Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission
(drug abuse/corticosterone/nucleus accumbens/individual differences/microdialysis)

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ABSTRACT An increase in the activity of mesencephalic dopaminergic neurons has been implicated in the appearance of pathological behaviors such as psychosis and drug abuse. Several observations suggest that glucocorticoids might contribute to such an increase in dopaminergic activity. The present experiments therefore analyzed the effects of corticosterone, the major glucocorticoid in the rat, both on dopamine release in the nucleus accumbens of freely moving animals by means of microdialysis, and on locomotor activity, a behavior dependent on accumbens dopamine. Given that glucocorticoids have certain state-dependent neuronal effects, their action on dopamine was studied in situations differing in dopaminergic tonus, including during the light and dark phases of the circadian cycle, during eating, and in groups of animals differing in their locomotor reactivity to novelty. Dopaminergic activity is increased in the dark period, further increased during food-intake, and is higher in rats defined as high responders to novelty than in low responders. Corticosterone, peripherally administered in a dose that approximates stress-induced plasma concentrations, increased extracellular concentrations of dopamine, and this increase was augmented in the dark phase, during eating, and in high responder rats. Corticosterone had little or no effects in the light phase and in low responder rats. Corticosterone also stimulated locomotor activity, an effect that paralleled the release of dopamine and was abolished by neurochemical (6-hydroxydopamine) depletion of accumbens dopamine. In conclusion, glucocorticoids have state-dependent stimulant effects on mesencephalic dopaminergic transmission, and an interaction between these two factors might be involved in the appearance of behavioral disturbances.

It is generally admitted that an increase in the activity of the mesencephalic dopaminergic (DA) neurons is related to the appearance of pathological behaviors. The major antipsychotic drugs are antagonists of DA receptors (1) and prolonged use of psychotropic compounds known to increase DA activity can induce psychotic symptoms (2). Furthermore, an enhanced DA activity in the nucleus accumbens is associated with an increased vulnerability to develop drug self-administration in laboratory rats (3).

Indirect observations suggest that glucocorticoids, the final product of the activation of the hypothalamus–pituitary–adrenal axis by stress, might be one factor capable of increasing the activity of mesencephalic DA neurons. DA neurons express corticosterone receptors (4) and dopamine-mediated behaviors are profoundly facilitated by glucocorticoids (5). Furthermore, increased glucocorticoid levels can induce behavioral changes similar to those attributed to enhanced DA activity. In humans, high levels of glucocorticoids can induce mood changes ranging from euphoria to psychosis (6, 7). In animals, glucocorticoids, within the range of stressor-induced plasma concentrations, increase the propensity to develop self-administration of drugs of abuse (8).

The experiments reported here examine the direct effect on dopamine of an increase in glucocorticoid levels mimicking that induced by a physiological stressor. Behavioral and biochemical indicators of the DA response to corticosterone were analyzed in experimental situations known to differ with respect to the activity of the midbrain DA system. This experimental setting was chosen because glucocorticoids have certain state-dependent neuronal effects. In particular, electrophysiological data suggest that glucocorticoid effects depend on background neuronal activity (9). For example, glucocorticoids modify the membrane potential of hippocampal CA1 cells in slice preparation when these neurons are in a depolarized state, but have no effect in resting conditions (9).

In the first experiment, behavioral and biochemical DA effects of glucocorticoids were compared in the light and dark phases of the circadian cycle, and DA activity was higher in the dark than in the light phase (10). In the second experiment, the effects of glucocorticoids were studied during eating and drinking, given that DA activity further increases during these behavioral activities (11). In the third experiment, the behavioral and biochemical effects of glucocorticoids were studied in groups of animals further differing in DA activity. For this purpose, rats separated on the basis of their behavioral reactivity to novelty were compared. As such, high responder rats (HRs) demonstrate a higher behavioral response to stress and psychostimulant drugs (12) and have higher DA activity in comparison with low responder rats (LRs) (13, 14).

In the experiments outlined above, corticosterone-induced changes in locomotion and dopamine release in the nucleus accumbens have been analyzed. A behavior at least partially dependent on nucleus accumbens dopamine (15), locomotion was studied in animals either with an intact or with a lesioned mesoaccumbens DA projection. Dopamine release was estimated by measuring changes in extracellular concentrations of dopamine in the nucleus accumbens of freely moving rats using the microdialysis technique.

METHODS AND MATERIALS

General Methods

Animals and Housing Conditions. Male Sprague-Dawely rats (Iffa Credo) weighing 280–300 g were individually housed with ad libitum access to food and water. The light/dark cycle (lights were on from 6 a.m. to 8 p.m.), temperature (22°C), and humidity (60%) were kept constant in the animal house.

Abbreviations: HR, high responder; LR, low responder; 6-OHDA, 6-hydroxy-dopamine; DA, dopaminergic.

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Animals were allowed to acclimate to the animal house for 1 week before experiments were started.

**Drugs and Drug Administration.** For all experiments, corticosterone-21-hemisuccinate (Agrar, Rome) was used and concentrations were expressed as corticosterone base. Corticosterone was administered either intravenously (0.75 mg/kg dissolved in a 0.9% NaCl saline solution) or orally (100 μg/ml dissolved in tap water). NaCl (0.9%) and tap water were also used as vehicles for matched controls.

**Locomotor Response to Corticosterone.** Locomotor response to corticosterone was evaluated in activity boxes (35 × 37.5 cm base, 50-cm high). A locomotor activity count was recorded each time a rat crossed the full distance (15 cm) between photocells located on each of the two long sides.

**Constitution of High and Low Responder Groups.** One day at 4 p.m., before any other experimental manipulation, animals were placed in a novel environment (a circular corridor, 10 cm wide and 70 cm in diameter). Locomotor activity scores (expressed in photocell counts) accumulated over 2 hr were used to divide rats into two groups: (i) HR (rats with activity scores above the mean of the whole group) and (ii) LR (the remainder of the rats). This selection criteria is identical to the one used to demonstrate differences between the HR and LR groups in DA activity (13, 14), psychostimulant-induced locomotion, and self-administration (12).

**Catheter Implantation.** Animals were implanted with intracardiac catheters (Silastic, 80 μl dead volume) under ether anesthesia and allowed at least 1 week of postoperative recovery. During this period, the catheter was filled with a heparin solution (100 units/ml).

**Lesion of the Meso-DA Terminals.** Under chloral hydrate anesthesia (500 mg/kg, i.p.), rats were placed in a stereotoxic apparatus and injected (over 4 min) bilaterally with either 1 μl of a 6-hydroxydopamine (6-OHDA) solution (4 μg/μl) or 1 μl of vehicle (0.9% NaCl containing ascorbic acid, 0.2 mg/ml). The coordinates of injection were A = 2.3 mm, L = 1.7 mm, V = 7.5 mm and were measured from bregma (16): the incisor bar was placed 5 mm above the interaural line. One hour before the lesion, rats were injected with desipramine (25 mg/kg, i.p.) to protect the noradrenergic system from the neurotoxin. Nucleus accumbens concentrations (nanograms per milligram of protein) of noradrenaline, dopamine, and serotonin were measured in brain tissue by high-pressure liquid chromatography (HPLC) with electrochemical detection.

**Microdialysis.** Under sodium pentobarbital anesthesia (50 mg/kg, i.p.), rats were implanted with a guide cannula (CMA/11-Sweden) that was lowered to 2 mm above the nucleus accumbens. The coordinates relative to bregma were A = 3.6 mm, L = 2.0 mm, V = 6.5 mm, at a lateral angle of 6° (16). After at least 10 days of recovery, the microdialysis probe (CMA/11, 2-mm membrane length) was inserted through the guide cannula. Two days later, the animal was transferred to the dialysis cage (31 × 31 cm base, 47 cm high), the probe was connected to a syringe pump (Harvard 22) via a two-channel swivel, and the perfusion (2 μl per min) started. Brain dialysis was performed with a fully automatic on-line system (13). HPLC coupled to a coulometric detector (Coulochem II, ESA, Bedford, MA) was used to detect dopamine (0.5 pg detection limit). Experimental manipulations were begun after three consecutive dialysate samples showed less than 10% variation in peak height. The average dopamine basal level of all experimental animals was 0.296 ± 0.15 pg/μl (1.926 nM). Results were expressed as percentages of baseline (the average of the last three pretreatment values). At the end of the experiments, cannula placements were verified histologically on 50 μm thionin-stained coronal sections.

**Statistics.** Locomotor activity scores, changes in extracellular dopamine concentrations, and corticosterone levels were compared by analyses of variance (ANOVA) for repeated measures. For the studies of corticosterone-induced locomotion after 6-OHDA lesion and HRs/LRs, a within-subject design was used. For all other analyses, the treatment (two levels: vehicle and corticosterone) was used as a between factor.

**Procedures.**

**DA Responses to Corticosterone in the Light and Dark Periods.** Corticosterone-induced locomotion. Animals were placed in the activity cages for a 2-hr habituation period and were infused afterward with either saline (n = 8) or corticosterone (0.75 mg/kg; n = 8) two times: once in the dark period (9 p.m.) and once in the light period (11 a.m.). 6-OHDA (n = 8) and sham-lesioned rats (n = 8) received infusions in the dark period, over 2 days, of either saline or corticosterone. For both experiments, a latin square design was used. Two additional groups of rats were infused in the light period with either 1.5 mg/kg corticosterone (n = 8) or saline (n = 8). Infusions were performed through the intracardiac catheter connected to a polyethylene tube (40 cm long), which was disconnected at the end of the infusion.

**Corticosterone-induced changes in extracellular concentrations of dopamine.** After 2 hr of habituation to the dialysis cage, groups of rats were infused as described above either in the light period (11 a.m.) [saline, n = 4; corticosterone (0.75 mg/kg), n = 4] or in the dark period (9 p.m.) [saline, n = 4; corticosterone (0.75 mg/kg), n = 5]. Extracellular concentrations of dopamine were measured for 2 hr over 20 min samples (40 μl).

**DA Response to Corticosterone During Eating and Drinking.** Animals were brought to the dialysis room in their home cage 48 hr before the test and connected to a dummy perfusion apparatus to allow habituation to the test condition. Two hours before the beginning of the dark period, the perfusion of the microdialysis probe started and drinking bottles were withdrawn. When three dopamine samples showed a variation of less than 10%, half of the animals (n = 8) received tap water and the other half (n = 10) received a corticosterone solution (100 μg/ml). The three samples preceding the start of drinking for each animal were considered the baseline, and variations of extracellular dopamine concentrations and the amount of fluid consumed were measured for 6 hr every 30 min. Corticosterone was dissolved in the drinking water to ensure that its administration was contingent upon eating and drinking, because they are strictly associated in the rodent (17). Furthermore, preliminary experiments have shown that spontaneous eating is disrupted by the manipulations necessary to perform a parenteral injection.

**DA Response to Corticosterone in HR and LR Rats.** Corticosterone-induced locomotion. Corticosterone-induced locomotion was studied only in the dark period. With a procedure identical to those previously described and after a latin square design, HR (n = 8) and LR (n = 8) animals were infused intravenously over 2 days with either saline or corticosterone (0.75 mg/kg).

**Corticosterone-induced changes in extracellular concentrations of dopamine.** Corticosterone was administered in the drinking water (100 mg/ml; HRs, n = 5; LRs, n = 5) according to a procedure identical to that previously described.

**Effects of Corticosterone Administration on the Plasmatic Levels of the Hormone.** Intravenous infusion. Animals were implanted with two intracardiac catheters, one in each jugular vein. One catheter was used to infuse either corticosterone (0.75 mg/kg; n = 8) or saline (n = 8), the other was used to withdraw two blood samples, the first immediately before the infusion (9 p.m.) and the second 20 min later.

**Oral administration.** One hour before the start of the dark period, animals were given either a solution of corticosterone (100 μg/ml; n = 6) or tap water (n = 6) in the drinking bottle of their home cage. The amount of liquid was recorded every 30 min and a blood sample was withdrawn from
the tail vein 4 h after the animals started drinking. The total amount of food intake over this period was also recorded. The sampling schedules of the two experiments were chosen because they correspond to the peak of corticosterone's effects on dopamine.

RESULTS

DA Responses to Corticosterone in the Light and Dark Periods. Corticosterone-induced locomotion. Infusion of corticosterone increased locomotor activity and this effect was dependent on the time of the day [day period \( \times \) treatment interaction; \( F(1,14) = 6.21, P < 0.02 \) (Fig. 1)]. A significant effect of corticosterone was found only in the dark period \( F(1,14) = 5.46, P < 0.05 \). The absence of effects in the light period cannot be due to lower plasmatic levels of corticosterone at this time. Infusion of a higher dose (corticosterone, 1.5 mg/kg) in the light period did not increase locomotion [corticosterone = 57.44 ± 29.14, vehicle = 59.37 ± 28.22 photocell counts, \( F(1,14) = 0.002, P > 0.95 \)]. 6-OHDA infusion in the nucleus accumbens induced an 80% depletion of dopamine content in this structure \( F(1,14) = 22.65, P < 0.001 \), but did not modify noradrenaline and serotonine contents. The lesion suppressed corticosterone-induced locomotion in the dark period [lesion \( \times \) treatment interaction, \( F(1,14) = 5.82, P < 0.05 \) (Fig. 1)]. Sham animals showed a higher locomotor response to corticosterone than to vehicle \( F(1,7) = 9.44, P < 0.01 \) (data not shown). In 6-OHDA lesioned rats, corticosterone- and vehicle-induced locomotion did not differ \( F(1,7) = 1.35, P > 0.3 \).

Corticosterone-induced changes in extracellular concentrations of dopamine. Changes in the extracellular concentrations of dopamine after the infusion of vehicle did not differ between the dark and the light periods \( F(1,6) = 0.45, P > 0.7 \). For this reason, dopamine responses to vehicle were cumulated in Fig. 2. The infusion of corticosterone increased extracellular dopamine \( F(1,13) = 7.03, P < 0.02 \), but this effect was dependent on the time of the day and changed over time [day period \( \times \) treatment \( \times \) time interaction \( F(5,65) = 3.32, P < 0.01 \) (Fig. 2)]. During the dark period, the increase in dopamine was higher in corticosterone- than in vehicle-infused rats \( F(1,11) = 8.21, P < 0.02 \), but the two groups did not differ significantly when infused in the light period \( F(1,10) = 1.8, P > 0.2 \).

DA Response to Corticosterone During Eating and Drinking. Animals drinking the corticosterone solution (100 μg/ml) contingent upon eating showed a higher increase in accumbens dopamine than animals drinking vehicle (tap water) \( F(1,16) = 7.66, P < 0.01 \) (Fig. 3). However, these animals did not differ for the total amount of fluid consumed [corticosterone = 15.2 ± 1.43 ml; tap water = 12.87 ± 1.3 ml; \( F(1,16) = 1.23, P > 0.25 \) or for its consumption over time \( F(11,176) = 0.47, P > 0.7 \). Drinking was equally distributed over the 6 hr of testing [mean intake per hr: corticosterone, 2.54 ± 0.4 ml; tap water, 2.14 ± 0.5 ml].

DA Response to Corticosterone in HR and LR Rats. Corticosterone-induced locomotion. HRs and LRs showed a different sensitivity to corticosterone [group \( \times \) treatment interaction, \( F(1,14) = 7.65, P < 0.01 \) (Fig. 4)]. HRs had a higher corticosterone-induced locomotion than LRs \( F(1,7) = 10.48, P < 0.01 \). Furthermore, in the LR group, corticosterone- and vehicle-induced locomotion did not differ \( F(1,7) = 0.058, P > 0.8 \).

Corticosterone-induced changes in extracellular concentrations of dopamine. HRs drinking the corticosterone solution (100 μg/ml) showed a faster [group \( \times \) time interaction \( F(1,11) = 2.93, P < 0.02 \) and higher \( F(1,18) = 4.65, P < 0.05 \) increase in dopamine than LRs (Fig. 5). The two groups, over the 6 hr of testing, did not differ in their total intake of the corticosterone solution [HRs = 15.8 ± 1 ml, LRs = 14.6 ± 2.8 ml; \( F(1,8) = 0.15, P > 0.7 \) or in its intake over time \( F(11,88) = 0.88, P > 0.5 \).

Effects of Corticosterone Administration on the Plasmatic Levels of the Hormone. Intravenous infusion of corticosterone (0.75 mg/kg) significantly raised plasmatic levels of this hormone to within the range induced by mild stressors (8) [before

![Fig. 1](image1.png)  
**Fig. 1.** Corticosterone-induced locomotion during the dark and light periods. Animals with an intact DA system (Intact) showed a higher locomotor response to corticosterone (0.75 mg/kg, i.v.) than to vehicle (0.9% NaCl) when infused in the dark (9 p.m.) but not in the light (11 a.m.) period. Corticosterone-induced locomotion was suppressed in animals in which the DA terminals in the nucleus accumbens had been lesioned (6-OHDA Lesion). * \( P < 0.05 \), ANOVA with respect to the matched vehicle group.

![Fig. 2](image2.png)  
**Fig. 2.** Corticosterone-induced changes in extracellular concentrations of dopamine in the nucleus accumbens during the dark and light periods. Animals infused with 0.9% NaCl in the dark (9 p.m.) and in the light (11 a.m.) periods did not differ and were cumulated in the vehicle group (Vehicle). Animals infused with corticosterone in the dark period (0.75 mg/kg, i.v.) [Corticosterone (dark)] showed a significant increase in dopamine. Infusion of corticosterone during the light period [Corticosterone (light)] had no significant effects.

![Fig. 3](image3.png)  
**Fig. 3.** Corticosterone-induced changes in extracellular concentrations of dopamine in the nucleus accumbens during eating. Animals drinking the corticosterone solution (100 μg/ml) in the dark period during eating (Corticosterone) showed a higher increase in dopamine than animals drinking tap water (Vehicle).
These findings confirm and extend previous data from the literature indicating that physiological effects of corticosterone may depend on the contingent activation of the central nervous system (9, 18, 19). This largely neglected feature of corticosterone physiology might in fact be a general principle governing the action of these hormones on behavior. Indeed, other behavioral effects of corticosterone, such as stimulation of eating behavior, seem to follow a similar principle (18).

The activity level of DA neurons at the time of the increase in plasmatic concentrations of corticosterone may be the factor determining the state-dependent effects of this hormone. It is known that DA activity is higher in the dark than in the light period (10), increases further during eating (11), and is higher in HR animals than in LRs (13, 14). This interaction between glucocorticoids and DA activity may explain previously reported contradictory results. Imperato et al. (20) performed their experiments during the light cycle, when DA activity is low, and found no effect of corticosterone on accumbens extracellular dopamine. In contrast, Mittleman et al. (21) performed their experiment under anesthesia, a condition that increases DA activity (22), and demonstrated a clear corticosterone-induced increase in accumbens extracellular dopamine.

Three principal mechanisms may mediate the elevation of extracellular concentrations of dopamine induced by corticosterone. First, glucocorticoids may modify the firing of DA neurons. Although there is no direct evidence for such an effect, modulation of membrane potential in other neuronal populations by glucocorticoids has been reported and these effects, consistently with our findings, depend on background electrical activity (9). Second, glucocorticoids may decrease dopamine catabolism by acting as a reversible monoamine oxidase inhibitor (23–25). This effect is consistent with the fact that administration of synthetic glucocorticoids decreases deaminated products of dopamine, such as HVA and DOPAC (25, 26), that depend on monoamine oxidase activity, while increasing 3MT levels (25). Third, glucocorticoids may decrease catecholamine reuptake (27, 28). This action of glucocorticoids seems consistent with the state dependency reported here, given the evidence that dopamine reuptake by striatal synaptosomes is decreased by glucocorticoids only if the synaptosomal preparation is K+ stimulated, but not in resting conditions (28).

On the basis of the results reported here, it is reasonable to suggest that glucocorticoids might act as endogenous psychostimulants. Glucocorticoids share the neurochemical actions of psychostimulants in that they both increase extracellular concentrations of dopamine (this paper) and both seem to exert this effect through inhibition of dopamine reuptake and inhibition of monoamine oxidase activity (23–29). At the behavioral level, corticosterone induces self-administration (19) and dopamine-dependent locomotor activity as do psychostimulants (30). Furthermore, repeated administration of glucocorticoids, like psychostimulants (3), can induce psychotic symptomatology in humans (6, 7) and behavioral sensitization in animals (31). However, glucocorticoids and psy-

†The state-dependent effects of glucocorticoids on dopamine reported here do not appear to be explained by differences in the plasma levels of corticosterone occurring during these different states. First, infusion during the light period of doses of corticosterone twice as high as the dose effective in the dark period was without significant effects. Second, oral and intravenous administrations of corticosterone, at the moment of their highest effects on dopamine, induced similar increases in the plasmatic levels of corticosterone. Third, HRs and LRs do not differ either with respect to their basal corticosterone levels or their levels after exogenous corticosterone administration in this and other experimental conditions (8, 19). In addition, administration of corticosterone in the drinking water does not seem to induce behavioral changes, as for example changes in drinking and eating, that may indirectly account for the higher increase in dopamine observed in this condition.
hostimulants differ in the degree to which their effects are state dependent. As demonstrated here, stimulant effects of glucocorticoids are evident only in situations associated with existing behavioral activation, which this hormone amplifies. In contrast, psychostimulants can trigger psychomotor activation in situations in which glucocorticoids cannot, for example, in the light period.

A higher sensitivity to the DA effects of glucocorticoids might be a relevant mechanism in the appearance of pathological behaviors. Indeed, HR animals, which have been demonstrated by these experiments to be more sensitive than LR rats to the DA effect of corticosterone, are also known to have higher behavioral reactivity to stress and propensity to develop psychostimulant self-administration (3, 8, 12). An interaction between glucocorticoids and dopamine might be particularly relevant for psychostimulant abuse given that the rewarding effects of these drugs have been attributed to their ability to increase nucleus accumbens dopamine (30). Clinical observations also support the theory that individual differences in the sensitivity to glucocorticoids may be involved in behavioral pathology. For example, administration of synthetic glucocorticoids induces behavioral disturbances, such as psychotic symptoms, in only some subjects (6, 7). In addition, a higher sensitivity to certain DA effects of these hormones has been found associated with schizophrenia (32); physiological concentrations of glucocorticoids increase DA receptors in the lymphocytes of schizophrenic patients but not in healthy controls (32).

In conclusion, the results of the experiments presented here demonstrate that glucocorticoids have state-dependent stimulant effects on the activity of mesencephalic DA neurons. In physiological conditions, through their action on DA transmission, these hormones might act as endogenous stimulants and enhance behavioral adaptation. In addition to this adaptive physiological role, however, an abnormal sensitivity to the DA effects of glucocorticoids in certain individuals might render this dopamine-hormone interaction a possible cause of behavioral disturbances.

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