

Effects of Cocaine on *c-Fos* and NGFI-B mRNA Expression in Transgenic Mice Underexpressing Glucocorticoid Receptors

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Numerous evidences suggest that stress and stress-related hormones can modulate the activity of the brain reward pathway and thus may account for individual vulnerability towards the reinforcing effects of drugs of abuse. Transgenic (TG) mice expressing an antisense mRNA against the glucocorticoid receptor (GR), which partially blocks GR expression, were used to assess the role of GR dysfunction on cocaine (COC)-induced *c-fos* and Nerve-Growth Factor Inducible-B (NGFI-B, or *Nur77*) gene expression. These two genes belong to different families of transcription factors and have been shown to be modulated by various dopaminergic drugs. TG and wild-type (WT) mice were both acutely and repeatedly treated with COC (20 mg/kg, i.p.). In the chronic experiment, mice received a 5-day treatment of COC and were challenged 5 days later with COC or vehicle. Locomotor activity was assessed during the entire chronic experiment in the mouse home cages. Animals were sacrificed 1 h after the last injection and NGFI-B and *c-fos* mRNA levels in the prefrontal cortex, the nucleus accumbens and the striatum were measured by *in situ* hybridization. Acute COC administration led to significantly smaller *c-fos* increases in TG mice compared to WT, whereas repeated COC treatment potentiated *c-fos* induction both in TG and WT mice to equivalent levels. TG mice displayed higher basal NGFI-B expression in the nucleus accumbens and the level of NGFI-B mRNA was differently modulated by COC in TG mice compared to WT mice. In accordance with data on *c-fos* expression, behavioral data indicate a blunted locomotor effect on the first COC injection in TG mice, a phenomenon corrected by the repeated COC treatment. These results suggest that an alteration of the hypothalamus–pituitary–adrenal axis can modify COC-induced regulation of the transcription factors *c-fos* and NGFI-B, and that these changes parallel those seen at the behavioral level. It also demonstrates that the differences at the behavioral and molecular levels noted between TG and WT mice after acute COC injection disappear following repeated COC administration, suggesting that repeated COC has a greater impact in TG mice underexpressing GRs.

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In some individuals repeated cocaine (COC) exposure will lead to addiction, a pathological state characterized by a compulsive drug-seeking and drug-taking behavior. It is thought that genetic predisposition as well as environmental factors could shape an individual vulnerability towards the reinforcing properties of drugs of abuse. Although the exact mechanisms implicated in this phenomenon are still unclear, data in the literature suggest a role for stress and the hypothalamus–pituitary–adrenal (HPA) axis (Piazza *et al*, 1996a). For example, it has been shown that stressful events can facilitate the acquisition, maintenance, and relapse of drug self-administration (Piazza and Le Moal,

1998; Shaham *et al*, 2000). It has also been demonstrated that chronic stress exposure potentiates COC-induced locomotor activity (Haile *et al*, 2001). There is numerous evidence that stress hormones can modulate dopaminergic function in the mesolimbic pathway. Corticosterone administration has been shown to increase basal release of dopamine (DA) in the nucleus accumbens (Piazza *et al*, 1996b), whereas adrenalectomy reduces basal extracellular DA levels and blunts COC-induced DA increase in the same brain structure (Barrot *et al*, 2000). Evidence of a direct interaction between mesolimbic DAergic system and the HPA axis is further emphasized by the fact that glucocorticoid receptors (GRs) are expressed by DAergic neurons (Harfstrand *et al*, 1986) and that a glucocorticoid-responsive element (GRE) is found in the promoter region of the mouse tyrosine hydroxylase gene (Hagerty *et al*, 2001a, b).

Transgenic (TG) mice bearing a transgene coding for a GR antisense RNA (Pepin *et al*, 1992), which partially blocks GR expression, have been previously used to investigate the consequence of impaired functioning of the GR on various

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behavioral (Spanagel *et al*, 1996; Rochford *et al*, 1997; Sillaber *et al*, 1998), neuroendocrine (Beaulieu *et al*, 1994; Stec *et al*, 1994) and neurochemical responses (Farisse *et al*, 2000; Linthorst *et al*, 2000). These mice present an approximate 30–50% reduction in GR mRNA levels in the brain (Pepin *et al*, 1992) and a 10-fold higher dose of dexamethasone is needed to suppress their circulating corticosterone levels when compared to normal mice (Barden *et al*, 1997). Of particular interest, these TG mice display higher locomotive activity when placed in a novel environment (Beaulieu *et al*, 1994), a characteristic hypothesized to predict an increased sensitivity towards the reinforcing effects of drugs of abuse (Piazza *et al*, 1989; Hooks *et al*, 1991). In addition, this TG mouse model shows a stronger locomotive response than control following an acute injection of amphetamine (Cyr *et al*, 2001).

Addiction is thought to rely on plastic neuronal adaptation initiated in the mesolimbic system by repeated administration of drugs of abuse (Hyman and Malenka, 2001; Nestler, 2001). Regulation of gene expression is one putative mechanism through which COC could initiate persistent neural and behavioral changes and in this way, transcription factors may play a crucial role. Levels of some transcription factors appear to increase in the central nervous system in response to acute and chronic COC administration (Young *et al*, 1991; Hope *et al*, 1992, 1994). Among the transcription factors studied so far, Fos members of the immediate-early gene family (*c-fos*, Fos-related antigens (FRAs) and Δ FosB) have been intensively investigated (Nestler, 2001). Nerve Growth Factor inducible-B (NGFI-B, also known as *Nur77*) is another transcription factor, which is closely associated with DA systems in the brain. This orphan ligand-activated nuclear receptor belongs to the steroid/thyroid hormone receptor family and is constitutively expressed in target structures of DA systems (Zetterström *et al*, 1996). NGFI-B mRNA expression can be modulated by DA and serotonergic drugs (Gervais *et al*, 1999). Furthermore, typical and atypical antipsychotic drugs induce contrasting patterns of NGFI-B expression after acute and chronic administration (Beaudry *et al*, 2000; Langlois *et al*, 2001). Of particular interest, other drugs of abuse such as morphine have been shown to modulate NGFI-B expression in the mesolimbic system (Werme *et al*, 2000a).

The aim of the present study was to assess the influence of GR dysfunction on COC-induced transcription factor mRNA expression in target structures of DA systems. We studied and compared the modulation of *c-fos* and NGFI-B, which belong to two families of transcription factors, after an acute COC injection, as well as in a paradigm of COC sensitization, in normal and TG mice underexpressing GRs. We also established a parallel between COC-induced gene expression and behavioral activity.

EXPERIMENTAL PROCEDURES

Animals and Treatments

Adult male wild-type (WT) B₆C₃F₁ mice (Charles River; St-Constant, Québec, Canada) and male homozygous TG mice underexpressing GRs were used. The TG mice bearing the

glucocorticoid antisense construct (an inverted 1.815 kb fragment of the 3' noncoding region of the GR coupled to a 2.3 kb *Eco*R1/*Hind*III human neurofilament gene promoter inserted in the genome of WT B₆C₃F₁ mice (Pepin *et al*, 1992)) were bred as homozygotes in our laboratory. Animals were kept in groups of five per cage and maintained in a standard 12 h dark/light cycle (lights on 08:00) with food and water available *ad libitum*. All experiments were conducted during the light portion of the cycle. At the time of killing, animals were about 8 weeks old. Handling of mice was performed in accordance with the Canadian Guide for The Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Care Committee of Laval University and all efforts were made to minimize the number of animals used and their suffering.

In a first series of experiments, WT and TG mice received a single injection of either the vehicle (NaCl 0.9%, i.p.) or COC (20 mg/kg, i.p.). Four groups were formed: WT/vehicle ($n=4$), WT/COC ($n=4$), TG/vehicle ($n=6$), and TG/COC ($n=6$). Injections occurred between 9:00 and 9:30 am. One hour following the injection animals were decapitated and their brains were frozen on dry ice before further processing. In the second series of experiments, four different groups of mice received a COC injection (20 mg/kg, i.p.) once a day for 5 days. Following the fifth administration on day 5, animals were left without treatment for 5 days. After this withdrawal period (on day 10), two groups of mice received the vehicle (NaCl 0.9%, i.p.) (WT/vehicle ($n=4$) and TG/vehicle ($n=4$)), whereas the other two groups received a COC challenge (20 mg/kg, i.p.) (WT/COC ($n=5$) and TG/COC ($n=4$)). This injection pattern has been shown to lead to the development of behavioral sensitization (Partridge and Schenk, 1999). All mice were decapitated, as indicated above, 1 h following the last drug injection.

Locomotor Activity

Locomotor activity was recorded continuously during the repeated COC treatment using telemetry (Data Sciences International, St Paul, MN, USA). We used this system to monitor locomotion before and after each injection, and that in the mouse home cages. The system included 17 wireless receivers (TA11-TA-C20) placed under each cage and getting signals from a biocompatible transmitter implant (RA1010) introduced into each mouse. Signals from the wireless receivers were sent to a computer via a consolidation matrix (BCM100) and data acquisition was ensured by a dedicated software (Dataquest A.R.T., version 2.0). Locomotion data were analyzed for 20 s every 10 min. Locomotion is expressed in locomotor counts and are based on the intensity of the signal received from the transmitter (for a detailed explanation of locomotor count calculation, see Clement *et al*, 1989). Results for each individual stem from an average of locomotor counts during 30-min periods (three locomotion data/30 min). For every injection during the repeated treatment with COC, locomotion data were computed for four succeeding blocks of 30 min, the first one being the one before the injection (–30–0, 0–30, 30–60, 60–90 min).

Transmitter Implantation

Transmitters (2.5 g; 1.4 cm²) were introduced into the mouse peritoneal cavity. Surgery was performed under isoflurane anesthesia (isoflurane 1.5%; O₂ 0.8 l/min) (Zeneca Pharma, Canada). Experiment involving repeated COC administration began 2 weeks after transmitter implantation, thus ensuring full recovery from the surgery.

In Situ Hybridization

After having been removed from the skull and frozen on dry ice, brains were cut into 12 µm-thick coronal sections with a cryostat, thaw-mounted on slides and kept at -80°C until further processing. Adjacent brain sections were hybridized with *c-fos* and NGFI-B probes. The antisense cRNA probe for *c-fos* was prepared from a 1.8 kb *EcoRI* fragment of a full-length rat *c-fos* cDNA subcloned into pBluescript SK-1 and linearized with *SmaI*. The antisense cRNA probe for NGFI-B stem from a 2.4 kb *EcoRI* fragment of a full-length rat NGFI-B cDNA subcloned into pBluescript SK-1 and linearized with *BamHI*. The single-stranded riboprobe complementary to the RNA of interest was synthesized and labeled using Promega riboprobe kit (Promega, Madison, WI), [³⁵S]UTP (Perkin-Elmer Inc., Canada), and the RNA polymerase T3 and T7 for NGFI-B and *c-fos*, respectively.

The hybridization technique was performed as previously described (Beaudry et al, 2000; Langlois et al, 2001). Briefly, brain sections were fixed in a 20-min paraformaldehyde bath (4% paraformaldehyde in 100 mM phosphate buffer) and then rinsed twice in phosphate buffer (100 mM) for 5 min. Subsequently, a triethanolamine incubation (TEA 100 mM, pH: 8.0) and a 10-min acetylation bath (0.25% acetic anhydride, TEA 100 mM) followed. Slides were finally rinsed in standard saline citrate (SSC) (NaCl 300 mM, sodium citrate 30 mM) and dehydrated in increasing concentrations of ethanol. The ³⁵S-labeled riboprobe was added to a concentration of 1 × 10⁷ cpm/ml in a hybridization mix (Denhart's solution, dextran sulfate and 50% deionized formamide) and heated at 65°C for 5 min. Each slide was covered up with 100 µl of the hybridization solution and a glass coverslip placed on the slide. The hybridization took place overnight at 58°C on a slide warmer. After hybridization, slides were soaked in SSC (NaCl 600 mM, sodium citrate 30 mM) for 30 min to remove coverslips and then washed in four subsequent SSC baths. Afterwards, slides spent 30 min in a 37°C incubation buffer with RNase A (2 mg/100 ml). A series of SSC baths with dithiothreitol (1 mM) follows, one of which being at 55°C for 30 min. Finally, tissues were dehydrated in ethanol, dried up, and apposed to a Biomax MR film (Kodak). Autoradiograms were developed approximately 48 h later.

Autoradiogram Quantification

Levels of radioautographic labeling on films were quantified by computerized densitometry. Digitized brain images were obtained with a CCD camera model XC-77 (Sony) equipped with a 60 mm f/2.8D (Nikon) magnification lens. Images were analyzed on a MacIntosh computer using the Image

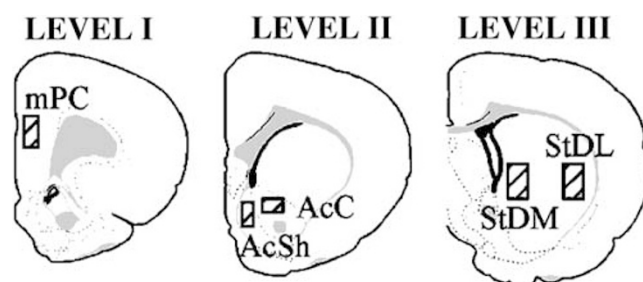


Figure 1 Schematic representation of the mouse forebrain regions selected for quantitative analysis of *c-fos* and NGFI-B mRNA expression. Levels I–III correspond to bregma 1.96, 1.18, and 0.5 levels, respectively. Specific brain areas sampled are shown as hatched boxes. AcC, nucleus accumbens core; AcSh, nucleus accumbens shell; mPC, medial prefrontal cortex; StDM, dorsomedial striatum; StDL, dorsolateral striatum.

software (Image 1.61, W Rasband, NIH). Optical density of the autoradiograms was translated in µCi/g of tissue using [¹⁴C] radioactivity standards (ARC 146A-¹⁴C standards, American Radiolabeled Chemicals Inc., St Louis). NGFI-B and *c-fos* mRNA labeling were measured in the medial prefrontal cortex (mPC), the shell (AcSh) and core (AcC) of the nucleus accumbens, and the striatum. This last structure was further subdivided into its dorsomedial (StDM) and dorsolateral (StDL) parts (Figure 1). The average level of labeling for each area was calculated from three adjacent brain sections of the same animal. Background intensity was subtracted from every measurements.

Statistical Analysis

For the *in situ* hybridization studies, differences between groups were assessed using a 2-factor (mice type × treatments) analysis of variance (ANOVA) and interactions between factors were further analyzed by the *post hoc* least significant difference (PLSD) Fisher's test. For the behavioral study, we performed ANOVA for repeated measures (mouse type × time course) and multiple comparisons between mouse type, using a PLSD Fisher's, when required. The *post hoc* comparisons were done at the time corresponding to the peak of the effect. If more than one comparison was needed for the same time course, a Bonferroni correction was taken into account.

RESULTS

Effects of an Acute COC Injection on *c-fos* Expression

Quantitative analysis of the film autoradiograms did not reveal any difference of basal *c-fos* expression between mouse types in control groups receiving vehicle injection (Figure 2, upper panels and Figure 3). In WT animals, a single injection of COC produced a significant increase in *c-fos* mRNA levels in all brain regions measured (Figure 3). In the nucleus accumbens, factorial ANOVA shows an effect of the treatment (AcSh, $F_{1,16} = 14.749$, $P < 0.01$; AcC, $F_{1,16} = 7.264$, $P < 0.05$) along with an effect of the mouse type (AcSh, $F_{1,16} = 6.561$, $P < 0.05$; AcC, $F_{1,16} = 5.850$, $P < 0.05$) and an interaction between the treatment and the mouse type in the AcSh subdivision (AcSh,

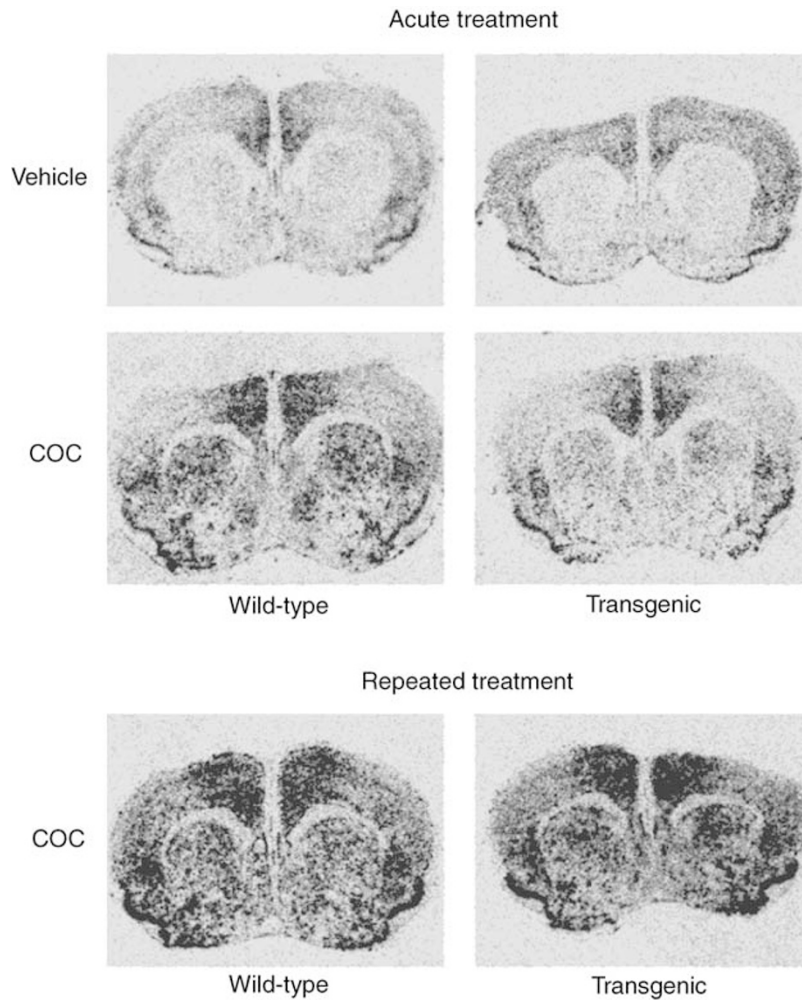


Figure 2 Representative autoradiograms showing *c-fos* mRNA levels radiolabeled by the [^{35}S]-antisense riboprobe in WT and TG mice receiving vehicle (upper panels); acutely treated with COC (20 mg/kg) (middle panels) or challenged with COC following a 5-day COC pretreatment (20 mg/kg) and a 5-day washout period (lower panels).

$F_{1,16} = 14.107$, $P < 0.01$; AcC, $F_{1,16} = 1.399$, $P = 0.254$). The *post hoc* test confirms that whereas the acute COC treatment increases *c-fos* mRNA levels in WT AcSh and AcC the same treatment fails to induce a significant elevation in TG mice (AcSh, $P = 0.948$; AcC, $P = 0.249$) (Figures 3a and b). In the striatum, COC administration produced a significant upregulation of *c-fos* mRNA in both WT and TG mice, although the increase was significantly smaller in the TG group when compared to WT animals (Figures 3c and d). For both structures, the ANOVA revealed significant differences of *c-fos* mRNA levels between the two mouse types (StDM, $F_{1,16} = 6.161$, $P < 0.05$; StDL, $F_{1,16} = 6.672$, $P < 0.05$), the treatment (StDM, $F_{1,16} = 28.642$, $P < 0.0001$; StDL, $F_{1,16} = 61.537$, $P < 0.0001$), and a significant interaction between these two factors (StDM, $F_{1,16} = 5.595$, $P < 0.05$; StDL, $F_{1,16} = 8.552$, $P < 0.01$). In the mPC, the ANOVA revealed a significant effect for the treatment ($F_{1,16} = 18.203$, $P < 0.005$) without any significant difference between the two mouse types ($F_{1,16} = 0.102$, $P = 0.753$) or an interaction between these factors ($F_{1,16} = 2.016$, $P = 0.175$) (Figure 3e).

Effects of a Repeated COC Treatment on *c-fos* Expression

After the 5-day COC pretreatment and a subsequent 5-day weaning period, the COC challenge led to significant increases in *c-fos* mRNA levels in all regions studied and without any difference between the two mouse types when compared to vehicle control groups from the repeated condition (Figure 3). In the AcSh, the factorial ANOVA did not reveal any significant difference between the two mouse types ($F_{1,13} = 0.510$, $P = 0.4878$) nor any interaction between this factor and the treatment ($F_{1,13} = 0.444$, $P = 0.5168$) with regard to the significant effect of the COC challenge ($F_{1,13} = 24.947$, $P < 0.001$) (Figure 3a). The absence of difference between mouse types in *c-fos* mRNA expression induced by repeated COC administration is also true for the AcC (Figure 3b). Furthermore, in both the StDM and StDL, a significant increase in *c-fos* mRNA levels was noted following the COC challenge on day 10 (STDM, $F_{1,13} = 131.737$, $P < 0.0001$; StDL, $F_{1,13} = 50.672$, $P < 0.0001$), again without any difference between mouse types (Figures 3c and d). In the mPC, COC challenge increases *c-fos* mRNA

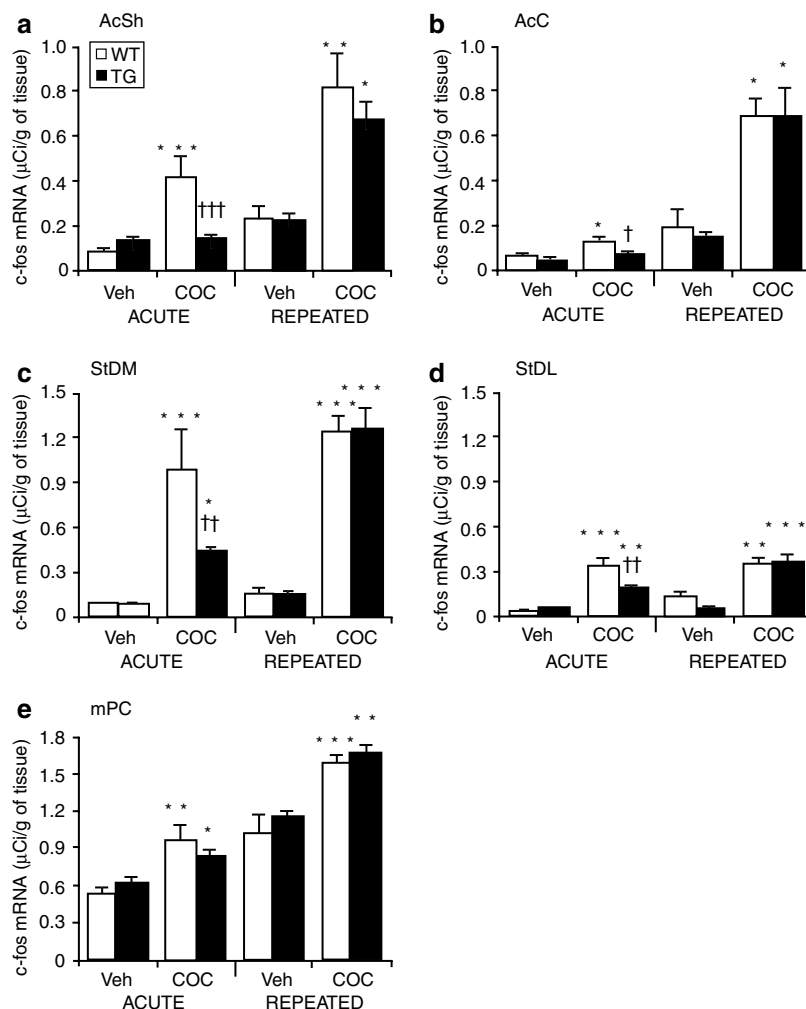


Figure 3 Histograms depicting *c-fos* mRNA levels after acute and repeated COC (20 mg/kg) administration. In the repeated experiment mice received vehicle or COC following a 5-day COC pretreatment (20 mg/kg). *C-fos* mRNA expression was measured in the (a) AcSh, (b) AcC, (c) StDM, (d) StDL, and (e) mPC. Results shown represent means \pm SEM. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ when compared to the same mouse type receiving the vehicle administration. ††† $P < 0.001$, †† $P < 0.01$, † $P < 0.05$ when compared to WT mice receiving the same treatment. These comparisons were done inside the same treatment, not between means from the acute and the repeated administration.

levels ($F_{1,13} = 39.743$, $P < 0.0001$) without difference between the two mouse types, here again when compared to vehicle control groups from the same condition (Figure 3e). Statistical analysis also revealed a potentiation of the effect of the COC challenge on *c-fos* induction when compared to the effect of an acute COC injection, and that in all structures measured without any regard to mouse types (AcSh, $F_{1,15} = 23.146$, $P < 0.001$; AcC, $F_{1,15} = 69.114$, $P < 0.0001$; StDM, $F_{1,15} = 13.747$, $P < 0.01$; StDL, $F_{1,15} = 5.905$, $P < 0.05$; mPC, $F_{1,15} = 83.199$, $P < 0.0001$).

Effects of an Acute COC Injection on NGFI-B Expression

In the AcSh, significantly higher basal NGFI-B mRNA levels were observed in TG mice compared to WT (ANOVA: $F_{1,16} = 10.248$, $P < 0.001$; Figure 4 upper panels and Figure 5a), whereas it did not vary significantly in the other brain regions studied. In this nucleus accumbens subdivision, acute COC administration led to a significant downregulation of NGFI-B mRNA levels in TG mice ($P < 0.05$), whereas

a nonsignificant increase was measured in WT ($P = 0.0567$) (Figure 5a). In the striatum, the levels of NGFI-B mRNA did not differ between mouse types receiving vehicle. It increased significantly in both striatal subdivisions in WT and TG mice following an acute COC injection (StDM, $F_{1,16} = 62.175$, $P < 0.0001$; StDL, $F_{1,16} = 26.891$, $P < 0.0001$) (Figures 5c and d). In contrast, NGFI-B mRNA levels were unaltered by an acute COC injection in both the AcC and the mPC (AcC, $F_{1,16} = 0.028$, $P = 0.87$; mPC, $F_{1,16} = 0.770$, $P = 0.393$). There was again no difference between WT and TG mice (AcC, $F_{1,16} = 1.403$, $P = 0.254$; mPC, $F_{1,16} = 0.69$, $P = 0.418$).

Effects of a Repeated COC Treatment on NGFI-B Expression

In the AcSh, the factorial ANOVA did not reveal any significant effect of the COC challenge ($F_{1,13} = 39.743$, $P = 0.5170$), the mouse type ($F_{1,13} = 1.751$, $P = 0.2086$) nor an interaction between these factors ($F_{1,13} = 3.210$,

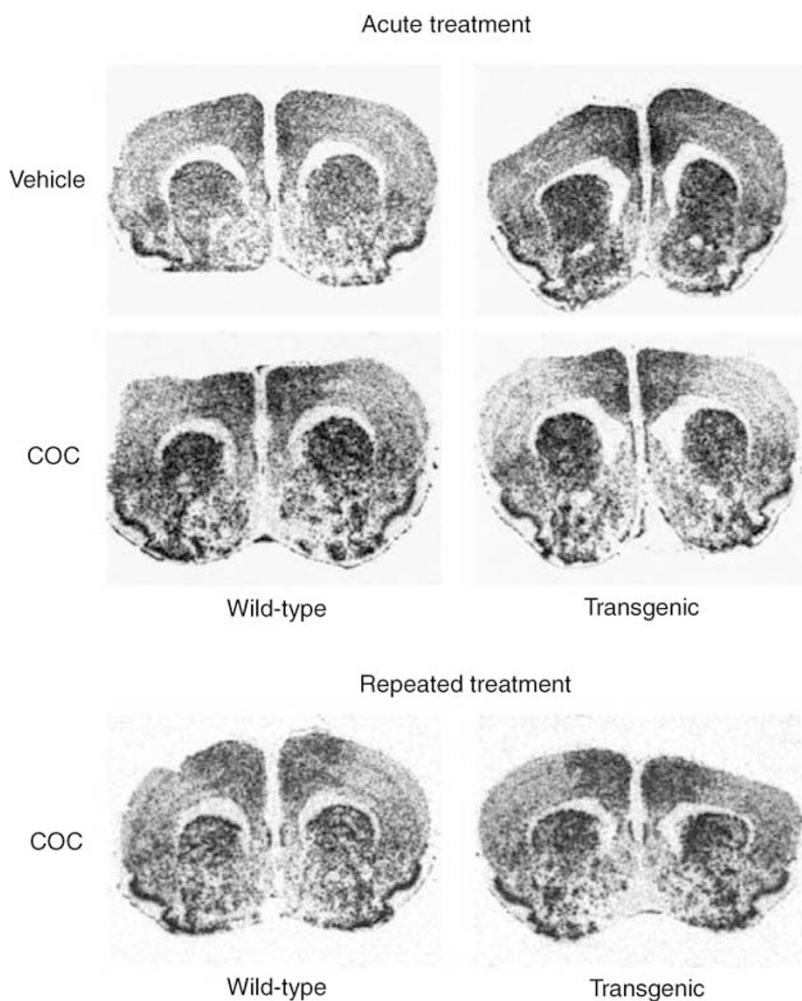


Figure 4 Representative autoradiograms showing NGFI-BmRNA levels radiolabeled by the [³⁵S]-antisense riboprobe in WT and TG mice receiving the vehicle (upper panels); acutely treated with COC (20 mg/kg) (middle panels) or challenged with COC following a 5-day COC pretreatment (20 mg/kg) and a 5-day washout period (lower panels).

$P = 0.0965$) when comparing the groups receiving the COC challenge to their vehicle controls from the same condition (Figure 5a). Noticeably, in TG mice, repeated COC administration maintains the NGFI-B mRNA decrease seen after the acute COC injection. In the StDM, a significant effect of the treatment ($F_{1,13} = 76.396$, $P < 0.0001$) and a difference between mouse types ($F_{1,13} = 12.642$, $P < 0.005$) were observed when analyzing means from the repeated condition (Figure 5c). TG mice consistently express more NGFI-B mRNA than WT mice and COC treatment significantly upregulated NGFI-B mRNA in both mouse strains. No effects were found in the StDL nor in the mPC (Figures 5d and e).

Effects of COC on Locomotor Activity

For the vehicle injection that occurred the day prior to the beginning of the repeated COC treatment, the ANOVA for repeated measures reveal an effect of the time course ($F_{3,45} = 43.683$, $P < 0.0001$), as well as a difference between the mouse types ($F_{1,15} = 4.664$, $P < 0.05$) and an interaction

between these two factors ($F_{3,45} = 7.592$, $P < 0.001$) (Figure 6a). The *post hoc* comparisons between periods $[-30-0]$ and $[0-30]$ showed a significant increase of locomotor response in WT animals ($P < 0.0001$), without any significant increase of locomotion in TG mice ($P = 0.1482$). On the first COC administration, both mouse types displayed an increased locomotor response ($F_{3,24} = 9.367$, $P < 0.001$) (Figure 6b). This effect appeared once again blunted in TG mice as suggested by the ANOVA for repeated measures ($F_{1,15} = 4.139$, $P = 0.06$). *Post hoc* comparison does in fact show a significant difference between mouse type at the peak of COC-induced locomotor response ($P < 0.05$) (Figure 6b). Following the subsequent administration of the same COC dose, there was a potentiation of COC-induced locomotor response in TG mice. There was a significant increase in locomotor response in TG animals at the third COC injection when compared to the first one ($P < 0.005$). No significant change was observed in COC-induced locomotor response of WT animals throughout the entire repeated treatment. On the third COC administration, the ANOVA for repeated measures indicate an effect on the

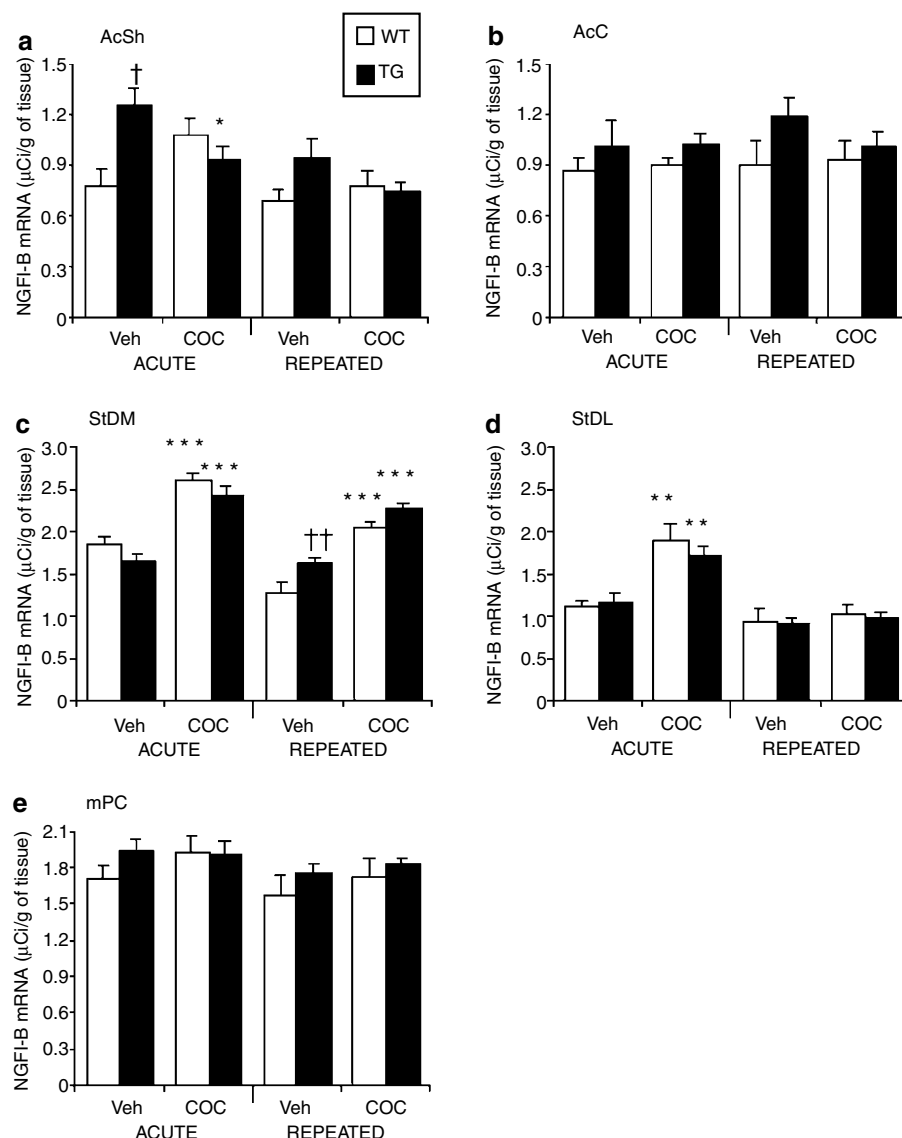


Figure 5 Histograms depicting NGFI-B mRNA levels after acute and repeated COC (20 mg/kg) administration. In the repeated experiment mice received vehicle or COC following a 5-day COC pretreatment (20 mg/kg). NGFI-B mRNA expression was assessed in the (a) AcSh, (b) AcC, (c) StDM, (d) StDL, and (e) mPC. Results shown represent means \pm SEM. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ when compared to the same mouse type receiving the vehicle. †† $P < 0.001$, † $P < 0.05$ when compared to WT mice receiving the same treatment. These comparisons were done inside the same treatment, not between means from the acute and the repeated administration.

time course ($F_{3,45} = 62.567$, $P < 0.0001$), as well as an interaction between the mouse type and the time course response ($F_{3,45} = 9.194$, $P < 0.0001$). The *post hoc* comparisons indicate a significant increase in COC-induced locomotor response in TG mice for the period 0–30 min when compared to WT ($P < 0.005$) (Figure 6c). The fourth (not shown), the fifth, and the last COC injections gave rise to identical time course responses between mouse types (Figure 6d and e), although the ANOVA for repeated measure indicates a nonsignificant interaction between factors ($F_{3,18} = 2.690$, $P < 0.0771$) on the last COC injection because of TG mice-increased basal activity (Figure 6e). Injection of the vehicle on the last day of the treatment produced an increase in locomotor response in both mouse types ($F_{3,18} = 10.202$, $P < 0.005$) (Figure 6f). This effect was,

however, stronger in TG animals ($F_{1,6} = 6.449$, $P < 0.05$). Once again we must take into account TG mice-increased basal activity ($P = 0.0126$).

DISCUSSION

This study shows that chronic alteration of the HPA axis can modulate the effect of an acute COC administration on the induction of transcription factors *c-fos* and NGFI-B in target structures of DA pathways. These changes at the molecular levels are paralleled by changes at the behavioral level. Our results also demonstrate that repeated COC administration can eliminate the differences between TG

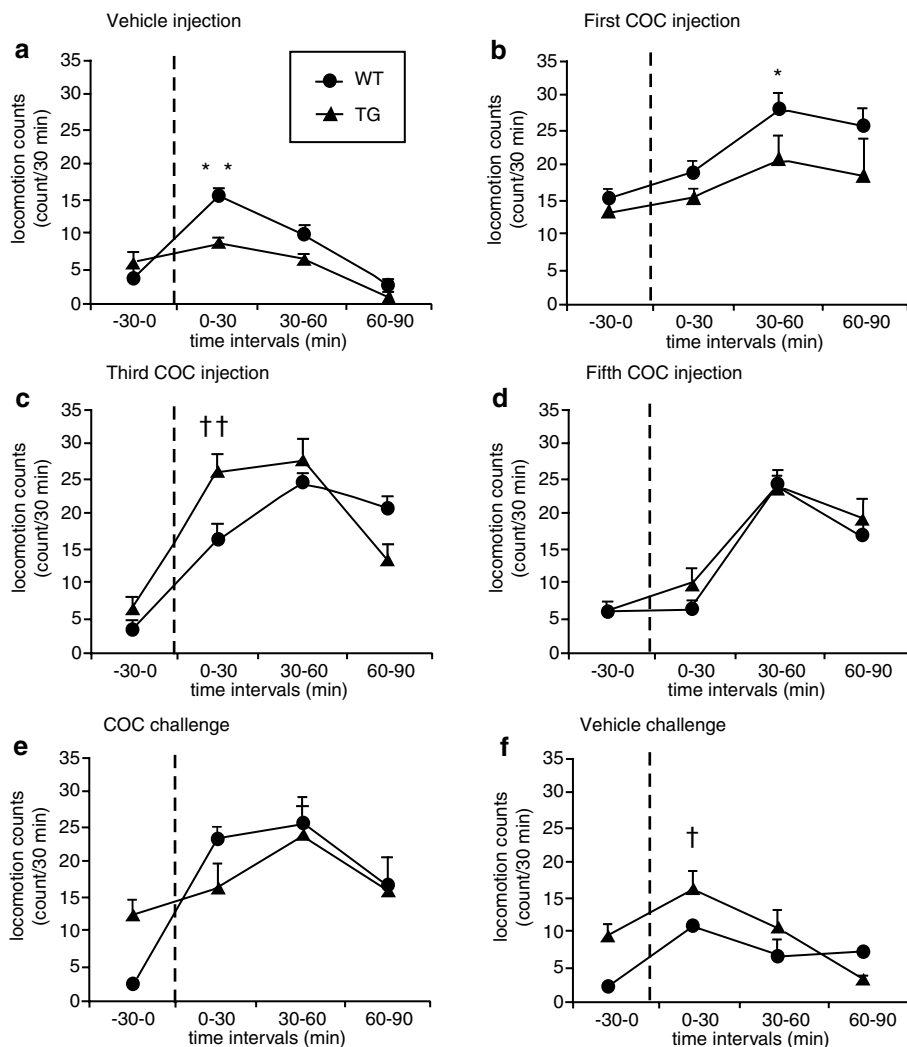


Figure 6 Line charts showing the time course of the locomotor response on different days during the repeated COC experiment: on the day prior to the beginning of the COC treatment, following the injection of the vehicle (a), on the first COC administration day (b), on the third COC administration day (c), on the fifth COC administration day (d) and on day 10, following the COC (e) or vehicle (f) challenge. Dashed lines correspond to the time of the injection. Results shown represent means \pm SEM. ** $P < 0.01$, * $P < 0.05$ when WT mice present higher locomotor response than TG for the corresponding 30-min period. †† $P < 0.01$, † $P < 0.05$ when TG mice present higher locomotor response than WT for the corresponding 30-min period.

and WT mice suggesting that repeated COC might have a greater impact in TG mice underexpressing GRs.

Modulation of *c-fos* Expression in TG Mice Following Acute COC Injection

In WT animals, all target structures of DA pathways displayed important increases in *c-fos* mRNA levels following both acute COC injection and repeated COC treatment. However, in mice with impaired GR function, acute COC administration had no or reduced effect on *c-fos* expression in both the nucleus accumbens and the striatum. In accordance with our results, it has been recently observed that adrenalectomy in rats reduces basal DA extracellular level and decreases COC-induced elevation of Fos-like proteins and extracellular DA in the AcSh (Barrot *et al*, 2000). Since our mice underexpress GR, some could

argue that DA elevation in response to COC was also blunted in TG mice, explaining the smaller *c-fos* increase reported here. Although the hypothesis of a hypoactive DA function would corroborate our acute data, it would be difficult to reconcile with previous studies done in these mice. Effectively, TG mice underexpressing GRs have higher basal DA synthesis levels (Cyr *et al*, 2001) and morphine-induced DA release is increased in the nucleus accumbens (Sillaber *et al*, 1998). Moreover, an increased amphetamine-induced locomotor response has been observed in these TG mice (Cyr *et al*, 2001). One explanation for the discrepancy between those results and both our behavioral and neurochemical data may stem from an alteration of DA reuptake reported in these TG mice. Cyr *et al* (2001) have observed lowered levels of DA transporter (DAT) binding sites in the striatum of these TG mice without any change in DAT mRNA levels in the substantia nigra pars compacta.

Whereas amphetamine elevates extracellular DA levels by acting both on its reuptake and its release (Sulzer and Rayport, 1990; Sulzer *et al*, 1993), COC rather acts solely by the former mechanism (Ritz *et al*, 1990a,b). Since *c-fos* induction after COC administration mostly depends on D₁ receptor stimulation (Drago *et al*, 1996; Das *et al*, 1997), DAT decrease levels in TG mice should then result in a decreased COC effect on D₁ receptor. A reduced effect of COC on DA levels in the striatum and the nucleus accumbens could also explain its blunted effect on locomotor response in TG mice. Accordingly, it has been shown that glucocorticoid suppression can also decrease DAT binding in the AcSh (Sarnyai *et al*, 1998). This hypothesis is supported by the fact that, amongst all DA target structures investigated in the present study, only the mPC was not influenced by the GR knockdown with regard to COC-induced *c-fos* expression. It has been shown previously that COC increases extracellular DA in the mPC mainly through its action on NA transporter (Tanda *et al*, 1997; Yamamoto and Novotney, 1998). This particular mechanism of action of COC in the mPC might explain the absence of difference between mouse strains in this brain region. Together, these results suggest that alteration of HPA functioning may result in an imbalance between DA reuptake in different parts of the brain, which could have an impact on COC addictive properties.

Modulation of *c-fos* Expression in TG Mice Following a Repeated COC Treatment

An interesting finding of our study is that the repeated COC pretreatment potentiated the effect of the COC challenge on *c-fos* expression. Although this effect appears to occur in both mouse types in the nucleus accumbens, it was more prominent in TG animals since it abolished differences in *c-fos* expression observed between mouse strains following acute COC administration. Repeated COC treatment also wipes out behavioral differences between mouse strains observed on the first COC administration. Not only COC pretreatment does potentiate the effect of a subsequent COC challenge, but it also potentiates the effect of the vehicle injection on *c-fos* induction in the mPC. Moreover, it produced a COC-conditioned hyperactivity in TG animals, as shown when comparing the effect of the vehicle injection before and after the COC treatment. COC-conditioned hyperactivity is also suggested by the increase in TG mice activity during the -30-0 min period on the last treatment day. The formation of Pavlovian conditioning is thus likely to have occurred by the association of neutral stimuli, such as the presence of the experimenter and the injection, and the unconditional rewarding effect of COC. Other studies have also reported a correspondence between COC-conditioned hyperactivity and enhanced Fos expression within the prefrontal cortex and the nucleus accumbens (Franklin and Druhan, 2000; Neisewander *et al*, 2000). Such observations may underlie the implication of those structures in the development of incentive motivation towards COC and other drugs of abuse.

The restoration of COC-induced *c-fos* expression noted here could result from COC's own pharmacological properties. In addition to its DA reuptake blocking properties, COC shares with antidepressants the capacity to increase

serotonin and noradrenaline levels at the synapse (Chen and Reith, 1994; Andrews and Lucki, 2001). The TG mouse model used in the present study faithfully reproduce many of the neuroendocrinological changes that are known to accompany depression (Beaulieu *et al*, 1994). Interestingly, treatment with the antidepressant moclobemide for 8 weeks normalizes both basal corticosterone and serotonin increases noted in TG mice underexpressing GR and it brings to control levels the exaggerated increase of DA and 5-HT measured in these TG mice following morphine administration (Sillaber *et al*, 1998). Chronic treatment with another antidepressant, amitriptyline, was also successful in correcting the decreased DAT levels in the same TG mice (Cyr *et al*, 2001). Thus, the capacity of the repeated COC treatment to restore *c-fos* induction in striatum and the nucleus accumbens in the present study may stem from its concomitant action on 5-HT and DA systems.

COC-Induced NGFI-B Expression in TG Mice

NGFI-B belongs to the nuclear receptor family of transcription factors and is closely associated with DA systems in the brain. Its mRNA level in the brain is modulated by various treatments interacting with DA neurotransmission (Gervais *et al*, 1999; Tremblay *et al*, 1999; Beaudry *et al*, 2000; Werme *et al*, 2000b; Langlois *et al*, 2001). Previous data in the literature indicate that its expression is increased by acute and chronic morphine and COC administration in rats although the effects are different in Lewis and Fisher rat strains (Werme *et al*, 2000a). In accordance with these results, we found that acute and chronic COC administration increase NGFI-B mRNA expression in the striatum of WT mice. However, TG mice had significantly higher basal NGFI-B levels in the AcSh compared to WT and surprisingly, acute COC administration decreased NGFI-B mRNA expression in this subdivision of the nucleus accumbens, an effect of COC opposed to the one observed in WT. This particular NGFI-B induction pattern suggests important alterations of DA function in the AcSh. Giving its important role in COC addiction, changes in AcSh plasticity could explain TG mice-increased behavioral sensitivity towards repeated COC injection.

The exact role of NGFI-B in target structures of the DA systems is not yet unraveled. We have previously shown that interaction between 5-HT_{1A} and DAergic drugs can lead to different pattern of NGFI-B expression (Gervais *et al*, 1999). Indeed D₁, D₂, or 5-HT_{1A} receptor agonists administered individually decrease NGFI-B expression, whereas the concomitant administration of D₁ and 5-HT_{1A} agonist increases NGFI-B mRNA levels in the striatum. In fact, complex interactions between DA and 5-HT could take place in TG mice underexpressing GR and may be responsible for the basal upregulation of NGFI-B in the AcSh, as well as for the opposite effect of COC on its regulation. Using microdialysis, Sillaber *et al* (1998) reported enhanced basal 5-HT levels in the nucleus accumbens of this TG mice strain compared to WT mice. We could hypothesize that NGFI-B mRNA elevation in the nucleus accumbens represents a neuroadaptive mechanism occurring in response to an altered 5-HT_{1A} tone in TG animals.

Behavioral Sensitization Following Repeated COC Administration

According to the behavioral sensitization paradigm (Wise and Bozarth, 1987), the enhanced locomotor response induced by the repeated administration of an addictive drug reflects neuronal adaptation in brain circuit mediating its rewarding effects. In the present study, a clear behavioral sensitization was not shown in WT animals and was only apparent in TG from days 1 to 3, after which the increase in locomotor response stayed relatively stable. This ceiling effect could in part be because of the dose used and the fact that COC was administered in the animal home cages. It has been shown that COC at a dose of 10–20 mg/kg dose would be inclined to give rise to stereotyped behaviors (Hiroi *et al*, 1997). Such behaviors, like sniffing and rearing, would have been difficult to detect with the telemetric system used. Another explanation for the lack of behavioral sensitization in WT animals could be related to the paradigm of behavioral recording employed in this study. There is some lines of evidence in the literature suggesting that behavioral sensitization is context-dependent (Badiani *et al*, 1995). As mentioned in the methodology, our animals were recorded and injected in their home cage throughout the entire COC repeated treatment. This particular testing paradigm restricts COC-related cue diversity to the presence of the experimenter and the act of injection. Nevertheless, TG mice displayed behavioral sensitization and COC-conditioned hyperactivity, suggesting a decreased threshold for behavioral sensitization in TG mice bearing an altered HPA axis.

Many lines of evidence argue for a role of stress and glucocorticoid in the attribution of an increased sensitivity towards the effects of drugs of abuse. Like high-responder rats (Piazza *et al*, 1989), mice underexpressing GR display higher locomotive activity when placed into a novel environment (Beaulieu *et al*, 1994). The intensity of the locomotive response produced by a novel environment predicts the behavioral response towards addictive substances. Accordingly, high-responder rats will display higher locomotive response following the administration of amphetamine and COC (Piazza *et al*, 1989; Hooks *et al*, 1991). Kabbaj *et al* (2000) have shown that high-responder rats present lower GR mRNA levels in the hippocampus. This decrease in GR mRNA was proposed to be responsible for their carefree-like behavior while exploring an unfamiliar environment since GR in the hippocampus plays a major role in the regulation of the stress response (Sapolsky *et al*, 1990). Interestingly, our TG mice underexpressing GR also present deficiency in their evaluation of dangerous environments. Indeed, TG mice spend more time than WT in the open arm of the elevated plus maze (Rochford *et al*, 1997; Strohle *et al*, 1998). According to these studies, we also reported here a blunted effect of the vehicle injection on TG mice locomotor response, whereas it produced a normal stress-induced locomotor activation in WT. This observation shows that TG mice do not respond properly to stressful events, further confirming their altered HPA axis state and the implication of such disturbance in COC addiction.

In summary, our results obtained with TG mice underexpressing GR showed that alteration of the HPA axis function can modulate acute COC-induced *c-fos* and NGFI-

B expression in brain structures thought to mediate the rewarding effects of drugs of abuse. An increased basal NGFI-B expression in the AcSh also suggests particular neuronal adaptations induced by GR knockdown in this structure. Finally, we showed that repeated COC administration could potentiate both *c-fos* induction and COC-induced locomotor response in TG mice so to abolish the differences initially noted between TG and WT mice. Despite this apparent similitude between mouse strains following repeated COC treatment, TG mice were the only ones to display COC-conditioned hyperactivity on the challenge day. Taken together, these observations emphasize the role of the HPA axis in COC addictive properties.

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