



# The role of glucocorticoids in the uncontrollable stress-induced potentiation of nucleus accumbens shell dopamine and conditioned place preference responses to morphine

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**Summary** Exposure to stressors can impact on the responsiveness to drugs of abuse, and glucocorticoid hormones (CORT) may interact with dopamine (DA) within the nucleus accumbens shell (NAcs) to mediate these responses. We have previously shown that the CORT response to morphine, but not to a previous uncontrollable stressor, is necessary for the stress-induced potentiation of morphine's rewarding effects. Here, we test (1) the necessity of CORT during inescapable stress (IS) and/or morphine for IS potentiation of morphine-induced NAcs DA and (2) the sufficiency of enhanced CORT, in the absence of prior IS, to potentiate morphine-induced NAcs DA as well as morphine conditioned place preference (CPP) in male Sprague-Dawley rats. In the first experiment, we administered the CORT synthesis inhibitors metyrapone and aminoglutethimide (100 mg/kg each, sc) to suppress the CORT response to either IS (100 mA tailshocks) or subsequent morphine (3 mg/kg, sc) treatment. Twenty-four hours after IS, microdialysis was performed and morphine was administered. In the next experiments, CORT (1 mg/kg, sc) was injected 20 or 30 min before morphine during either microdialysis or CPP testing, respectively, in non-stressed rats. We found that IS potentiated subsequent morphine-induced NAcs DA and this was completely blocked by CORT suppression before morphine, but not before IS. However, elevated levels of CORT concurrent with morphine, but in the absence of a stressor, failed to potentiate NAcs DA or CPP. These results suggest that the CORT response to morphine is necessary, but not sufficient in the absence of prior IS, for sensitized NAcs DA and CPP responding to morphine, and provide further evidence that CORT is involved in the expression, but not the induction, of this sensitization. © 2006 Elsevier Ltd. All rights reserved.

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## 1. Introduction

Glucocorticoid hormones (cortisol in humans, corticosterone (CORT) in rats) are released as a result of hypothalamic-pituitary-adrenal axis activation in response to both stressful (Carrasco and Van de Kar, 2003) and positively reinforcing stimuli such as drugs of abuse (Piazza and Le Moal, 1997). Dopamine (DA), particularly within the shell of the nucleus accumbens (NAcs), can also be regulated by stressors (Thierry et al., 1976; Abercrombie et al., 1989) and is argued to mediate the anticipatory effects of drugs of abuse (Koob and Nestler, 1997; Berridge and Robinson, 2003; Adinoff, 2004; Di Chiara et al., 2004), such that both types of stimuli tend to elevate NAcs DA. The fact that both CORT and DA are sensitive to both classes of environmental stimuli suggests that the interaction between these two chemical messengers may be involved in mediating the differential responding to positively reinforcing drugs following a single or repeated stressful experience. Indeed, there is high comorbidity between stress-related disorders and substance abuse in humans (Kilpatrick et al., 2000; Jacobsen et al., 2001; Sinha, 2001), and a variety of environmental stressors have been shown to increase the rewarding effects of potentially addictive drugs in animals as well (Piazza and Le Moal, 1998; Lu et al., 2003). However, the exact roles of CORT and DA in stress-induced potentiation of the effects of drugs of abuse are not entirely clear.

Drugs such as opiates and psychostimulants are commonly self-administered in rodents and can elevate CORT (Piazza and Le Moal, 1997) and NAcs DA (Pontieri et al., 1995; Barrot et al., 1999). Both the behavioral and DA responses to drugs may be mediated in part by CORT as surgical or pharmacological adrenalectomy (without basal replacement) attenuates self-administration of and locomotor responding to cocaine (Goeders and Guerin, 1996; Deroche et al., 1997; Marinelli et al., 1997) as well as the NAcs DA response to morphine and cocaine (Barrot et al., 2000). As already noted, a variety of acute or chronic stressors can enhance the rewarding effects of drugs. However, the mechanisms underlying these effects are not entirely clear, and may involve the regulation of NAcs DA neurotransmission, and thus drug responding, by either stress- or drug-induced CORT increases.

We have previously shown that exposure to an acute uncontrollable stressor prior to drug administration potentiates the (1) rewarding effects of morphine as measured by conditioned place

preference (CPP) (Will et al., 1998) and psychomotor responding (Will et al., 2002), (2) CORT response to morphine (Der-Avakian et al., 2005), and (3) NAcs DA response to morphine (Bland et al., 2004b). The behavioral response is mediated in part by CORT, as both adrenalectomy (with basal replacement) and pharmacological suppression of the CORT response to morphine, but not to the stressor, blocks the potentiation of morphine CPP by uncontrollable stress (Der-Avakian et al., 2005). However, it is not known whether acute stressor sensitization of the NAcs DA response to a rewarding drug is, in general, mediated by CORT or, more specifically, whether a potentiated CORT response is involved in the uncontrollable stress-induced potentiation of DA efflux to morphine. In the following study, we used *in vivo* microdialysis to determine whether the CORT response during either the stressor or the drug administration is necessary for the potentiated NAcs DA response to morphine that is produced by the previous uncontrollable stressor (inescapable stress, IS).

Although some studies have shown that CORT suppression blocks the basic reinforcing effects of drugs, fewer studies have reported whether simply elevating CORT in the absence of a stressful stimulus would affect drug responding. This type of manipulation addresses the issue of whether CORT by itself is sufficient to enhance responding to drugs, or whether it acts in a permissive manner in the presence of a stressor. Repeated administration of stress-induced levels of CORT has been shown to enhance the locomotor properties and self-administration of psychostimulants (Deroche et al., 1992b; Mantsch et al., 1998; Patacchioli et al., 1998). Furthermore, acute CORT administration has been shown to enhance NAcs DA in a state-dependent manner (Piazza et al., 1996b). However, little is known regarding the effects of acute CORT administration on drug-induced NAcs DA and drug reward, or the effects of CORT on responding to opiates. Therefore, we also examined the sufficiency of acute stress-induced levels of exogenous CORT administered concurrently with morphine, but in the absence of stress, to enhance both the neurochemical (NAcs DA) and behavioral (CPP) measures of morphine reward.

## 2. Materials and methods

### 2.1. Subjects

Adult male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 280-400 g

were housed in pairs in Plexiglas cages with food and water available *ad libitum*. The subjects were maintained in a climate-controlled colony room at 22 °C on a 12 h light-dark cycle, and all experiments were conducted during the light phase (0600-1800 h). All subjects were naive and allowed a minimum of 1 week adaptation prior to surgery or testing. All animal care and experimental procedures were in accord with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

## 2.2. Apparatus

### 2.2.1. Stressor

The stressor environment was a dimly lit room with dimensions of approximately 3.0×2.5×2.5 m. IS occurred in Plexiglas restraint tubes that measured 17.5 cm in length and 7.0 cm in diameter. The front end of the tube was blocked by a Plexiglas plunger containing several air holes. Each rat's tail extended from the rear of the tube and was taped to a Plexiglas rod measuring 4.0 cm in length. Two copper strips were coated with a small amount of electrode paste, wrapped 4.0 cm apart around the midsection of the tail, and attached to two electrodes. Tailshocks were delivered using a Precision-Regulated Animal Shocker with Graphic State 3.0 software (Coulbourn Instruments, Allentown, PA).

### 2.2.2. HPLC

DA in the dialysates was determined using an ESA 5600A Coularray detector with an ESA 5014B analytical cell and an ESA 5020 guard cell connected to an ESA HR80 column (C<sub>18</sub>, 3 μm, 80×3 mm) which was maintained at 30 °C. The mobile phase was 150 mM sodium dihydrogen phosphate monohydrate, 4.76 mM citric acid monohydrate, 3 mM sodium dodecyl sulfate, 50 μM EDTA, 10% methanol, and 15% acetonitrile, pH=5.6 with sodium hydroxide. The potentials were set at -75 and +220 mV, and the guard cell potential was set at +250 mV. Injections were performed with an ESA 542 autosampler using an injection volume of 27 μl. Quantitative comparisons were made with external standards (Sigma-Aldrich, St Louis, MO) that were run each day.

### 2.2.3. Conditioned place preference

The Plexiglas place preference apparatus measured 72×30×30 cm (length, width, height) and was composed of two distinct conditioning environments and a neutral area. Each conditioning environment measured 30×30×30 cm. One environment was

striped horizontally with alternating 2 cm black and white electrical tape on the walls, while the other environment was striped vertically in the same manner. The floor of the apparatus was black sanded Plexiglas with a 2 cm wire grid on the horizontal side and a 3 mm wire mesh on the vertical side. The neutral area measured 12×30×30 cm, was painted gray, and had no wire mesh or grid on the floor. During the conditioning treatments, vertically and horizontally striped Plexiglas partition walls were inserted on the respective sides of the neutral area to restrict the animals to their designated conditioning environment.

The activity of each subject was monitored by a Philips TC352A video camera (Lancaster, PA) mounted 1.5 m above the center of the CPP apparatus. The camera relayed the information of the subject's location to the Chromotrack Version 4.02 tracking software (Prototype Systems, Ltd, Boulder, CO) run on a PC compatible computer located in a separate room. The SA-3 tracker (San Diego Instruments, San Diego, CA) measured the subject's time spent within each of the three compartments.

## 2.3. Drugs

Morphine sulfate (NIDA) was dissolved in 0.9% sterile saline and injected at a dose of 3.0 mg/kg. Metyrapone and aminoglutethimide (Sigma-Aldrich, St Louis, MO) were dissolved in propylene glycol and injected simultaneously at a dose of 100 mg/kg each. Metyrapone, an inhibitor of 11-β hydroxylase (Sonino, 1982), and aminoglutethimide, an inhibitor of cholesterol side-chain cleavage (Touitou et al., 1973), were used in conjunction to be assured of complete suppression of endogenous CORT levels. Although the half-life of metyrapone is short (20-26 min), its derivatives (e.g. metyrapol) are as effective in inhibiting CORT synthesis for a longer period, and enzyme activity is normalized by 8 h after a single administration (Sonino, 1982). The pharmacokinetics of aminoglutethimide are not as well characterized, however, for the purposes of this study, we have verified that both inhibitors block the CORT responses to the stressor and drug manipulations described below, and the doses used here were identical to those previously used for the same purpose (Der-Avakian et al., 2005). Exogenous CORT (Sigma-Aldrich, St Louis, MO) was dissolved in propylene glycol and injected at a dose of 0, 0.5, and 1.0 mg/kg in the second experiment and 1.0 mg/kg in the final two experiments. All drugs

were injected subcutaneously at a volume of 1.0 ml/kg body weight.

## 2.4. Procedure

### 2.4.1. Experiment 1: effect of temporary CORT suppression on the IS potentiation of NAcS DA in response to morphine

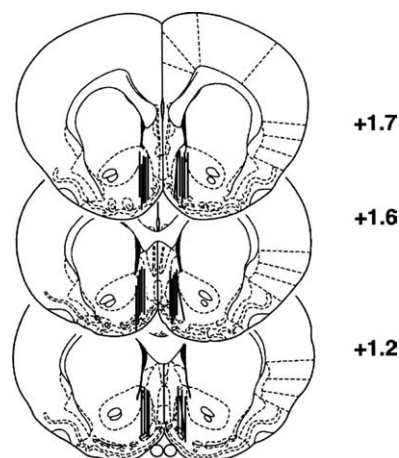
**2.4.1.1. Surgery.** Microdialysis guide cannula implantation was performed under halothane anaesthesia. CMA 12 guide cannulae (CMA Microdialysis) were aimed at either the right or left NAcS (AP = +1.7, LM = ±0.8, DV = -6.0) in a counter-balanced fashion. Coordinates were from bregma using the atlas of Paxinos and Watson (1998). The guide cannulae and a tether screw (CMA Microdialysis) were anchored to the skull with three jeweler's screws and dental cement. Rats were individually housed after surgery and allowed to recover for one week.

**2.4.1.2. Inescapable stress (IS).** Rats were randomly assigned to either receive IS or remain in their home cages (HC) as non-stressed controls. One-third of the rats exposed to either treatment received metyrapone and aminoglutethimide (MA; 100 mg/kg each, sc) and two-thirds received vehicle 1 h before initiation of IS. We have previously used this procedure to block the CORT response to IS and have verified that this response is indeed blocked (Der-Avakian et al., 2005). The stressor consisted of 100 tail shocks (1.0 mA, 5 s each) delivered on a 1 min variable-interval schedule (30-90 s ITI). Stressor exposure took place between 0900 h and 1100 h, and rats were returned to their home cages immediately following the session.

**2.4.1.3. Microdialysis.** On the afternoon before microdialysis, and approximately 4 h after the end of the IS treatment, rats were transferred to the dialysis room that was on the same light-dark cycle as the colony room. Microdialysis probes (CMA 12, MW cut-off 20,000 Da, 2 mm active membrane) were inserted into the guide cannulae and rats were placed in separate Plexiglas infusion bowls with food and water available ad libitum. Ringers solution (147 mM NaCl, 2.97 mM CaCl, 4.02 mM KCl; Baxter) was perfused through the probes using a CMA infusion pump at a flow rate of 0.2 µl/min overnight. The flow rate was increased to 1.5 µl/min the next morning and, after a 2 h equilibration period, sample collection began and dialysates were collected manually every 20 min and immediately placed in -80 °C until analysis.

Collection tubes were pre-filled with 3 µl of 0.02% EDTA (anti-oxidant) in 1% ethanol. After collection of three baseline samples, MA or vehicle was administered in the same manner as described above. We have also previously used this procedure to block the CORT response to morphine and have verified that this response is blocked as well (Der-Avakian et al., 2005). Rats that received MA before IS or HC treatment 24 h earlier received vehicle during microdialysis. Rats that received vehicle the previous day received either MA or vehicle during microdialysis. Three more samples were collected, followed by morphine administration (3.0 mg/kg, sc) and subsequent sampling for 120 min. We have previously observed that saline administration does not affect either medial prefrontal cortex serotonin (Bland et al., 2003) or NAcS DA (Bland et al., 2004b) in either IS or HC rats, so this group was not included. Thus, the experimental design was a two (IS vs. HC) × 3 (MA before IS/HC, before morphine, before neither) × 12 (time) factorial. Total sample sizes were five to seven in each group. Dialysates were analyzed by HPLC within 2 weeks of collection.

**2.4.1.4. Probe verification.** To verify probe placement, rats were euthanized with 65 mg/kg ip sodium pentobarbital. The brains were removed, frozen in chilled isopentane, and cryostat sectioned (40 µm) at -20 °C. Sections were mounted on gelatin-treated slides, stained with cresyl violet, and coverslipped. Only rats with probes placed within the NAcS were included in the analysis (Fig. 1).



**Figure 1** Histological representation of microdialysis probe placements in the nucleus accumbens shell. Coordinates are from bregma (mm) and are adapted from the atlas of Paxinos and Watson (1998). Not all probes are shown due to overlapping placements from both microdialysis experiments.

#### 2.4.2. Experiment 2: plasma CORT levels after exogenous CORT administration

**2.4.2.1. Plasma collection.** Adrenally intact rats were injected with either CORT (0.5 or 1.0 mg/kg, sc) or vehicle (0 mg/kg, sc) in their home cages at 1030 h. Serial blood samples were then taken 30, 60, 90, 120, and 240 min following the injection, beginning at 1100 h. Blood samples were obtained by taking the rat from its home cage, wrapping it gently in a towel, and restraining it with a Velcro strap, leaving the tail exposed. A small nick was made in the lateral tail vein and the tail was stroked until 200  $\mu$ l of whole blood was collected in a microfuge tube. This entire procedure lasted no longer than 1.5 min per rat. Blood samples were then spun in a centrifuge at 4 °C and 50  $\mu$ l of plasma was aliquoted and stored at -20 °C until assayed. Thus, the design was a three (CORT dose)  $\times$  5 (time) factorial. Total sample sizes were three to four in each group.

**2.4.2.2. RIA.** Total plasma CORT was measured by radioimmunoassay. Plasma samples (20  $\mu$ l) were diluted in 0.01 M PBS (1 ml) and corticosteroid binding globulin was inactivated by heat at 75 °C for 1 h. Samples and standards (25-2000 pg/tube) were then incubated overnight with antiserum (rabbit antibody B3-163; Endocrine Sciences, Inc., Tarzana, CA) and [<sup>3</sup>H] CORT (20,000 cpm/tube). Free CORT was separated from antibody-bound CORT with 500  $\mu$ l dextran-coated activated charcoal. Antibody-bound CORT was then mixed with scintillation cocktail (3 ml) and counted with a liquid scintillation counter (Packard, 1600TR). The assay sensitivity was approximately 0.5  $\mu$ g/ml for a 20  $\mu$ l plasma sample. The intraassay coefficient of variability was less than 11%.

#### 2.4.3. Experiment 3: effect of CORT enhancement on the NAcS DA response to morphine

Surgery and probe verification (Fig. 1) were performed exactly as above. One week following surgery, microdialysis was performed as mentioned above with the following exceptions. Following the third baseline sample, half the rats received CORT (1.0 mg/kg, sc) while half received vehicle. This dose was used since the plasma CORT response to the injection, as measured in the second experiment, most closely resembled the animals' CORT response to 3.0 mg/kg sc morphine 24 h following IS (Der-Avakian et al., 2005). One more sample was collected, followed by morphine (3.0 mg/kg, sc) or saline administration. Sampling continued for 160 min. Thus, the design was a two (CORT vs. vehicle)  $\times$  2 (morphine vs. saline)  $\times$  12 (time)

factorial. Total sample sizes were six to eight in each group.

#### 2.4.4. Experiment 4: effect of CORT enhancement on morphine CPP

One day prior to testing, a 2 $\times$ 2 cm piece of reflective tape was attached to a rat collar (BAS, West Lafayette, IN), which was then loosely collared around each rat's neck for detection by the tracking system. On day 1, between 1130 and 1300 h, all subjects were individually exposed to the CPP apparatus. Subjects were initially placed in the neutral area and allowed to explore the entire preference apparatus for 20 min. This day served to assess the subjects' initial preferences and any possible environmental biases. Any rat which spent less than 4 min (20% of total time) in either environment was eliminated from the study. On day 2, all rats remained in the colony room. On day 3, all subjects were weighed in the morning and given random counter-balanced conditioning assignments. Conditioning treatments occurred at 1000 and 1400 h for 45 min each. All rats received two conditioning treatments on this day, half with morphine and half without. The morphine-conditioned group received the drug (3.0 mg/kg, sc) during one treatment and was placed in one of the two environments. At the other time of day, the same animals received saline and were placed in the second environment. Conditioning assignments were counter-balanced with regard to time of morphine and saline injections and conditioning environment paired with morphine. The saline-conditioned group received saline during both treatments. Of the rats conditioned with morphine, half received an injection of CORT (1.0 mg/kg, sc) and half received vehicle in the colony room immediately before being transported to the CPP room and 30 min prior to only the morphine injection. Of the rats conditioned with saline, half received an injection of CORT and half received vehicle in the same manner as described above prior to one of the two daily conditioning treatments. These control groups were included to assess any possible reinforcing effects of CORT in the absence of morphine. On day 4, animals were conditioned in the same manner as on day 3, except the order of presentation was reversed. On day 5, testing of CPP was conducted between 1130 and 1300 h exactly as was performed on day 1. Subjects were simply placed in the neutral area of the preference apparatus and their presence in each compartment was measured for 20 min. Thus, the design was a two (CORT vs. vehicle)  $\times$  2 (morphine vs. saline conditioned) factorial. Total sample sizes were 10-11 in each group.

## 2.5. Statistical analysis

The microdialysis experiment involving MA was analyzed using a mixed design ANOVA between stressor (IS vs. HC) and between time of MA administration (before IS/HC vs. before morphine vs. before neither), with sampling time as a repeated measure. The CORT response experiment was analyzed using a mixed design ANOVA between dose (0, 0.5, 1.0 mg/kg), with sampling time as a repeated measure. The microdialysis experiment involving CORT was analyzed using a mixed design ANOVA between pre-treatment (CORT vs. vehicle) and between drug (morphine vs. vehicle), with time as a repeated measure. The CPP experiment was analyzed using a  $2 \times 2$  ANOVA between pre-treatment (CORT vs. vehicle) and between conditioning (morphine vs. saline). DA data are expressed as a percentage of the average of the baseline samples. CPP data are expressed as the difference in time (s) spent in the drug-paired environment between the pre- and post-conditioning test sessions. Positive scores indicate an increase in preference during the post-conditioning test for the previously morphine-paired environment. When appropriate, each ANOVA was followed by post hoc Newman-Keuls tests (alpha set at 0.05).

## 3. Results

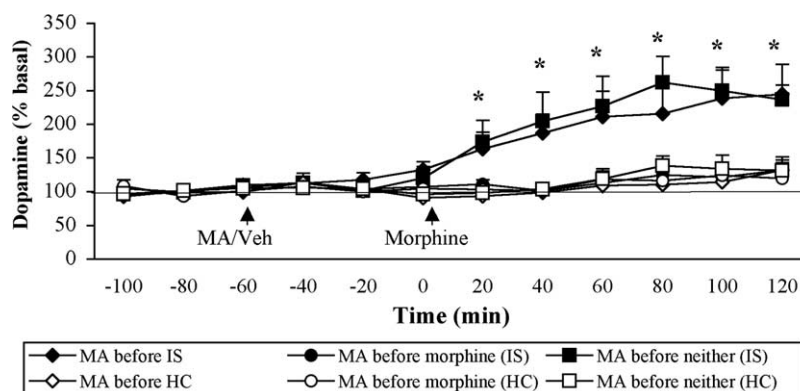
### 3.1. Experiment 1: effect of temporary CORT suppression on the IS potentiation of NAcS DA in response to morphine

DA levels as a percentage of baseline (mean + SEM) are shown in Fig. 2. HC rats showed a modest

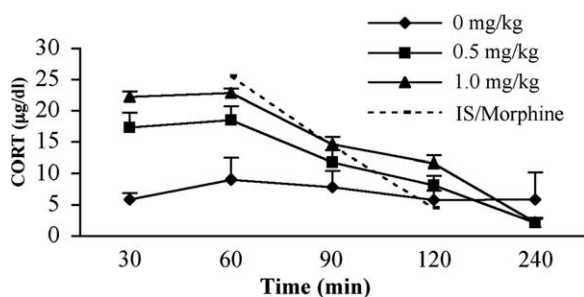
and delayed increase in DA efflux in the NAcS in response to morphine, and previous IS significantly potentiated this response. Inhibiting the CORT response to IS, but not to morphine, did not affect the potentiated DA response. Rats receiving prior IS, but with a suppressed CORT response to the stressor, still exhibited an enhanced DA response to later morphine. However, inhibiting the CORT response to morphine, but not to IS, completely blocked the potentiated DA response. Therefore, rats receiving prior IS, but with a suppressed CORT response to later morphine, showed only a modest increase in DA, similar to HC controls. There was a significant interaction between stressor, time of MA administration, and time [ $F(22,319)=1.77, p < 0.05$ ]. Post hoc tests revealed that IS rats with a suppressed CORT response to morphine had significantly less DA efflux in response to the drug compared to the other two IS groups. The levels for this group were similar to those observed in HC rats. Rats with a suppressed CORT response to IS had similar DA levels compared to vehicle treated rats that received IS. Also, average basal DA levels did not differ between groups and were:  $2.22 \pm 0.75$  pg/ $27 \mu\text{l}$  (HC, MA before morphine),  $2.31 \pm 1.17$  (HC, MA before HC),  $1.27 \pm 0.29$  (HC, MA before neither),  $1.54 \pm 0.50$  (IS, MA before morphine),  $1.98 \pm 0.74$  (IS, MA before IS),  $1.75 \pm 0.85$  (IS, MA before neither).

### 3.2. Experiment 2: plasma CORT levels after exogenous CORT administration

CORT levels (mean + SEM) were stable across the repeated testing in controls (Fig. 3). The lower



**Figure 2** Nucleus accumbens shell dopamine (DA) responses, expressed as a percentage of baseline (mean + SEM), to morphine (3 mg/kg, sc) 24 h following inescapable stress (IS) or home cage (HC) treatment. One IS and one HC group each received metyrapone and aminoglutethimide (MA; 100 mg/kg, sc) 1 h before these treatments and vehicle (Veh) before morphine administration (MA before IS or HC). Another IS and HC group received vehicle before these treatments and MA 1 h before morphine administration (MA before morphine (IS or HC)). The final IS and HC groups received vehicle before all treatments (MA before neither (IS or HC)). \*Different from 'MA before morphine (IS)' group and all HC groups ( $p < 0.05$ ).



**Figure 3** Plasma corticosterone (CORT) levels (mean + SEM) following an injection of either 1.0 or 0.5 mg/kg sc CORT or vehicle (0 mg/kg) in adrenalectomized intact animals. Serial tail bleeds were performed beginning 30 min after CORT administration. The dashed line represents CORT levels following morphine administration (3.0 mg/kg, sc) in animals that received inescapable stress (IS) 24 h earlier (Der-Avakian et al., 2005).

dose of CORT (0.5 mg/kg, sc) resulted in elevated plasma levels at 30 and 60 min following the injection. However, 1.0 mg/kg sc CORT also resulted in elevated plasma levels at 30 (22.091 + 0.818 µg/dl) and 60 (22.665 + 0.766 µg/dl) min, and these values closely resemble endogenous CORT levels in response to morphine in rats that received IS 24 h earlier (Der-Avakian et al., 2005). There was a main effect of dose [ $F(2,8)=7.443$ ,  $p<0.05$ ] and time [ $F(4,32)=44.355$ ,  $p<0.0001$ ], and a significant interaction between the two [ $F(8,32)=8.026$ ,  $p<0.0001$ ]. Also, CORT levels returned to baseline 4 h after the injection. In experiment 4, half the rats received a CORT injection prior to the morning conditioning session. These results verify that plasma CORT will have returned to basal levels by the afternoon conditioning session 4 h later. Therefore, the 1.0 mg/kg dose of CORT was used in experiments 3 and 4.

### 3.3. Experiment 3: effect of CORT enhancement on the NAcS DA response to morphine

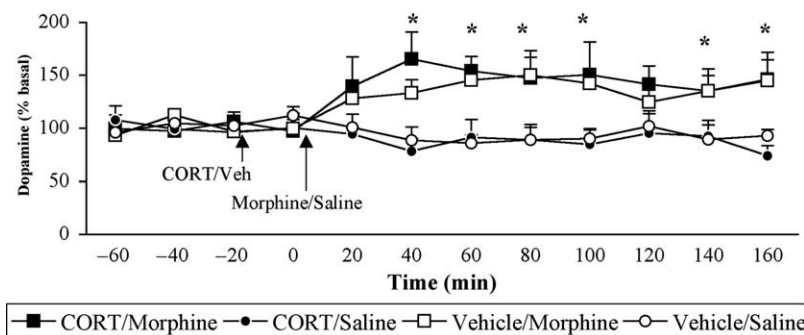
Morphine increased DA efflux in the NAcS, whereas saline did not (Fig. 4). This increase in response to morphine was not affected by pre-treatment with CORT. Moreover, administration of CORT alone failed to increase DA levels in saline-treated rats. There was a main effect of drug [ $F(1,22)=15.98$ ,  $p<0.001$ ], with morphine elevating DA levels equally regardless of pre-treatment. The analysis also revealed a significant interaction between drug and time [ $F(11,242)=4.47$ ,  $p<0.001$ ]. In addition, average basal DA levels did not differ between groups and were: 1.31 + 0.22 pg/27 µl (veh/saline), 1.09 + 0.17 (veh/morphine), 1.53 + 0.16 (CORT/saline), 1.36 + 0.19 (CORT/morphine).

### 3.4. Experiment 4: effect of CORT enhancement on morphine CPP

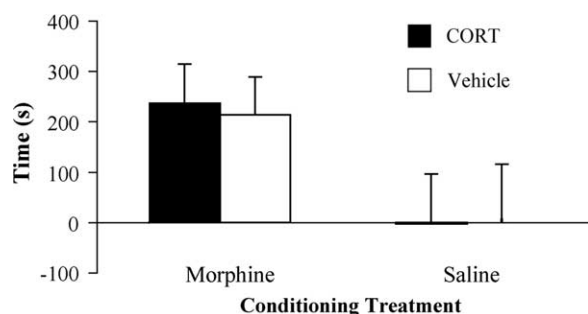
Morphine conditioning resulted in an increase in preference for the drug-paired environment, but CORT administered just prior to morphine failed to enhance this effect (Fig. 5). Also, saline conditioning resulted in virtually no change in preference, and CORT failed to alter this effect as well, indicating the lack of a rewarding effect of CORT as measured by CPP. Thus, a 2 × 2 ANOVA revealed only a main effect of conditioning [ $F(1,39)=5.896$ ,  $p<0.05$ ], with morphine conditioning leading to an equally elevated CPP regardless of pre-treatment.

## 4. Discussion

These results confirm that prior exposure to an uncontrollable stressor potentiates the NAcS DA response to morphine and indicate that this



**Figure 4** Nucleus accumbens shell dopamine (DA) response, expressed as a percentage of baseline (mean + SEM), to corticosterone (CORT; 1.0 mg/kg sc) or vehicle (Veh) followed 20 min by morphine (3.0 mg/kg, sc) or saline administration. \*Morphine-injected groups different from saline-injected groups ( $p<0.05$ ).



**Figure 5** Conditioned place preference response (mean ± SEM) to either morphine (3.0 mg/kg, sc) or saline conditioning. Conditioning sessions were preceded 30 min by either corticosterone (CORT; 1.0 mg/kg, sc) or vehicle injections. Data are expressed as the difference in time (s) spent in the drug- or saline-paired environment before and after conditioning sessions.

uncontrollable stress-induced sensitization is dependent upon the glucocorticoid response to the drug, but not to the stressor. This is consistent with previous findings showing that the potentiated CPP to morphine following uncontrollable stress is also dependent upon drug-induced, but not stressor-induced, glucocorticoids (Der-Avakian et al., 2005). However, an acute increase in CORT, similar to levels observed in response to morphine given after uncontrollable stress, but in the absence of the stressor (Der-Avakian et al., 2005), is not sufficient to produce an enhanced neurochemical (NAcs DA) or behavioral (CPP) response to morphine. Thus, the potentiated CORT response to morphine would appear to be necessary, but not sufficient, to produce these effects.

A number of studies have shown that adrenalectomy without basal CORT replacement blocks many aspects of drug responding, including the locomotor responses to morphine (Marinelli et al., 1994), cocaine (Marinelli et al., 1994; Marinelli et al., 1997), and amphetamine (Cador et al., 1993; Mormede et al., 1994) as well as self-administration of cocaine (Goeders and Guerin, 1996; Deroche et al., 1997). Other studies have shown that blocking CORT also attenuates the NAcs DA response to morphine and cocaine (Piazza et al., 1996a; Barrot et al., 2000). Elevated NAcs DA has been argued to mediate the anticipatory effects of positively reinforcing drugs (Koob and Nestler, 1997; Berridge and Robinson, 2003; Adinoff, 2004; Di Chiara et al., 2004). However, it should be noted that not all aspects of drug responding are dependent upon CORT, such as stress-induced reinstatement of alcohol- and heroin-seeking behaviors (Shaham et al., 1997; Le et al., 2000). Interestingly, in the cases where drug responding is dependent upon CORT, administration of basal levels of the hormone

restores many of these responses (Cador et al., 1993; Marinelli et al., 1994; Mormede et al., 1994; Piazza et al., 1996a; Marinelli et al., 1997; Barrot et al., 2000), suggesting that basal levels of CORT mediate the behavioral and neurochemical effects of rewarding drugs. More importantly, the stress-induced sensitization of the locomotor responses to morphine, cocaine, and amphetamine and self-administration of cocaine is also blocked by surgical or pharmacological adrenalectomy, but with basal replacement (Deroche et al., 1992a; Deroche et al., 1993a; Deroche et al., 1994; Prasad et al., 1998; Campbell and Carroll, 2001), suggesting that stress levels of the hormone may be required to produce these exaggerated responses. However, our previous findings indicate that the potentiated CPP to morphine following an uncontrollable stressor is not dependent upon the CORT response to the stressor (Der-Avakian et al., 2005). Rather, this potentiation is completely blocked only when the CORT response to morphine is suppressed. Stressors, such as food restriction and restraint stress, are known to sensitize the mesolimbic DA response to various psychostimulant drugs (Rouge-Pont et al., 1995; Pacchioni et al., 2002; Cadoni et al., 2003), and it has been hypothesized that the sensitizing effect of stress on these midbrain DA neurons facilitates the subsequent exaggerated behavioral response to drugs (Kalivas and Stewart, 1991). However, the role of CORT during this response is not entirely clear, and the first experiment presented here addressed this issue. Metyrapone and aminoglutethimide treatment, which temporarily blocks the synthesis of CORT in response to stressful or positively reinforcing stimuli, but leaves basal levels intact, attenuated the NAcs DA response to morphine given 24 h after uncontrollable stress only when the treatment was administered prior to morphine. Therefore, stress-induced CORT has no effect on the stress-induced potentiation of NAcs DA efflux in response to morphine, whereas morphine-induced CORT is necessary. These findings confirm a clear dichotomy between the CORT responses to an uncontrollable stressor and to morphine during the stress-induced potentiation of drug responding.

It should be noted that the effects of CORT during the stress sensitization of drug responding has been differentiated in a previous report. Rouge-Pont and colleagues (1995) showed that chronic metyrapone treatment before exposure to the stressor or the drug attenuated stress-induced potentiation of the NAcs DA and psychomotor responses to cocaine. However, not only was metyrapone administered chronically over 8 days, but it was done so to block the endocrine response to food restriction stress. It is currently unknown whether or not food restriction

would sensitize the DA response to morphine as the stressor used here does. Despite the inhibitory actions of metyrapone on the synthesis of CORT, it should also be noted that this compound has been argued to itself act as an acute pharmacological stressor (Rotllant et al., 2002). Metyrapone has also been shown to reinstate heroin self-administration, much like an acute physical stressor (Shaham et al., 1997). However, in the present study, metyrapone seems to have exerted its effects despite its stressful properties, not because of them. For example, one might expect an acute stressful experience to enhance the NAcS DA response to morphine, not attenuate it. Despite this possible limitation of the use of metyrapone, adrenalectomy would have an even more severe limitation here, namely that the CORT response to the stressor and to the drug could not be blocked selectively. Thus, along with their widespread use in studies examining the various effects of adrenal steroids, synthesis inhibitors proved to be the most useful option to temporarily suppress glucocorticoid release in the present study.

While the exaggerated CORT response to morphine seems to be required for the potentiation of NAcS DA and CPP following uncontrollable stress, it remained unclear whether elevated CORT was sufficient, in the absence of stress, to produce the same effects. Thus, this question was addressed here. In a previous study, we showed that the CORT response to morphine was potentiated when drug administration was preceded 24 h earlier by an uncontrollable stressor (Der-Avakian et al., 2005). In the present study, we administered exogenous CORT to raise plasma levels to those observed in our earlier study. Consequently, we administered the optimal dose from Experiment 2 (1.0 mg/kg, sc) prior to morphine administration during microdialysis and CPP conditioning trials to mimic the hormonal change in response to the drug, but in the absence of the stressor. Morphine produced a mild increase in NAcS DA as expected, however, an acute injection of CORT just prior to drug administration failed to alter this response. It should be noted that the modest DA response to morphine during this experiment is statistically similar to the response observed in non-stressed controls during the first experiment, and both are within the range that we typically observe in response to this low dose (Bland et al., 2004a; Bland et al., 2004b). Also, the same dose of CORT injected just prior to each morphine conditioning trial failed to alter the modest increase in CPP produced in response to morphine alone. In both cases, CORT itself in the absence of morphine failed to elevate NAcS DA or produce a CPP.

The role of exogenous CORT administration on NAcS DA neurotransmission and behavioral

responding to drugs is somewhat controversial. Previous studies have primarily focused on the effects of chronically administered CORT on the subsequent behavioral responses to psychostimulants, possibly because chronic stressors are commonly used to produce a robust sensitization of self-administration and locomotor responding to these drugs. In these cases, chronic administration of stress-induced levels of CORT has been shown to increase cocaine self-administration (Mantsch et al., 1998) and locomotor responding to cocaine (Patacchioli et al., 1998) and amphetamine (Deroche et al., 1992b). The literature regarding acute CORT manipulation is not as clear. A clinical study has indicated that acute CORT administration has no effect on the physiological or behavioral responses to amphetamine (Wachtel et al., 2001). However, acute CORT administration increases amphetamine self-administration in rats that initially produced a low CORT response to a novel environment, and decreases self-administration in rats that initially produced a high response to novelty (Piazza et al., 1991). This distinction between low and high responders was not made in the study by Wachtel et al. (2001) or in the study here. It should be noted that CORT itself, at stress-induced levels, is also self-administered, suggesting that high levels of the hormone are positively reinforcing (Deroche et al., 1993b; Piazza et al., 1993). The rewarding effects of CORT were not observed here using a different measure of reward (CPP), although the dose of CORT administered was significantly lower than the total doses self-administered in the studies mentioned above.

There is evidence suggesting that DA tone may be critical in mediating the effects of CORT on the NAcS DA or behavioral responses to drugs. Interestingly, CORT administration does not alter DA efflux when testing is conducted at a time of day when basal DA is relatively low (Imperato et al., 1991). On the other hand, in cases when basal DA is elevated, such as during the dark cycle or feeding, CORT administration does lead to DA efflux (Piazza et al., 1996b). The basal state of DA ergic activity may explain why CORT alone had no effect on DA efflux here. As in the study of Piazza et al. (1996b), exogenous CORT did not facilitate a NAcS DA response in animals tested during the light cycle, when DA tone should be relatively low. Similarly, CORT at this time of day may not facilitate DA efflux in response to a drug, such as morphine, for the same reason. Even so, the data presented here suggest that either stress-induced CORT does not have a facilitatory effect on NAcS DA neurotransmission when DA tone is low, or that the uncontrollable stress-induced sensitization of the neurochemical and behavioral responses to

morphine are mediated by cells other than midbrain DA neurons.

Given that both stressful experiences and drugs of abuse can affect both CORT and DA neurotransmission, it is natural to consider that a possible interaction between the two affects the behavioral impact of stress on drug responding. However, given that a variety of stressors, along with the subsequent neuroendocrine responses to each, may influence different aspects of drug responding, the effects of CORT on DA neurotransmission are likely not the only mediators of stress-induced sensitization of drug reward. Likewise, cases in which there is a high comorbidity between stress-related disorders and drug abuse do not always involve the occurrence of drug intake during or immediately following a stressful experience (Kilpatrick et al., 2000; Jacobsen et al., 2001; Sinha, 2001). Uncontrollable stress produces a variety of long lasting neurophysiological responses, other than a change in neuroendocrine function, that could affect later drug responding (Maier and Watkins, 2005).

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