The Medial Prefrontal Cortex Determines the Accumbens Dopamine Response to Stress through the Opposing Influences of Norepinephrine and Dopamine

Although the medial prefrontal cortex (mpFC) appears to constrain stress responses, indirect evidences suggest that it might determine the stress response of the mesoaccumbens dopamine (DA) system. To test this hypothesis, we first evaluated the dynamics of norepinephrine (NE) and DA release in the mpFC and of DA release in the nucleus accumbens (NAc) of acutely stressed rats. Then, we tested the effects of selective depletion of NE or DA in the mpFC (by local 6-hydroxydopamine infusion following desipramine or 1-[2-(4-fluorophenyl)methoxyethyl]-4-(3-phenylpropyl)piperazine (GBR 12909) on stress-induced changes in mesoaccumbens DA release. Rats experiencing restraint stress for 240 min showed an initial, short-lived increase of NE outflow in the mpFC and of DA in the NAc. These responses were followed by a sustained increase of DA in the mpFC and by a decrease to below resting levels of DA in the NAc. Moreover, selective prefrontal NE depletion eliminated the increase of NE in the mpFC and of DA in the NAc, and selective depletion of mesocortical DA eliminated the enhancement of mpFC DA as well as the inhibition of mesoaccumbens DA, without affecting basal catecholamines outflow. These results demonstrate that the opposing influences of mpFC NE and DA determine mesoaccumbens DA response to stress and suggest that alterations of this mechanism may be responsible for some major psychopathological outcomes of stress.

Keywords: appraisal, coping, dopamine, medial prefrontal cortex, norepinephrine, nucleus accumbens

Introduction

Stress is a major source of environmental determinants of psychopathology. Indeed, it influences the development, the course and outcome, as well as the recurrence or relapse after period of remission of several psychiatric disorders, including schizophrenia, depression, and addiction (Laruelle 2000; Shamah et al. 2000; Lieberman et al. 2001; Marinelli and Piazza 2002; Charney 2004; Hasler et al. 2004; McFarland et al. 2004; Brady and Sinha 2005; Maier and Watkins 2005). Thus, the identification of central stress mechanisms is fundamental to the understanding of disease processes and to the development of increasingly effective therapies.

Much evidence indicates that the medial prefrontal cortex (mpFC) controls the hormonal stress response, that is, the activation of the hypothalamic–pituitary–adrenocortical axis culminating in release of corticoids hormones, in an inhibitory way (see Herman et al. 2003). Moreover, very recent results indicate that mpFC exerts an inhibitory influence on stress-induced activation of serotonin neurons in the dorsal raphe nucleus when stressful conditions are controllable (susceptible to coping efforts) by the organism (Amat et al. 2005). Finally, dopamine (DA) in the mpFC exerts an inhibitory influence on DA release in the nucleus accumbens (NAc) and depletion of mesocortical DA facilitates stress-induced activation of mesoaccumbens DA release (Deutch et al. 1990; Doherty and Gratton 1996; King et al. 1997).

These observations suggest that, in stressful conditions, the main role of mpFC is to restrain neurophysiological stress responses and, hence, that pathological outcomes of stress develop when these responses overcome the inhibitory control of mpFC. The ability of mesocortical DA to restrain activation of NAc DA release in stressful conditions is of special interest in preclinical research because disturbances of mesoaccumbens DA transmission have been implicated in different psychopathologies susceptible of stress influences such as schizophrenia (Laruelle 2000), depression (Rossetti et al. 1993; Cabib and Puglisi-Allegra 1996; Grappi et al. 2003; Dailly et al. 2004; Salamone et al. 2005), and addiction (Di Chiara 2002; Everitt and Wolf 2002; Marinelli and Piazza 2002; Robinson and Berridge 2003; Salamone et al. 2003; Cardinal and Everitt 2004; Kelley 2004; Stewart 2004; Vezina 2004; Kalivas and Volkow 2005).

However, research in human subjects has long supported the idea that coping requires cognitive evaluation of the stressful condition and of responses outcomes (Ursin and Eriksen 2004) and the mpFC, due to its “supervisory” functions such as attention to stimulus features and action–outcome rules (Cardinal et al. 2002; Dalley et al. 2004; Toates 2004), should play a major role in this evaluation. Moreover, accumbens DA release shows biphasic changes in response to a novel stressor because the initial short-lived enhancement of DA release is followed by a reduction of released DA below resting levels that lasts as long as the stressful experience (Cabib et al. 1998, 1994; Puglisi-Allegra et al. 1991, 1993; Ventura et al. 2002). There is consistent evidence that this inhibitory phase is not due to habituation or to depletion of DA pools by excessive release (Imperato, Angelucci, et al. 1992; Imperato et al. 1993) and that it requires intact mesocortical DA transmission (Ventura et al. 2002). Finally, recent reports pointing to a major role for prefrontal cortical norepinephrine (NE) transmission in both psychostimulant- and opioid-induced mesoaccumbens DA release (Darracq et al. 1998; Ventura et al. 2003, 2005) suggest a facilitating influence of mpFC NE on subcortical DA release. Because all known experimental stressors stimulate NE release in the mpFC (Finlay et al. 1995; Dalley et al. 1996; Goldstein et al. 1996; Jedema et al. 1999; Kawahara et al. 1999; McQuade et al. 1999; Fenstera et al. 2000; Page and Lucki 2002), it is conceivable that stress-induced stimulation of mesoaccumbens DA release might involve enhanced NE release in the mpFC.

Based on this evidence, it is possible to hypothesize that during novel stressful experiences the mpFC determines, rather than constrains, mesoaccumbens DA response through the
opposing effects of NE and DA. If confirmed, this hypothesis could explain why stress may be involved in different pathological conditions. Indeed, the balanced action of the 2 catecholamines in the mpFC may be required for healthy coping, whereas unbalanced action may promote hyper- or hyporesponding by mesoaccumbens DA, leading to different and even opposite behavioral disturbances.

Thus, in the present experiments, we tested this hypothesis by 1) evaluating the dynamics of NE and DA release in the mpFC of rats submitted to restraint, a prototypic psychogenic stressor (Doherty and Gratton 1996; Herman et al. 2003; Morilak et al. 2005) that promotes biphasic DA release in the rat NAc (Puglisi-Allegra et al. 1991; Imperato et al. 1993) and 2) assessing the ability of selective prefrontal cortical NE or DA depletions to affect the dynamics of the catecholaminergic stress responses.

Materials and Methods

Animals

Male Sprague-Dawley rats (250–350 g; Charles River Labs, Calco, Como, Italy) were housed 3 to cage with food and water ad libitum in animal facility where temperature was kept between 22°C and 23°C and lights were on from 7:00 AM to 7:00 PM. Rats were allowed at least 1 week to acclimate to the colony room before any treatment. During this time, rats were handled routinely. All surgery and experiments were carried out between 9:00 AM and 5:00 PM. All experiments were conducted according to the Italian national law (DL 116/92) on the use of animals for research based on the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Drugs

From Sigma-Aldrich (Milano, Italy), 6-hydroxydopamine (6-OHDA), desipramine hydrochloride (DMI), GBR-12909 dihydrochloride (GBR), xylazine hydrochloride, and tetrodotoxin (TTX) were purchased. Ketamine (Ketavet 100) was purchased from Intervet (Peschiera Borromeo, Italy). DMI, GBR, ketamine, and xylazine were dissolved in saline (0.9% NaCl) and injected intraperitoneally (i.p.) in a volume of 3 ml/kg. 6-OHDA was dissolved in saline containing Na metabisulfite (0.1 M) was dissolved in artificial cerebrospinal fluid (CSF) for reverse microdialysis experiments.

Microdialysis

Rats were anesthetized with ketamine (85 mg/kg) and xylazine (10.5 mg/kg) i.p. and mounted on a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and implanted unilaterally with a guide cannula (stainless steel; shaft outer, 0.38 mm; Metalant, Stockholm, Sweden) in the mpFC or in the NAc. The length of the guide cannula was 3 mm for mpFC and 7 mm for NAc. Each guide cannula was fixed with epoxy glue and dental cement, and the skin was sutured. The coordinates from bregma (measured according to Paxinos and Watson 1998) were as follow (in mm): mpFC: +3.7 anterior-posterior (AP), −0.9 L; NAc: +1.5 Anteroposterior (AP), −0.8 L. The probe (dialysis membrane length 2 mm for mpFC and NAc; OD: 0.24 mm, MAB 4 cuprophane microdialysis probe, Metalant) was introduced 24 h after the implantation of the guide cannula in intact rats, whereas in Sham rat (Sham) and catecholamine-depleted animals it was introduced 6 days after guide cannula. The rats were lightly anesthetized to facilitate manual insertion of the microdialysis probe into the guide cannula. Animals were then returned to their home cages, and the outlet and inlet probe tubing were protected by locally applied parafilm. The membranes were tested for in vitro recovery of DA and NE on the day before use to verify recovery. The microdialysis probe was connected to a CMA/100 pump (Carnegie Medicine, Stockholm, Sweden) through PE-20 tubing and an ultralow torque dual channel liquid swivel (Model 375/D/22QM, Instech Laboratories, Inc., Plymouth Meeting, PA) to allow free movement. Artificial CSF (in mM: NaCl 140.0; KCl 4.0; CaCl2 1.2; MgCl2 2 1.0) was pumped through the dialysis probe at a constant flow rate of 2 ml/min. Experiments were performed 22–24 h after probe placement.

Following the start of the dialysis perfusion, rats were left undisturbed for approximately 2 h before the collection of baseline samples. The mean concentration of the 3 samples collected immediately before treatment (<10% variation) was taken as basal concentration. All experimental groups were then subjected to restraint in a plexiglas box (9 × 7 × 15 cm) provided with a sliding surface allowing rats to be gently handled during both restraining and releasing procedures (Imperato et al. 1991, 1992a, 1993; Puglisi-Allegra et al. 1991). The dialyzate samples were collected every 20 min for 240 min. Placements were judged by methylene blue staining. In Figure 1 is represented the location of microdialysis probes in the mpFC and in the NAc. Only data from rats with correctly placed cannula have been reported. Five rats were discarded from the whole experimental groups. Twenty microliters of each dialyzate sample were analyzed by high-performance liquid chromatography (HPLC). The remaining 20 µl were kept for possible subsequent analysis. Concentrations (pg/20 µl) were not corrected for probe recovery.

The HPLC system consisted of an Alliance (Waters Corporation, Milford, MA) system coupled to a coulometric detector (model 5200® Coulochem II; ESA, Chelmsford, MA) provided with a conditioning cell (M 5021) and an analytical cell (M 5011). The conditioning cell was set at 400 mV, electrode 1 at 200 mV, and electrode 2 at −250 mV. A Nova-Pack C18 column (3.9 × 150 mm; Waters Corporation) and a Sentry Guard Nova-Pack C18 precolumn (3.9 × 20 mm) maintained at 35°C were used. The mobile phase was described previously (Westerink et al. 1998). The detection limit of the assay was 0.1 pg.

Figure 1. Location of microdialysis probes in the mpFC (upper panel) and in the NAc (lower panel) of rat brain. Silhouettes of probe tracks were drawn onto representative sections of the rat brain adapted from the atlas Paxinos and Watson (1998). The numbers indicate millimeters rostral to bregma. For details, see Materials and Methods.
NE and DA Depletion in the mpFC
Anesthesia and surgical set are described in the preceding paragraph. During surgery, rats were also implanted with the guide cannula for microdialysis. Rats from NE-depleted group were injected with GBR (15 mg/kg, i.p.) 30 min before 6-OHDA in order to protect dopaminergic terminals. Two bilateral injections of 6-OHDA (4 mg/0.6 ml/4 min for each side) were made into the mpFC (coordinates: +3.7 AP, –0.9 L; –2.5 V; –4.2 V with respect to bregma) through a stainless steel cannula (0.15 mm OD; UNIMED, Lausanne, Switzerland) connected to a 1-ml syringe by a polyethylene tube and driven by a CMA/100 pump. The cannula was left in place for 2 min after the injections. Rats from the DA-depleted group followed the same treatment described in the preceding paragraph except for the fact that these rats were injected with desipramine (25 mg/kg, i.p.) 30 min before 6-OHDA microinjection in order to protect noradrenergic terminals. Sham was subjected to the same treatment of NE- or DA-depleted rats but received intracerebral vehicle. Rats were used for microdialysis experiment 7 days after surgery. Samples of chromatograms of basal NE and DA outflow in the mpFC of NE- and DA-depleted rats are presented in Figure 2.

Moreover, in additional groups of rats, we assessed that steady NE and DA outflow levels in NE (n = 3) or DA (n = 3)-depleted animals were susceptible to be reduced by fast sodium channel blocker TTX infused through the microdialysis probe reduced in the mpFC. For reverse microdialysis experiments, TTX (3 μM) was perfused in the mpFC for 60 min, after baseline collection, through the microdialysis probe (Feenstra and Botterblom 1996).

NE and DA tissue levels in the mpFC were assessed to evaluate the amount and the extent of depletion. The brains were fixed vertically on the freeze plate of a freezing microtome. Punches of both hemispheres were obtained from brain slices (coronal sections) no thicker than 500 μm, by a stainless steel tube of 2.3 mm inner diameter. The coordinates were measured according to the atlas of Paxinos and Watson (1998) as follows (coronal sections as mm from bregma): 3 slices from 4.70 to 3.20. The punches were stored in liquid nitrogen until the day of analysis. On the day of analysis, frozen samples were weighed and homogenized in HClO4 0.05 M. The homogenates were centrifuged at 10 000 × g for 20 min at 4°C. Aliquots of the supernatant were simultaneously using an HPLC procedure coupled with Coulochem electrochemical detection. The HPLC system was described in the preceding paragraph, with the potentials being set at +450 and +100 mV at the analytical and the conditioning cell, respectively. The column, a Nova-Pack Phenyl column (3.9 × 150 mm) and a Sentry Guard Nova-Pac precolumn (3.9 × 20 mm), were purchased from Waters Corporation. The flow rate was 1 ml/min. The mobile phase consisted of 3% methanol in 0.1 M Na phosphate buffer, pH 3, 0.1 mM Na3EDTA, and 0.5 μM 1-octane sulfonic acid Na salt (Sigma).

Experimental Protocol and Statistics
Two groups of intact rats (n = 7) were used to evaluate the effects of restraint on mpFC NE and DA outflow and NAc DA outflow (CONTROL). Additional 8 groups of rats (n = 7) were used to evaluate the effects of selective NE or DA depletion on NAc DA outflow as well as prefrontal cortical NE and DA outflow during a restraint experience (Sham, NE depleted and DA depleted). Finally, a group of rats (n = 5) was left undisturbed in the microdialysis cages for 240 min following baseline collection in order to control for stress-unrelated changes in DA outflow in the NAc.

Statistical analyses were always carried out on raw data (concentrations: pg/20 μl). Data were presented in figures as percent changes from baseline levels. Finally, because the stress responses of Sham groups were identical, the pooled data are represented in figures. Data on the effect of restraint on DA outflow in the NAc and on NE and DA outflow in the mpFC were statistically analyzed by repeated-measures analysis of variance (ANOVA) (time, 13 levels: 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 min of restraint). The effects of prefrontal NE or DA depletion on NE and DA release in the mpFC or on DA outflow in the NAc of rats subjected to restraint were analyzed by 2-way ANOVAs for repeated measure (treatment as between factor: 2 levels = Sham and NE or DA depleted and time, as within factor = 13 levels: 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 min of restraint).

Simple effects were assessed by 1-way ANOVA at each time point. Individual between group comparisons were carried out, where appropriate, by post hoc test (Duncan multiple range test).

The effects of prefrontal catecholamine depletion on tissue levels of either NE or DA in the mpFC were analyzed by 1-way ANOVA (treatment, 2 levels = Sham and NE or DA depleted).

Results
Mesoaccumbens and Mesocortical Responses to Restraint
The effects of restraint on DA outflow in the NAc of CONTROL rats are shown in Figure 3 (upper panel). Statistical analyses revealed a significant effect of time (F12,72 = 3.24, P < 0.001). Restraint produced a significant increase in accumbal DA outflow between 20 and 40 min, followed by a decrease below baseline levels that was significant at 160 min and lasted through the end of the experience.

The effects of restraint on DA outflow in the mpFC of CONTROL rats are shown in Figure 3 (middle panel). Statistical analyses revealed a significant effect of time (F12,72 = 5.26, P < 0.001). The restraint produced a significant initial increase in DA outflow at 20 min and a subsequent larger increase that was significant from 80 min onwards.

The effects of restraint on NE outflow in the mpFC of CONTROL rats are shown in Figure 3 (lower panel). Statistical analyses revealed a significant effect of time (F12,72 = 11.89, P < 0.001). Restraint promoted an immediate significant increase in NE outflow (20–80 min) followed by a return to basal levels.

The basal outputs in CONTROL rats was DA in the NAc = 3.30 ± 0.39 pg/20 ml; DA in the mpFC = 1.35 ± 0.29 pg/20 ml; and NE in the mpFC = 3.11 ± 0.30 pg/20 ml.

Finally, statistical analysis of data obtained in rats left undisturbed in the microdialysis cages for 240 min following
collection of baseline samples did not reveal significant changes in DA outflow in the NAc ($F_{12,48} = 0.997, P = 0.48$).

**Effects of Selective NE or DA Depletion on Stress-Induced Changes in DA or NE Release in the mpFC**

The effects of prefrontal NE or DA depletion on stress-induced DA outflow in the mpFC are shown in Figure 4 (upper panel). Because Sham groups of the 2 experiments did not present any statistical difference and were substantially overlapping, 1 curve only for Sham is represented in the figure. Statistical analyses revealed a significant treatment × time interaction ($F_{12,144} = 12.683, P < 0.0001$) for DA depletion only. In Sham, restraint produced an initial increase in DA outflow that was significant at 20 min and a subsequent larger increase that was significant from 100 min throughout. The response of NE-depleted rats was identical to that observed in the Sham group. Instead, in DA-depleted rats restraint promoted an initial short-lasting increase of DA release and a subsequent reduction of DA outflow that was significant at 60 min and lasted through the end of the experience.

No significant differences were found in DA basal levels (Sham = $1.20 ± 0.26$ pg/20 ml; DA depleted = $1.21 ± 0.05$ pg/20 ml; Sham = $1.20 ± 0.18$ pg/20 ml; NE depleted = $1.28 ± 0.11$ pg/20 ml).

The effects of prefrontal NE or DA depletion on NE outflow in the mpFC of rats subjected to restraint are shown in Figure 4 (lower panel), where, as for previous experiment, 1 curve for Sham groups is shown in the figure. Statistical analyses revealed a significant treatment × time interaction ($F_{12,144} = 6.100, P < 0.001$) for NE depletion only. In Sham, restraint promoted an immediate significant increase in NE outflow that lasted 100 min. Selective DA depletion did not affect this response, whereas selective NE depletion abolished it and led to a significant reduction of NE release to below basal levels (100–240 min).

No significant differences were found in NE basal levels (Sham = $3.02 ± 0.28$ pg/20 ml; DA depleted = $3.08 ± 0.34$ pg/20 ml; Sham = $3.13 ± 0.28$ pg/20 ml; NE depleted = $2.90 ± 0.30$ pg/20 ml).

**Effects of Selective Prefrontal NE or DA Depletion on Stress-Induced Changes in DA Release in the NAc**

The effects of prefrontal NE or DA depletion on DA outflow in the NAc of rats subjected to restraint are shown in Figure 5. Because Sham groups of the 2 experiments did not present any statistical difference and were substantially overlapping, 1 curve only for Sham is represented in the figure. Statistical analyses revealed a significant treatment × time interaction for both NE depletion ($F_{12,144} = 2.577, P < 0.001$) and DA depletion ($F_{12,144} = 1.918, P < 0.05$). In Sham of both experiments, restraint produced a significant increase in DA outflow between 20 and 60 min, followed by a decrease below baseline levels that was significant at 140 min and lasted to the end of the restraint experience.

Selective mpFC NE depletion eliminated the initial restraint-induced increase in DA outflow but did not affect the subsequent decrease of DA outflow. Instead, selective DA depletion in the mpFC enhanced the initial increase and abolished the following inhibition of DA release.

Neither DA nor NE depletion in the mpFC affected basal DA outflow (Sham = $3.1 ± 0.41$ pg/20 ml; DA depleted = $3.32 ± 0.56$ pg/20 ml; Sham = $3.05 ± 0.20$ pg/20 ml; NE depleted = $3.02 ± 0.30$ pg/20 ml).

---

**Figure 3.** Effects of 240 min of restraint on DA outflow in the NAc (upper panel), and DA (middle panel) and NE (lower panel) in the mpFC of control rats ($n = 7$ per group). Results are expressed as percent changes (means ± standard error) from basal values. Statistical analyses were performed on raw data. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with basal values.
Effects of Selective NE or DA Depletion on Tissue Levels of DA and NE in the mpFC

Prefrontal NE depletion promoted a significant ($F_{1,12} = 155.1; P < 0.0001$) decrease in NE tissue levels (Sham = 449.3 ± 47.5; NE depleted = 45.3 ± 3.9 ng/g wet tissue), whereas it spared DA tissue levels (Sham = 186.9 ± 39.1; NE depleted = 183.0 ± 15.8 ng/g wet tissue). Instead, prefrontal DA depletion produced a significant ($F_{1,12} = 115.8; P < 0.0001$) reduction of DA tissue levels in the mpFC (Sham = 188.5 ± 23.2; DA depleted = 16.9 ± 2.8 ng/g wet tissue), whereas it spared NE tissue levels (Sham = 436.5 ± 58.0; DA depleted = 397.4 ± 35.7 ng/g wet tissue).

Discussion

The results of the present experiments demonstrate that mpFC governs mesoaccumbens DA release during stressful experiences through the opposing influences of NE and DA. Thus, an initial, short-lived increase in NE release in the mpFC determines a temporary activation of mesoaccumbens DA release. Conversely, subsequent sustained activation of cortical DA release leads to a profound inhibition of mesoaccumbens DA release.

The results of the first experiment indicate that exposure to a novel stressful experience promotes an initial, short-lived increase of both NE and DA release in the mpFC and of DA release in the NAc. These responses have been repeatedly reported by studies on the central effects of different types of short-lasting stressful experiences (Thierry et al. 1976; Deutch et al. 1990; Dalley et al. 1996; Doherty and Gratton 1996; Goldstein et al. 1996; King et al. 1997; Jedema et al. 1999; Kawahara et al. 1999; McQuade et al. 1999; Cabib et al. 2000; Page and Lucki 2002; Bland, Hargave, et al. 2003; Bland, Twining, et al. 2003; Stevenson and Gratton 2003). Moreover, DA release in the NAc decreased slowly to below basal levels during the stressful experience, as previously described (Puglisi-Allegra et al. 1991; Imperato et al. 1993). Finally, at the same time, cortical NE outflow
decreased to basal levels and remained stable thereafter, whereas cortical DA outflow showed a further and significant increase that lasted throughout the stressful experience.

To our knowledge, this is the first observation of different dynamics of prefrontal cortical NE and DA responses to stress. On the other hand, very few studies have evaluated the dynamics of catecholamine outflow in the mpFC during a prolonged stressful experience, and none has followed the evolution of DA and NE outflows in these conditions. Moreover, these few previous studies used transversal microdialysis that does not target the cortical areas tested in the present experiments (Imperato et al. 1991).

The changes in DA and NE outflow observed during exposure to the stressor are unlikely to be ascribed to the termination of the stress reaction or to the reduction of rats’ motor responses. Indeed, the first hypothesis predicts a return to basal levels of all catecholaminergic responses. Instead, only mpFC NE outflow returned to basal levels following 120 min of restraint, whereas DA levels in the mpFC and NAc were still significantly higher and lower, respectively, than basal levels at 240 min of restraint. As for the second hypothesis, rats left undisturbed for 240 min following the collection of baseline samples did not show the decrease of NAc DA outflow observable in stressed rats, and these rats were mostly asleep.

On the other hand, reduced mesoaccumbens DA release has been reported in rats and in mice exposed to different types of stressful experiences (Cabib et al. 1988; Puglisi-Allegra et al. 1994; Puglisi-Allegra et al. 1991; Rossetti et al. 1993, Chrapusta et al. 1997; Grappi et al. 2003) and in rats during the early phase of withdrawal from psychostimulants (Imperato, Mele, et al. 1992; Diana et al. 1993). Moreover, using a paradigm in which pairs of animals are subjected to a series of electric shocks, with only 1 animal being able to interrupt shock delivery for both, it was demonstrated that whereas the small initial increase of mesocortical DA release and the earlier activation of mesoaccumbens DA outflow are observable in both experimental groups, the subsequent larger release in mesocortical DA and the decrease in mesoaccumbens DA was present only in animals that could not interrupt shock delivery (Cabib and Puglisi-Allegra 1994; Bland, Hargave, et al. 2003; Bland, Twining, et al. 2003). These considerations support the view that the pattern of catecholamine outflow observed during prolonged exposure to restraint was indeed related to the stress response. Moreover, they suggest a strong relationship between the initial mpFC NE stress response and the temporary increase in mesoaccumbens DA release and between later stress-induced increase in mesocortical DA release and the inhibition of mesoaccumbens DA outflow.

In the second experiment, we confirmed this hypothesis by the effects of selective depletion of prefrontal cortical NE or DA. Selective depletion of prefrontal NE DA afferents were obtained by 6-OHDA infusion in the mpFC following protection of either DA or NE by selective uptake inhibitors. This approach allowed a profound depletion of tissue levels of the targeted catecholamine to be produced, leaving the levels of the protected one virtually unaffected. The basal outflow of both targeted and protected catecholamines was unaffected by the treatment, thus suggesting that spared noradrenergic afferents develop a compensatory response that leads to an extracellular NE outflow similar to that of Sham, in agreement with previous studies based on nonselective NE depletion in rats (Abercrombie and Zigmond 1989; Hughes and Stanford 1998) and on previous data based on selective depletion in mice (Ventura et al. 2003, 2005). Similarly, compensation in extracellular DA outflow adequate to maintain normal neurotransmitter levels in the resting state despite extensive neuronal degeneration is consistent with a body of literature (Abercrombie and Zigmond 1989 for a review). Finally, it should be pointed out that NE and DA outflow in catecholamine-depleted cortices were TTX sensitive.

Nonetheless, selective depletion of prefrontal NE eliminated both stress-induced NE outflow in the mpFC and stress-induced enhanced DA release in the NAc. These data demonstrate that stress-induced stimulation of mesoaccumbens DA release is promoted by the enhanced NE transmission in the mpFC. It should be pointed out that this is the first demonstration of a control by cortical NE transmission on mesoaccumbens DA release in drug-free organisms. On the other hand, pharmacological evidence supports the view that mesoaccumbens DA release promoted by addictive drugs is dependent on mpFC NE transmission (Darracq et al. 1998; Ventura et al. 2003, 2005). This homology between the central effects of stress and addictive drugs may be relevant for understanding the influence of stress on relapse into drug taking (Lu et al. 2003; Sanchez et al. 2003; McFarland et al. 2004; Stewart 2004; Ventura et al. 2005).

By contrast, selective depletion of mesocortical DA eliminated both stress-induced DA outflow in the mpFC and stress-induced inhibition of mesoaccumbens DA release. These results demonstrate that stress-induced inhibition of mesoaccumbens DA release is dependent on the large stress-induced increase of DA outflow in mpFC observable in the later phase of the stressful experience. The present results are the first direct demonstration of this mechanism although previous ex vivo studies in mice had suggested an involvement of mesocortical DA in stress-induced inhibition of mesoaccumbens DA release (Ventura et al. 2002). Moreover, in line with previous studies (Deutch et al. 1990; King et al. 1997), we observed an increase of the initial stress-induced enhancement of mesoaccumbens DA release in prefrontal DA-depleted rats.

In addition, our results indicate that, in stressful conditions, NE and DA release in the mpFC are independent. Indeed, selective NE depletion did not affect stress-induced DA release and selective DA depletion did not affect stress-induced NE release. These data appear at odds with the results indicating an interaction between NE and DA release in mpFC (Linner et al. 2002; Pan et al. 2004; Devoto et al. 2005; Carboni et al. 2006). This discrepancy may be explained by the different effects of pharmacological, physiological, and lesion manipulations. Indeed, it has been reported that lesioning of the noradrenergic neurons does not affect handling-induced release of DA in the mpFC (Kawahara et al. 1999) and that lesioning of dorsal noradrenergic bundle has no effect on basal DA release (Valentini et al. 2004).

Further, our results clearly demonstrate the opposing influence of NE and DA transmission in the mpFC on mesoaccumbens DA release. Thus, mesocortical DA depletion enhanced mesoaccumbens DA release only in the first phase of stress response, when stress enhanced NE outflow in the mpFC. This observation indicates that the small initial increase of DA release in the mpFC contrasted the effects of NE outflow. Moreover, the inhibitory effects of prefrontal DA on the subcortical response was only observable following the return of NE to basal levels, indicating that enhanced NE transmission...
may reduce the inhibitory effects of prefrontal DA on accumbal dopaminergic transmission.

The opposing influence of prefrontal cortical NE and DA on mesoaccumbens DA release could be promoted by their action on the same or on different pathways. Indeed, recent results indicate that DA receptors of the D2 type and beta-adrenergic receptors, colocalized on prefrontal cortical neurons, exert opposite influences on cyclic adenosine monophosphate, a marker of postsynaptic activation (Montezinho et al. 2006). On the other hand, mpFC NE and DA might activate 2 different pathways to regulate mesoaccumbens DA release in opposite ways: an "activating pathway" provided by indirect glutamatergic projections onto the DA cells (Omelchenko and Sesack 2005) and an "inhibitory pathway" provided by prefrontal glutamatergic efferents to midbrain GABAergic interneurons and striatomesencephalic γ-aminobutyric acid neurons (Carr et al. 1999, Carr and Sesack, 2000; Sesack and Carr 2002).

Finally, the present results may contribute to the understanding of some psychopathogenic effects of stress. Indeed, beside the already discussed homology between some central effects of addictive drugs and the effects of stress observed in the present experiments, preclinical results point to disturbances of cortical NE and subcortical DA transmission in depression (Cabib et al. 2002; Rossetti et al. 1993; Ressler and Nemeroff 2001, 1984; Ventura et al. 2002; Grappi et al. 2003) and to facilitation of stress-induced cortical NE release by chronic treatment with antidepressants (Page and Lucki 2002). Further, mood stabilizers have been shown to influence the interaction between D2-like and beta-adrenergic receptors in the prefrontal cortex (Montezinho et al. 2006).

In conclusion, it should be pointed out that stress responses are not pathological per se; indeed they are necessary to allow psychophysiological coping with environmental challenges. Stress research in human subjects has long supported the idea that coping requires cognitive evaluation of the stressful condition and of response outcomes (Ursin and Erikson 2004), and few indications can be also found in animal research (D’Angio et al. 1988). The mpFC, due to its "supervisory" functions such as attention to stimulus features and action-outcome rules (Cardinal et al. 2002; Dalley et al. 2004; Toates 2004), should play a major role in this evaluation. On the other hand, mesoaccumbens DA transmission is required to energize behavioral output and is implicated in behavioral activation and effort-related decision-making (Salamone et al. 2005). Therefore, enhanced DA release in the NAC is certainly needed in active behavioral coping. Instead, inhibition of DA release in the NAC may be required in situations where active behavioral coping is ineffective and complete withdrawal and inactivity may serve a "replenishing" function (Bonne et al. 2004). Finally, the control of mesoaccumbens DA release by the opposing action of prefrontal cortical NE and DA may be required to tie behavioral output to cognitive evaluation of the stressful condition and of response outcomes.

Notes
We thank Dr E. Catalfamo for his skillful assistance. This research has been supported by Ministero della Ricerca Scientifica e Tecnologica (PRIN 2003, 2005), Università "La Sapienza" Ateneo (2003/2004), and Ministero della Salute (Progetto Finalizzato RF03.182P). Conflict of Interest: None declared.

Address correspondence to Simona Cabib, PhD, Department of Psychology, Università "La Sapienza," via dei Marsi 78, Rome I-00185, Italy. Email: simona.cabib@uniroma1.it.

References


Sesack SR, Carr DB. 2002. Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. Physiol Behav. 77:513-517.


