ± 1.1	32.7 ± 1.9	30.3 ± 0.7
D2	SNc	35.2 ± 0.6 #	37.1 ± 0.5	42.3 ± 0.2 ***	42.9 ± 0.6 ***
	VTA	33.2 ± 0.4	34.2 ± 0.4	36.1 ± 0.5 **	35.5 ± 0.3 **

B

		C57BL/6		DBA/2	
		ADX	SHAM	SHAM	ADX
D1	NAc core	28.4 ± 1.3	27.4 ± 1.1	31.5 ± 1.2 *	28.8 ± 1.2
	NAc shell	23.3 ± 1.5	20.1 ± 1.0	25.8 ± 1.5 *	24.9 ± 2.4
	rCP	33.9 ± 1.1	34.4 ± 1.0	37.9 ± 1.5 *	35.4 ± 1.2
	cCP Lat	34.3 ± 0.9	34.8 ± 0.9	33.6 ± 1.5	31.4 ± 1.2
	cCP DM	32.3 ± 0.8	32.9 ± 0.8	35.4 ± 2.1	33.5 ± 1.5
	cCP VM	30.7 ± 1.2	31.7 ± 0.9	33.3 ± 1.9	30.9 ± 1.3
D2	NAc core	23.6 ± 1.7 **	22.5 ± 0.8 *	16.6 ± 2.2	16.6 ± 1.3
	NAc shell	19.3 ± 2.5	17.6 ± 1.8	13.5 ± 1.1	14.9 ± 1.3
	rCP	33.3 ± 1.4 *	34.4 ± 0.7 *	30.7 ± 1.1	28.6 ± 1.6
	cCP Lat	41.7 ± 1.6	40.8 ± 1.5	47.0 ± 2.6 *	46.8 ± 2.7
	cCP DM	32.2 ± 2.2	32.4 ± 1.6	34.1 ± 2.4	31.8 ± 1.7
	cCP VM	29.6 ± 1.2	28.5 ± 1.9	31.0 ± 1.6	29.1 ± 1.9

Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression and D1 and D2 receptor binding in the somatic (A) and dendritic (B) dopaminergic subfields of drug naïve C57BL/6 and DBA/2 mice 3 weeks after adrenalectomy (ADX) or SHAM surgery. Data represent optical density on film (arbitrary units) ± SEM. n=5-12 animals/group. *** $p<0.001$, ** $p<0.01$, * $p<0.05$ vs. mice of the other strain subjected to similar surgery. # $p<0.05$ vs. SHAM operated mice of the same strain (Fisher's LSD). SNc: substantia nigra pars compacta, VTA: ventral tegmental area, NAc: nucleus accumbens, (r/c) CP (Lat,DM,VM): (rostral/caudal) caudate putamen (lateral, dorsomedial, ventromedial regions).

vs. C57BL/6: NAc core 38.3 vs. 28.0, $p<0.05$, NAc shell 38.8 vs. 29.0, $p<0.01$, rCp: 39.5 vs. 34.8, ns.). There were no strain differences in D1 binding or mRNA expression in the three caudal subdivisions of the CP. By contrast, C57BL/6 mice exhibited greater D2 receptor binding in NAc core and the rCP. In experiment 1, a similar effect was observed in the DM subdivision of the cCP, but this could not be replicated in experiment 2. In the NAc shell, there was no significant strain difference in D2 binding, although there was a tendency towards higher densities in the C57BL/6 strain. In the Lat cCP by contrast, D2 binding was consistently found to be higher in DBA/2 when compared to C57BL/6 mice.

The effects of ADX on the dopamine system of drug naïve mice

Table 3 depicts the dopaminergic markers measured in drug naïve SHAM and ADX mice of both strains. D2 receptor binding was slightly reduced by ADX only in the SNc of the C57BL/6 strain ($F[strain]_{1,30}=134.245$, $p<0.001$, $F[surgery]_{1,30}=1.222$, $p=0.279$, $F[strain \times surgery]_{1,30}=5.080$, $p<0.05$, post hoc: $p<0.05$). The strain differences in D1 binding in the NAc and rCP were attenuated by ADX, but a significant surgery effect was not revealed in any of the subregions. No other effects of ADX on the dopaminergic markers were detected in either strain.

The effects of ADX and cocaine sensitisation on the dopamine system

In mice subjected to the sensitisation regimen, there were strain- and surgery-specific adaptations in D2 receptor binding (figures 2 and 3) and TH and DAT mRNA expression (table 4) in subfields of the nigrostriatal system. In the DBA/2 strain, D2 receptor binding was reduced in the NAc core and rCP of ADX mice resistant to behavioural sensitisation when compared to sensitised SHAMs, and a similar trend was observed in the NAc shell (core: $F[surgery \times treatment]_{1,37}=4.220$, $p<0.05$, shell: $F[treatment]_{1,37}=4.358$, $p<0.05$, rCP: $F[treatment]_{1,36}=5.048$, $p<0.05$, $F[surgery \times treatment]_{1,36}=4.138$, $p<0.05$, post hoc: core and rCP: $p<0.05$, shell: $p=0.155$ vs. SHAM/COC). Furthermore, in the DBA/2 strain, cocaine treatment was associated with an increase in TH and DAT mRNA expression in the SNc (TH: $F[treatment]_{1,36}=5.539$, $p<0.05$, DAT: $F[treatment]_{1,36}=4.873$, $p<0.05$, post hoc: $p<0.05$ vs. SHAM/SAL) and a reduction in D2 binding in the DM subdivision of the cCP ($F[treatment]_{1,38}=4.965$, $p<0.05$, post hoc: $p<0.05$ vs. SHAM/SAL), which were prevented by ADX. The effects of treatment and ADX on D2 binding in the DM cCP were also observed in the C57BL/6 strain ($F[treatment]_{1,30}=7.008$, $p<0.05$, post hoc: $p<0.05$ vs. SHAM/SAL). There were no other effects of treatment or surgery in either strain (data not shown).

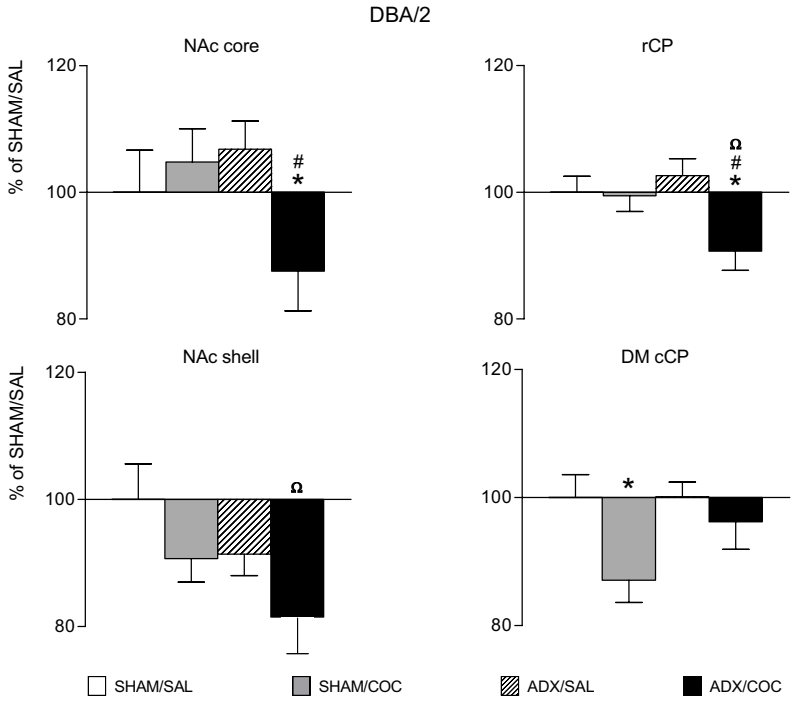

Figure 2: *D2 binding in DBA/2 mice subjected to the sensitisation regimen.*
D2 receptor binding in adrenalectomised (ADX) or SHAM operated DBA/2 mice subjected to the cocaine sensitisation regimen. Previously, animals received 15.0 mg/kg cocaine (COC) or saline (SAL) for 9 consecutive days, followed by a 5 day withdrawal interval and a 7.5 mg/kg cocaine challenge. Animals were sacrificed 24 hours after the drug challenge. Data are presented as percentage of SHAM/SAL (%) ± SEM. n=6-13 animals/group.
* $p<0.05$ vs. SAL-treated mice subjected to similar surgery, # $p<0.05$ vs. SHAM/COC, and Ω $p<0.05$ vs. SHAM/SAL (Fisher's LSD). NAc: nucleus accumbens, (r/c) CP (DM): (rostral/caudal) caudate putamen (dorsomedial region).

DISCUSSION

This study shows strain differences in the effects of ADX on neuroadaptations in the midbrain dopamine system in mice that were subjected to a 9 day cocaine sensitisation regimen. The DBA/2 strain was susceptible to adrenal hormones on measures for dopamine in the nigrostriatal system and the NAc core under these conditions of behavioural sensitisation, whereas ADX only marginally affected the dopamine system in the C57BL/6 strain. This confirms our hypothesis that the DBA/2 strain displays parallel susceptibility to the impact of adrenal hormones on behavioural and neurochemical responsiveness to cocaine. Therefore, these results suggest

Table 4: *TH and DAT mRNA expression in the SNc of mice subjected to the sensitisation regimen.*

			SHAM		ADX	
			SAL	COC	SAL	COC
TH	**SNc**	**C57BL/6**	100.0 ± 2.6	100.6 ± 2.4	94.6 ± 3.8	96.3 ± 1.7
		DBA/2	100.0 ± 2.3	106.1 ± 1.6 *	98.8 ± 1.8	101.6 ± 1.8
DAT	**SNc**	**C57BL/6**	100.0 ± 4.3	104.4 ± 3.2	103.0 ± 3.9	95.1 ± 3.4
		DBA/2	100.0 ± 2.0	111.9 ± 3.2 *	99.0 ± 4.6	103.2 ± 3.5

Tyrosine hydroxylase (TH) and dopamine transporter (DAT) mRNA expression in the substantia nigra pars compacta (SNc) of adrenalectomised (ADX) or SHAM operated C57BL/6 and DBA/2 mice subjected to the cocaine sensitisation regimen. Previously, animals received 15.0 mg/kg cocaine (COC) or saline (SAL) for 9 consecutive days, followed by a 5 day withdrawal interval and a 7.5 mg/kg cocaine challenge. Animals were sacrificed 24 hours after the drug challenge. Data are presented as percentage of SHAM/SAL (%) ± SEM. n=7-11 animals/group. * $p<0.05$ vs. SAL-treated mice subjected to similar surgery.

that adrenal stress hormones influence psychostimulant sensitivity in a genotype-dependent manner through adaptations in the midbrain dopamine system.

Compared to the C57BL/6 strain, the DBA/2 strain is less sensitive to the neurochemical-, rewarding- and behavioural effects of amphetamine [65,71,124,447,611,700,701,760], and the locomotor-stimulant effect of cocaine [145]. This may result from strain differences in both pre- and/or post-synaptic transmission in the midbrain dopamine system, aspects of which were investigated using the dopaminergic markers.

The greater number of TH-positive neurons in the DBA/2 strain would imply greater dopaminergic input in the NAc and CP from the A9/A10 cell groups. By contrast, DBA/2 mice exhibit lower amphetamine-induced dopamine release in the NAc [701,760] whereas there are no strain differences in basal dopamine release in this brain region [760]. The apparent discrepancy between the midbrain dopaminergic cell numbers and the dopamine release indicates that there must be strain differences in presynaptic dopaminergic function. Indeed, the number of dopaminergic neurons can [212], but does not necessarily [753], correlate with mesencephalic TH activity. In the present study, TH mRNA expression was increased to a lesser extent (12-20%) than the number of dopaminergic cells (20-32%) in the DBA/2 compared to C57BL/6 strain, which is suggestive of a relatively lower TH function in the former strain. However, from mRNA expression it is not possible to draw a definitive conclusion regarding TH protein content and activity [373].

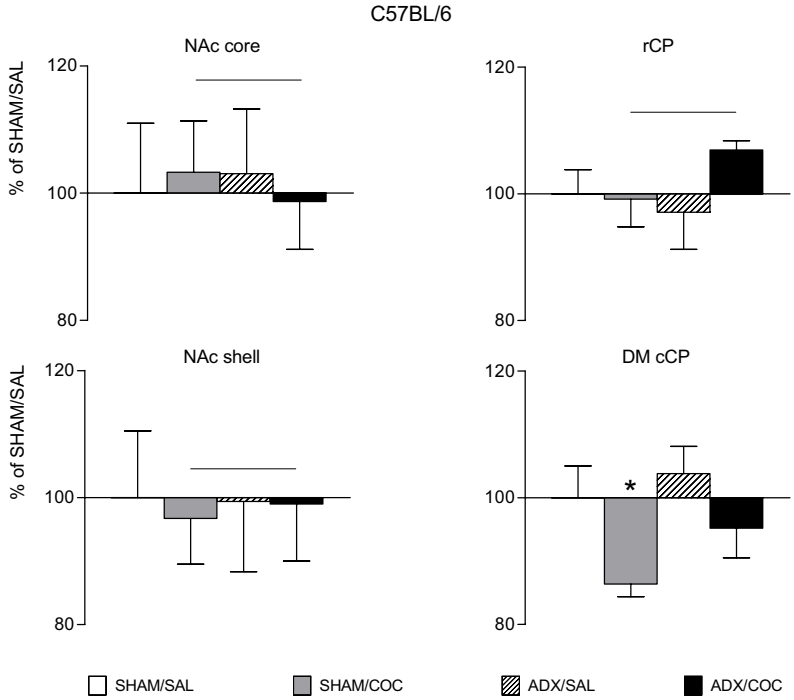

Figure 3: *D2 binding in C57BL/6 mice subjected to the sensitisation regimen.* D2 receptor binding in adrenalectomised (ADX) or SHAM operated C57BL/6 mice subjected to the cocaine sensitisation regimen. Previously, animals received 15.0 mg/kg cocaine (COC) or saline (SAL) for 9 consecutive days, followed by a 5 day withdrawal interval and a 7.5 mg/kg cocaine challenge. Animals were sacrificed 24 hours after the drug challenge. Data are presented as percentage of SHAM/SAL (%) ± SEM. n=6-8 animals/group. * $p<0.05$ vs. SAL-treated mice subjected to similar surgery (Fisher's LSD). NAc: nucleus accumbens, (r/c) CP (DM): (rostral/caudal) caudate putamen (dorsomedial region).

Factors that control dopaminergic transmission, possibly in a genotype-dependent manner, include regulation by afferent projections and by autoreceptors of the D2 receptor family. Indeed, when compared to the C57BL/6 strain, the DBA/2 strain displays lower drug-induced dopamine release in the NAc as a consequence of higher dopamine release in the prefrontal cortex [700,701]. Furthermore, DBA/2 mice have been proposed to exhibit greater autoreceptor-mediated inhibition of dopaminergic transmission, due to the higher D2 receptor availability in the VTA and SN [69,470]. However, the observed difference in cell numbers suggests that the higher D2 receptor binding in the somatic regions of the DBA/2 strain reflects a greater number of neurons, rather than a higher density of receptors per cell. Further studies linking dopaminergic cell counts to D2 receptor numbers and function will be

necessary to evaluate the capacity for autoinhibition of dopaminergic transmission in the two strains. Collectively, these findings suggest that presynaptic regulation of dopaminergic neurotransmission may be a crucial factor in restraining the higher dopaminergic output that would be expected to arise from the greater numbers of TH positive neurons in the DBA/2 strain.

Pronounced strain differences were also found for dopamine receptor binding in the terminal fields, which are in agreement with most previous findings [69,199,470]. Most remarkable is the opposite strain difference in D1 and D2 binding in the NAc and rCP. How the regionally differentiated dopamine receptor binding contributes to the strain differences in behavioural responsiveness to psychostimulants remains to be investigated. One avenue of further research could involve the implication of the various dopamine receptor subtypes of the D1 and D2 families, that have partially overlapping but also distinct functional characteristics.

We have demonstrated previously, that ADX does not alter the locomotor response of C57BL/6 and DBA/2 mice to a single administration of cocaine [145]. In parallel, no major changes were observed in the basal dopamine system following ADX. By contrast, studies in several strains of rats have indicated that depletion of adrenal glucocorticoids attenuates basal and cocaine-induced dopamine release and psychostimulant-induced locomotion [77,166,420,423,508,580] and substantially reduces D1 and D2 receptor binding in subdivisions of the dopamine system [47,136]. The discrepancy between the present findings in mice and the observations in rats may be related to the residual corticosterone after ADX that circulates in mice, but not in rats [300]. Indeed, it has been demonstrated in rats that corticosterone in the range of diurnal concentrations is sufficient to restore the effects of ADX on basal dopamine release and on neurochemical and behavioural responsiveness to an acute psychostimulant exposure [30,77,423,508].

ADX prevented behavioural sensitisation in the DBA/2, but not the C57BL/6 strain [145]. Strain- and surgery-specific adaptations were also observed in the midbrain dopamine system of mice subjected to the sensitisation regimen. Given that a single psychostimulant exposure can induce long-term behavioural, neuroendocrine, and neurochemical sensitisation [694], it should be noted that none of the groups were drug naïve (all animals were exposed to the cocaine challenge one day prior to sacrifice).

In the DBA/2 strain, TH and DAT mRNA expression were increased in the SNc of sensitised SHAM mice, suggesting an enhanced tone of the DA system, which is in agreement with previous studies employing comparable withdrawal durations (<1 week) [33,406,623,633,713]. The enhanced expression of the presynaptic markers, which we interpret as increased capacity of nigrostriatal dopaminergic neurons under basal

conditions, was fully prevented by ADX in the DBA/2 strain, thus correlating with behavioural responsiveness to the drug challenge. Although it cannot be concluded whether the increases in TH and DAT mRNA are critical for expression of sensitisation, it does reflect the plasticity of the presynaptic component of the dopamine system in the DBA/2 strain. In the C57BL/6 strain, by contrast, sensitisation did not correlate with enhanced expression of presynaptic markers, which might indicate that this neural adaptation is not required for behavioural sensitisation in the C57BL/6 background. Alternatively, given the marked time-dependency in neuroadaptations associated with psychostimulant sensitisation, the presynaptic adaptations could occur after a different withdrawal duration in the C57BL/6 strain [497,520].

The finding that D2 family receptors are subject to regulation by adrenal hormones in a genotype-dependent manner, is in line with previous observations that D2 receptors engage in gene x environment interactions, both in humans and in laboratory rodents [41,69,198]. The sensitisation-resistant ADX mice of the DBA/2 strain exhibited reduced D2 receptor binding in the NAc core and rCP, with a similar trend in the NAc shell. As discussed previously, D2-like receptors within the terminal regions may represent both pre- and postsynaptic receptors, the former functioning as inhibitory autoreceptors. Given the lack of behavioural sensitisation in ADX mice of the DBA/2 strain, it is tempting to speculate that the decrease in D2 binding reflects a deficit in postsynaptic signalling. Previous studies have demonstrated that supersensitivity of postsynaptic D2 receptors may play a role in long-term expression of behavioural sensitisation to amphetamine [306,675,695]. Furthermore, concurrent activation of D1 and D2 receptors is required for expression of neural- and behavioural phenotypes of cocaine sensitisation [87]. Therefore, the reduction in D2 binding in the NAc and CP might precipitate the sensitisation deficit of DBA/2 mice in which the adrenals were surgically removed.

By contrast, in sensitised SHAM mice of both strains, D2 binding was reduced in the DM subdivision of the cCP. Previous studies have demonstrated subsensitivity of axonal and somatodendritic D2 dopamine autoreceptors during the first few days of withdrawal from repeated psychostimulant administration [3,736,744,748]. The decrease in D2 binding associated with a history of cocaine treatment may therefore represent such an autoreceptor subsensitivity. The observation that ADX prevented the reduction in D2 binding in the DM cCP of *both* strains, suggests that the adaptation in this brain region is not critical for the impact of adrenal hormones on behavioural sensitisation. Clearly, the role of the regional changes in D2 receptor binding in behavioural sensitisation requires further investigations. Furthermore, it will be necessary to distinguish between the D2 and D3 receptors, given that activation of D3 receptors is thought to suppress, rather than enhance, locomotion and behavioural sensitisation [543] (but see also [23]).

Collectively, these findings indicate that in the DBA/2 strain, adrenal hormones modulate dopaminergic transmission, presumably at the level of both pre- and post-synaptic neurotransmission. Further studies are required to investigate the importance of the individual neural adaptations in psychostimulant sensitisation, e.g. by regional overexpression of TH and DAT or by downregulation of D2 receptors in the NAc and CP. Interestingly, the neural adaptations were most evident in the nigrostriatal dopamine system and NAc core. This contradicts the notion that the mesocorticolimbic pathway, and particularly the NAc shell, plays a critical role in initiation and expression of psychostimulant sensitisation [210,519]. However, it has become increasingly evident that the mesocorticolimbic and nigrostriatal projections are both anatomically and functionally intertwined, and evidence is emerging for a role of the latter in behavioural sensitisation. Indeed, expression of psychostimulant sensitisation has been associated with structural changes in the NAc core, but not the shell, subregion [390] and one study reported sensitisation of dopamine transmission specifically in the NAc core [74]. Furthermore, alterations in cellular activity associated with expression of amphetamine sensitisation have been reported to occur in the CP, but not the NAc [696]. Finally, the dorsal PFC-NAc core connection (via glutamatergic efferents) plays an increasingly more prominent role in expression of drug-induced adaptations, under more habitual circumstances [329].

In conclusion, the present data suggest that adrenal hormones modulate behavioural sensitisation to cocaine through adaptations in the nigrostriatal dopamine system and the NAc core, in a genotype-dependent manner. The DBA/2, but not the C57BL/6 strain, appeared vulnerable to the impact of adrenal stress hormones on cocaine sensitivity at the level of both dopaminergic neurotransmission and, as previously described, behavioural responsiveness [145]. The 'susceptible' DBA/2 phenotype therefore provides a good model for further research into the interaction between stress, the brain dopamine system and vulnerability to psychostimulant drugs.

ACKNOWLEDGEMENTS

This research was supported by NWO/INSERM/ZON grants 985-10-014 and 985-10-504 and by the Royal Netherlands Academy of Arts and Sciences. We gratefully acknowledge dr. S. Smits for kindly providing the D1 receptor riboprobe, drs. J.C. de Greef for technical support and dr. O.C. Meijer for critically reading the manuscript.