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Biochemical Adaptations in the Mesolimbic Dopamine System in Response to Repeated Stress

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We have demonstrated previously that chronic administration of morphine, cocaine, or ethanol produces some common biochemical adaptations in the ventral tegmental area (VTA) and nucleus accumbens (NAc), components of the mesolimbic dopamine system implicated in the reinforcing and locomotor activating properties of these drugs of abuse. Because this neural pathway is also regulated by stress, and because stress has been shown to influence an animal's behavioral responses to drugs of abuse, it was of interest to determine whether repeated exposure to stress results in similar biochemical adaptations. By use of immunoblot analysis, we show here that a course of chronic "unpredictable" stress, like chronic drug exposure, increased levels of immunoreactivity of tyrosine hydroxylase and glial fibrillary acidic protein and decreased levels of immunoreactivity of neurofilament proteins in the VTA. Chronic unpredictable stress also increased levels of cyclic AMP-dependent protein kinase activity and decreased levels of immunoreactivity of the G protein subunit, Giα, in the NAc. These effects required long-term exposure to stress and were in most cases not seen in the substantia nigra and caudate-putamen, components of the nigrostriatal dopamine system studied for comparison. The biochemical effects of chronic stress in the VTA and NAc differed among three strains of rat studied. Fischer 344 rats were the most responsive in that they exhibited all of the aforementioned adaptations, whereas Lewis rats were the least responsive in that they exhibited none of these adaptations; Sprague-Dawley rats exhibited an intermediate number of responses. Taken together, the results of the present study demonstrate that chronic exposure to stress results in biochemical adaptations in the mesolimbic dopamine system that resemble the chronic actions of several drugs of abuse. These adaptations could contribute to the convergent behavioral effects induced by treatments that are mediated via the VTA-NAc pathway. **[*Neuropsychopharmacology 14:443–452 1996*]**

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Important advances have been made in recent years in understanding the mechanisms by which drugs of abuse exert their effects on brain function. There is a growing consensus that the acute reinforcing and locomotor activating properties of these drugs are mediated in large part by the mesolimbic dopamine system (Wise 1990; Kuhar et al. 1991; Fibiger et al. 1992; Kalivas and Samson 1992; Koob 1992; Dworkin and Smith 1993; Robinson and Berridge 1993; Self and Nestler 1995).

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This neural pathway consists of dopaminergic neurons in the ventral tegmental area (VTA) and their various targets, notably the nucleus accumbens (NAc). The mesolimbic dopamine system may also be an important site in the brain where drugs of abuse produce adaptations after long-term administration that underlie sensitization to their locomotor activating properties, as well as changes in the drug-reinforcement mechanisms (e.g., drug craving) that characterize drug addiction.

Over the past several years, interactions between drugs of abuse and stress have been increasingly well established. The mesolimbic dopamine system is one of the most sensitive stress-responsive pathways in brain; acute exposure to stress, like acute exposure to drugs of abuse, increases extracellular levels of dopamine in the NAc and related regions as assessed by in vivo microdialysis (Deutch and Roth 1990; Kalivas and Stewart 1991; Imperato et al. 1992; Sorg and Kalivas 1993). There is also growing evidence for some of the same long-term consequences of stress and drugs of abuse in the mesolimbic dopamine system. Repeated exposure to stress, like repeated exposure to most drugs of abuse, can result in locomotor sensitization (Kalivas and Stewart 1991; Sorg and Kalivas 1993). Moreover, stress and the various drugs of abuse exhibit "cross-sensitization." For example, prior exposure to opiates or cocaine (or a related stimulant) can sensitize an animal to the locomotor activating effects of the other drug as well as to stress (Vezina and Stewart 1990; Kalivas and Stewart 1991; Cunningham and Kelley 1992; Hamamura and Fibiger 1993; Sorg and Kalivas 1993). Conversely, prior exposure to stress can sensitize an animal to these and other drugs of abuse. Prior exposure to stress also increases the acquisition of drug self-administration behavior, indicating that stress may regulate both the reinforcing and the locomotor properties of drugs of abuse (Piazza et al. 1991; Ramsey and Van Ree 1993; Fahlke et al. 1994; Goeders and Guerin 1994; Shaham and Stewart 1994; Shaham et al. 1994). Together, the results suggest that chronic exposure to stress and to drugs of abuse may result in common adaptations in the mesolimbic dopamine system that underlie these behavioral phenomena.

In previous works, we and others have identified proteins in the VTA and NAc that are subject to similar regulation by chronic opiate, cocaine, or ethanol administration. In the VTA chronic exposure to these drugs increases levels of tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of dopamine (Beitner-Johnson and Nestler 1991; Sorg et al. 1993; Vrana et al. 1993; Ortiz et al. 1995b) and decreases levels of the neurofilament proteins, NF-200, NF-160, and NF-68, the three major neuron-specific intermediate filament proteins expressed in brain (Beitner-Johnson et al. 1992; Ortiz et al. 1995b). The three drugs also regulate glial fibrillary acidic protein (GFAP) in the VTA, a glial-specific intermediate filament protein, although in different ways: morphine and ethanol increase levels of GFAP immunoreactivity, whereas cocaine increases back phosphorylation levels of the protein (Beitner-Johnson et al. 1993; Ortiz et al. 1995b). Chronic morphine and cocaine treatments also produce similar effects in the NAc, with decreased levels of the inhibitory G protein, Giα, and increased levels of adenylyl cyclase and cyclic AMP-dependent protein kinase (protein kinase A) (Terwilliger et al. 1991). Chronic ethanol administration results in a similar increase in protein kinase A in this brain region (Ortiz et al. 1995b). These effects of morphine, cocaine, and ethanol require chronic exposure to the drugs, are specific to the mesolimbic dopamine system, and are not seen in response to chronic exposure to several classes of psychotropic drugs that lack reinforcing properties.

Based on these common chronic actions of opiates, cocaine, and ethanol in the mesolimbic dopamine system and the view that this neural pathway is a common neurobiological substrate for stress as well as drugs of abuse, it was of interest to determine whether chronic exposure to stress was associated with some of the same biochemical adaptations in the VTA and NAc. We show here that, as predicted, certain forms of repeated stress produce many of the same adaptations in TH, NFs, GFAP, protein kinase A, and Giα in the VTA-NAc pathway as do the drugs of abuse.

We also compared stress regulation of these proteins in two inbred rat strains, the Fischer 344 and Lewis rat. These strains are known to differ in basal levels of these proteins, specifically in the VTA and NAc, under drug-naive conditions. The VTA of the Lewis rat displays higher levels of TH and GFAP and lower levels of NFs compared to the VTA of the Fischer rat (Beitner-Johnson et al. 1991, 1993; Guitart et al. 1992). In addition, the NAc of the Lewis rat displays higher levels of protein kinase A and lower levels of Giα compared to the NAc of the Fischer rat (Guitart et al. 1993). Thus, the Lewis rat, compared to the Fischer rat, resembles Sprague-Dawley rats treated chronically with a drug of abuse. Moroever, chronic exposure to a drug of abuse normalizes these inherent biochemical differences between Fischer and Lewis rats, because Fischer rats show biochemical adaptations similar to those observed in Sprague-Dawley rats, whereas Lewis rats show no detectable responses (Guitart et al. 1992, 1993). We show here that the Fischer and Lewis strains display similar differential biochemical responses to repeated stress.

Together, the results of the present study provide further evidence for the converging effects of stress and drugs of abuse at the level of the mesolimbic dopamine system and indicate the types of biochemical changes that may contribute to the common behavioral actions of these treatments.

MATERIALS AND METHODS

Stress Treatments and Isolation of Brain Regions

Male Sprague-Dawley rats (150–200 g) were obtained from CAMM (Wayne, NJ); Fischer and Lewis rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in groups of three, with food and water ad libitum in a 12-hour light/dark cycle (lights on at 7:00 A.M.). Chronic restraint stress involved placing rats in restraint bags for 45 minutes daily for 10 days as described (Morinobu et al. 1995); rats were used 24 hours after the last treatment. Chronic unpredictable stress involved exposing rats to several types of stresses, which varied from day to day, for a period of 10 days. The following paradigm was used; it was based on published methods (Sapolsky et al. 1984; Willner 1984, 1991) and was scheduled with the aid of random numbers generated in a microprocessor:

Day 1 12:00 P.M., cage rotation, 50 minutes; 1:00 P.M., swim stress, 4 minutes

Day 2 11:00 A.M., cold (4°C) isolation, 60 minutes; 7:00 P.M., lights on, overnight

Day 3 12:00 P.M., lights off, 3 hours; 3:00 P.M., cold isolation, 15 minutes

Day 4 7:00 P.M., cage rotation, 50 minutes; 7:00 P.M., food/water deprivation, overnight

Day 5 1:00 P.M., swim stress, 3 minutes; 7:00 P.M., isolation housing, overnight

Day 6 11:00 A.M., restraint stress, 60 minutes; 3:00 P.M., lights off, 2 hours

Day 7 10:00 A.M., swim stress, 4 minutes; 4:00 P.M., restraint stress, 60 minutes

Day 8 7:00 P.M., lights on, overnight; 7:00 P.M., food/water deprivation, overnight

Day 9 10:00 A.M., cage rotation, 20 minutes; 7:00 P.M., lights on, overnight

Day 10 7:00 P.M., isolation housing, overnight; 7:00 P.M., food/water deprivation, overnight

Day 11 10:00 A.M., animals killed

In one experiment, animals were treated with unpredictable stress for 3 days only, as follows:

Day 1 9:00 A.M., cage rotation, 50 minutes; 1:00 P.M., restraint stress, 60 minutes

Day 2 11:00 A.M., swim stress, 6 minutes; 3:00 P.M., cold isolation, 60 minutes

Day 3 11:00 A.M., cage rotation, 50 minutes; 3:00 P.M., restraint stress, 60 minutes; 7:00 P.M., lights on, overnight

Day 4 10:00 A.M., animals killed

Brains were removed rapidly from decapitated rats and cooled in ice-cold physiological buffer (final concentrations: 126 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, pH 7.4). The VTA and substantia nigra (SN) were obtained from 1 mm coronal cross sections of brain stem by use of a 15-gauge syringe needle as described (Terwilliger et al. 1991); bilateral punches (about 1.5 mg wet weight) were pooled from individual rats. The NAc was obtained by use of a 12-gauge syringe needle (pooled bilateral punches weighed about 5 mg), and caudate/putamen was removed either by 12-gauge punches or gross dissection; the two dissections yielded equivalent results. All biochemical analyses were performed on brain samples obtained from individual rats.

Immunolabeling of TH, NFs, GFAP, and Giα

Brain samples were homogenized (10 mg wet weight/ml) in 1% SDS, and protein levels were determined by the method of Lowry. Samples were adjusted to contain (final concentrations): 50 mM Tris pH 6.7, 4% glycerol, 4% SDS, 2% 2-mercaptoethanol, with bromophenol Blue as a marker, and then boiled for 2 minutes. Blot immunolabeling of TH, NFs, GFAP, and Giα was carried out based on published procedures (Beitner-Johnson and Nestler 1991; Terwilliger et al. 1991; Beitner-Johnson et al. 1992, 1993), except that immunoreactivity was detected by chemiluminescence and exposure to hyperfilm (Amersham). These experiments utilized 5 to 20 μg of protein per sample and the following antibodies: a rabbit polyclonal antiserum prepared against TH (diluted 1:10,000, kindly provided by Dr. John Haycock, Louisiana State University), monoclonal antibodies directed against the various NF proteins [anti-NF 200 (clone N52, 1:20,000, Sigma), anti-NF-160 (clone NN18, 1:3,000, Sigma), anti-NF-68 (clone NR4, 1:160,000, Boehringer Mannheim)], a mouse monoclonal anti-GFAP antibody (clone GA5,1:50,000 Sigma); and a rat polyclonal anti-Giα antibody (anti-Giα1/2, 1:8,000, New England Nuclear).

The levels of immunoreactivity of the various proteins were quantified by computerized laser densitometry of resulting autoradiograms. The arbitrary densitometric values of protein immunoreactivity obtained for stress-treated samples were then compared to those for control animals. Under the immunolabeling conditions used, levels of immunoreactivity of TH, NFs, GFAP, and Giα were linear over at least a threefold range of tissue concentration.

Protein Kinase A Activity Assay

Brain samples were homogenized (10 mg/ml) in ice-cold "homogenization buffer" containing 50 mM Tris, pH 7.4/1 mM dithiothreitol/1 mM EGTA/10 μg per milliliter of leupeptin/50 kallikrein units per milliliter of aprotinin. Aliquots of homogenates were centrifuged

Table 1. Biochemical Effects of Repeated Stress in the VTA of Sprague-Dawley Rats (% of control ± SEM)

	Restraint Stress	Unpredictable Stress	
	10 days	3 days	10 days
TH	96 ± 8 (6)	96 ± 6 (6)	147 ± 11 (6)*
NF200	99 ± 13 (6)	94 ± 13 (6)	72 ± 5 (6)*
NF160	103 ± 9 (6)	89 ± 8 (6)	75 ± 8 (10)*
NF68	100 ± 11 (6)	93 ± 4 (6)	63 ± 6 (6)*
GFAP	94 ± 7 (6)	89 ± 13 (5)	101 ± 10 (6)

*$p < .05$ vs. control (nonstressed) by student's t-test.

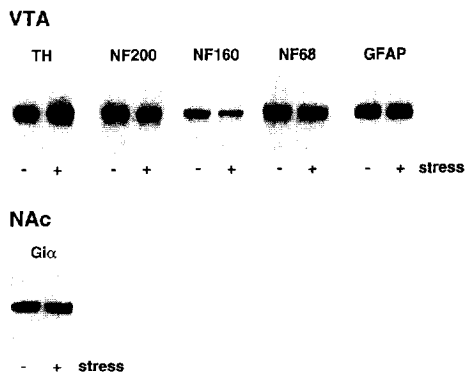

Figure 1. Autoradiograms showing the effect of chronic unpredictable stress on levels of TH, NF, and GFAP immunoreactivity in the VTA and on levels of Giα immunoreactivity in the NAc of Sprague-Dawley rats. VTA or NAc extracts from control and chronic (10 days) stress-treated rats were subjected to one-dimensional SDS-polyacrylamide gel electrophoresis, to blot immunolabeling for TH, NF-200, NF-160, NF-68, GFAP, or Giα, and to autoradiography as described in Methods.

in a Sorvall microultracentrifuge at 150,000 × g for 10 minutes at 4°C. The supernatants were designated the soluble fractions; the pellets were resuspended in the original volume of homogenization buffer and were designated the particulate fractions. Duplicate aliquots of the fractions (containing 2–5 μg of protein) were assayed for protein kinase A activity by use of a filter paper assay with purified histone as substrate exactly as described (Terwilliger et al. 1991). Protein kinase A activity was calculated as the difference in histone phosphorylation observed in the presence of cyclic AMP and that measured in the presence of a specific inhibitor of protein kinase A, called protein kinase inhibitor or PKI (Sigma). Under the assay conditions used, protein kinase A activity was linear over a fivefold range of tissue concentration and between 1 and 5 minutes of incubation. The specific activities of protein kinase A in particulate and soluble fractions of brain regions from control animals were similar to values reported previously (Terwilliger et al. 1991).

RESULTS

Effect of Stress on TH, NFs, and GFAP in the VTA of Sprague-Dawley Rats

As a first step in evaluating the influence of chronic exposure to stress on specific proteins in the VTA, we analyzed the VTA from Sprague-Dawley rats subjected to daily restraint stress for 10 days. This stress paradigm, commonly used to study the effects of chronic stress (Sapolsky et al. 1984; Wolfgang et al. 1994; Morinobu et al. 1995), resulted in no detectable changes in levels of TH, NFs, or GFAP (Table 1). Since animals can become habituated to repeated exposure to the same stressful stimulus (see Discussion), we next examined the influence of a different stress paradigm, one involving different stresses applied in an unpredictable manner. Such unpredictable stress has been shown to produce more potent effects on an animal's behavioral and neurochemical responses (Sapolsky et al. 1984; Willner 1984, 1991). Indeed, exposure of Sprague-Dawley rats to a 10-day period of unpredictable stress resulted in significant changes in several biochemical parameters in the VTA. As illustrated in Figure 1, chronic unpredictable stress increased levels of TH and decreased levels of NF-200, NF-160, and NF-68 in this brain region, whereas no effect of stress was apparent on levels of GFAP. These results are shown quantitatively in Table 1. The ability of chronic unpredictable stress to regulate TH and NFs in the VTA required chronic treatment, as exposure of animals to a short-term (3 days) treatment paradigm did not influence levels of these proteins (Table 1).

As a way of studying the regional specificity of stress regulation of TH and NFs in the VTA, we examined the influence of chronic unpredictable stress on levels of these proteins in the SN, another major dopaminergic nucleus in brain not generally implicated in drug-reinforcement mechanisms. It was found that the stress treatment was without significant effect on levels of TH, NFs, and GFAP in the SN, although there was a tendency for a decrease in NF-160 (Table 2).

Effect of Stress on TH, NFs, and GFAP in the VTA of Fischer and Lewis Rats

We next studied the influence of stress on levels of TH, NFs, and GFAP in the VTA of Fischer and Lewis rats. In Fischer rats chronic restraint stress significantly increased levels of TH in the VTA and tended to decrease levels of NF-68 (Table 3). More dramatic effects were obtained with chronic unpredictable stress. As shown in Table 3, this treatment regimen was found not only to increase

Table 2. Biochemical Effects of Repeated (10 days) Unpredictable Stress in the SN of Sprague-Dawley and Fischer Rats (% of control ± SEM)

	Sprague-Dawley	Fischer
TH	109 ± 15 (6)	92 ± 7 (6)
NF200	131 ± 23 (6)	93 ± 7 (6)
NF160	76 ± 7 (6)†	95 ± 10 (6)
NF68	88 ± 6 (6)	99 ± 6 (6)
GFAP	90 ± 14 (6)	92 ± 4 (6)

†$p < .1$ vs. control (nonstressed) by student's t-test.

Table 3. Biochemical Effects of Repeated (10 days) Stress in the VTA of Fischer and Lewis Rats (% of control ± SEM)

	Fischer		Lewis
	Restraint Stress	Unpredictable Stress	Unpredictable Stress
TH	122 ± 9 (6)*	128 ± 8 (11)*	103 ± 10 (6)
NF200	97 ± 8 (6)	78 ± 6 (11)*	103 ± 11 (6)
NF160	96 ± 7 (6)	79 ± 9 (11)*	114 ± 14 (6)
NF68	83 ± 8 (6)†	86 ± 8 (11)*	93 ± 4 (6)
GFAP	97 ± 10 (6)	118 ± 5 (11)*	101 ± 7 (6)

*$p < .05$ vs. control (nonstressed) by student's t-test.
†$p < .1$ vs. control (nonstressed) by student's t-test.

significantly levels of TH but also to decrease significantly levels of all three NF proteins as well as to produce a small, but significant, increase in levels of GFAP. Stress regulation of these proteins was specific to the VTA, in that no effects were seen in the SN (Table 2).

Different results were obtained for Lewis rats. In contrast to effects seen in Sprague-Dawley and Fischer rats, chronic unpredictable stress had no influence on levels of TH, NFs, or GFAP in the VTA of Lewis rats (Table 3).

Effect of Stress on Protein Kinase A and Giα in the NAc of Sprague-Dawley, Fischer, and Lewis Rats

Because chronic exposure to drugs of abuse also regulates protein kinase A and Giα in the NAc, we next examined the influence of chronic stress on these biochemical end points. It was found that 10 days of unpredictable stress increased levels of protein kinase A activity in the soluble fraction of the NAc of Fischer rats, with a tendency for an increase in the particulate fraction (Table 4). This increase is similar to that observed previously for morphine, cocaine, or ethanol (Terwilliger et al. 1991; Ortiz et al. in press). This effect was not seen in the caudate putamen (data not shown), a target region of the SN not generally implicated in drug-reinforcement mechanisms either. In contrast, chronic unpredictable stress had no effect on protein kinase A activity in the NAc of Sprague-Dawley or Lewis rats (Table 4).

Similar results were obtained for Giα regulation. Chronic unpredictable stress decreased levels of this G protein subunit in the Fischer NAc (Table 4), similar to decreases observed with chronic morphine or cocaine treatments (Terwilliger et al. 1991; Striplin and Kalivas 1993). In contrast, chronic unpredictable stress had no effect on Giα levels in the Fischer caudate putamen (data not shown). Moreover, chronic stress failed to alter levels of this G protein subunit in the NAc of Sprague-Dawley and Lewis rats (Table 4).

Effect of Stress on Weight Gain in Sprague-Dawley, Fischer, and Lewis Rats

As a general indicator of the effect of stress treatments on the physiological state of Sprague-Dawley, Fischer, and Lewis rats, the weight gain exhibited by these animals was monitored during the course of the experiments. The results are shown in Table 5. All three types of rat showed reduced weight gain following stress treatments, with chronic unpredicable stress resulting in a more pronounced reduction in weight gain compared to chronic restraint stress. Moreover, the Lewis rats seemed to be less responsive to the stress treatments in that they tended to show less of a reduction in weight gain compared to the Sprague-Dawley and Fischer rats.

Table 4. Biochemical Effects of Repeated (10 days) Unpredictable Stress in the NAc of Sprague-Dawley, Fischer, and Lewis Rats (% of control ± SEM)

	Sprague-Dawley	Fischer	Lewis
Protein kinase A			
Soluble	105 ± 6 (6)	151 ± 8 (6)*	103 ± 10 (6)
Particulate	98 ± 3 (6)	116 ± 4 (6)†	95 ± 8 (6)
Giα	101 ± 11 (6)	78 ± 10 (6)*	100 ± 10 (6)

*$p < .05$ vs. control (nonstressed) by student's t-test.
†$p < .1$ vs. control (nonstressed) by student's t-test

Table 5. Effects of Repeated Stress on Weight Gain in Sprague-Dawley, Fischer, and Lewis Rats[a] (% weight gain ± SEM)

	Sprague-Dawley	Fischer	Lewis
Unpredictable stress (10 days)			
Control	29 ± 1 (6)	28 ± 3 (6)	27 ± 1 (6)
Stress	12 ± 1 (6)	11 ± 2 (6)	18 ± 1 (6)
Restraint stress (10 days)			
Control	32 ± 1 (6)	32 ± 2 (6)	
Stress	23 ± 1 (6)	17 ± 10 (6)	

[a] The starting weights of all animals were between 170 and 185 g and did not differ among the various control and stress groups tested. Data represent the percent increase in weight over the course of the 10-day stress treatments.

DISCUSSION

One major finding of the present study is that repeated exposure of rats to stress results in many of the same biochemical adaptations in the mesolimbic dopamine system as demonstrated previously for opiates, cocaine, and ethanol after long-term administration. Moreover, the responses to chronic stress depend on the genetic background of the rats, in that clear differences were observed between two inbred rat strains, the Fischer and Lewis rat. In the VTA chronic unpredictable stress increased levels of TH and decreased levels of NF proteins in Fischer rats as well as in outbred Sprague-Dawley rats. In the Fischer VTA this stress treatment also increased levels of GFAP. In contrast, no changes in these proteins were detected in the Lewis VTA. In the NAc chronic unpredictable stress increased levels of protein kinase A and decreased levels of Giα in Fischer rats only, with no changes oberved in Sprague-Dawley or Lewis rats. These biochemical effects of chronic unpredictable stress in the mesolimbic dopamine system, which resemble the chronic actions of morphine, cocaine, and ethanol treatments in these brain regions, were seen after prolonged (10 days) exposure to stress but not after a shorter (3 days) treatment period. These findings indicate that either the shorter treatment is inadequate to produce these effects or that the effects of an initial exposure to stress require several more days to develop into a measurable change. The biochemical effects of chronic stress in the mesolimbic dopamine system did not occur in most cases in the nigrostriatal dopamine system, which is anatomically related to the mesolimbic system but generally not implicated in drug-reinforcement mechanisms. Although chronic unpredictable stress was found to have many of the same biochemical effects as chronic exposure to drugs of abuse, we know that the drug effects are not the result of the stress associated with involuntary drug treatments per se, because self-administration of heroin has been shown recently to result in the same pattern of biochemical adaptations in the VTA and NAc (Self et al. 1995).

It is apparent from this study that a paradigm of unpredictable stress is more effective at producing these biochemical adaptations in the mesolimbic dopamine system than repeated restraint stress. Although repeated restraint stress is commonly employed in stress studies, there is growing appreciation of the fact that rats can quickly habituate to the repeated application of the same stressful stimulus. One biochemical indicator of such habituation comes from studies of c-Fos expression. c-Fos is the product of an immediate early gene that is rapidly and transiently induced in the nervous system in response to a wide variety of stimuli, including stress (Morgan and Curran 1991). Whereas c-Fos is induced robustly in response to acute exposure to several types of stress, these responses desensitize with repeated exposures (Campeau et al. 1991; Melia et al. 1994). In contrast, application of different stressful stimuli at irregular intervals would be expected to reduce the degree of habituation or desensitization that would result in response to any particular stimulus. This has been demonstrated directly in studies wherein repeated unpredictable stress has been shown to produce biochemical and behavioral changes not seen with repeated predictable stress (Sapolsky et al. 1984; Willner 1984, 1991). As a result, unpredictable stress has been proposed to represent a better animal model with which to study the long-term consequences of stress.

In the current study Fischer rats exhibited the most responses to chronic stress, with respect to biochemical adaptations in the mesolimbic dopamine system; Lewis rats showed the fewest responses, with outbred Sprague-Dawley rats showing an intermediate number of responses to chronic stress. The interpretation that the Fischer rat was most responsive to chronic stress is supported by the observation that unpredictable stress produced changes in GFAP levels in the VTA and in protein kinase A and Giα levels in the NAc of this strain, whereas Sprague-Dawley rats exhibited no such adap-

tations. Moreover, Fischer rats showed some, albeit limited, responsiveness to repeated restraint stress, whereas Sprague-Dawley rats showed none. The interpretation that the Fischer rat is particularly responsive to chronic stress is also supported by behavorial studies, in which Fischer rats were shown to exhibit exaggerated stress responses compared to Sprague-Dawley rats (Rosecrans et al. 1986). On the other hand, the stress-induced adaptations in TH and NFs in the VTA observed in the present study were of similar magnitude in Fischer and Sprague-Dawley rats.

Previous studies have demonstrated clear Fischer-Lewis strain differences in specific biochemical parameters in the mesolimbic dopamine system under drug-naive conditions, with the Lewis rat, compared to the Fischer rat, resembling Sprague-Dawley rats treated chronically with morphine, cocaine, or ethanol (see Introduction). Moreover, chronic exposure to morphine normalizes these baseline biochemical differences, because Fischer rats show the same types of biochemical adaptations as observed in Sprague-Dawley rats, whereas Lewis rats show no detectable adaptations to drug exposure (Guitart et al. 1992, 1993). The results of the present study indicate a similar strain difference in responsiveness to chronic stress. Fischer rats exhibited the full complement of biochemical adaptations in the VTA and NAc in response to chronic unpredictable stress, as observed in Fischer and Sprague-Dawley rats in response to chronic morphine treatment. In contrast, Lewis rats exhibited no detectable adaptations in any of the biochemical parameters studied in response to stress or morphine treatment.

The mechanisms by which stress produces these biochemical adaptations in the mesolimbic dopamine system remain unknown. Chronic administration of glucocorticoids has been shown to increase levels of TH immunoreactivity in the VTA of Fischer, but not Lewis, rats (Ortiz et al. 1995a). These results suggest that systemic glucocorticoids, released in response to repeated stress, may contribute to some of the biochemical effects of stress on the mesolimbic dopamine system. This view is consistent with evidence that the hypothalamic-pituitary-adrenal axis can influence, and may even mediate, some of the reinforcing and locomotor-activating effects of drugs of abuse (Cole et al. 1990; Kalivas and Stewart 1991; Piazza et al. 1991; Goeders 1992; Marinelli et al. 1994). It is also consistent with the large differences in plasma levels of glucocorticoids between Fischer and Lewis rats observed under baseline and stress-induced conditions (Sternberg et al. 1989; Griffin and Whitacre 1991; Glowa et al. 1992; Dhabbar et al. 1993; Ortiz et al. 1995a). However, glucocorticoid treatment has been shown to have no influence on levels of NF or GFAP immunoreactivity in the VTA of either the Fischer or Lewis strain (Ortiz et al. 1995a), suggesting that other mediators, in addition to glucocorticoids, are also involved. Indeed, stress is known to affect a large number of neurotransmitter systems and circulating factors, which could conceivably also contribute to the biochemical effects of stress in the VTA and NAc, as well as to Fischer-Lewis strain differences in these effects. For example, the corticotropin releasing factor (CRF) system is known to be activated by stress, and large Fischer-Lewis differences have been documented in brain and pituitary levels of CRF (Sternberg et al. 1989). As another example, stress is associated with increases in levels of several cytokines in the periphery and central nervous system (Shintani et al. 1995). Moreover, local infusion of one cytokine, CNTF (ciliary neurotrophic factor), directly into the VTA has been shown recently to mimic the effects of stress and drugs of abuse on levels of TH and GFAP in this brain region (Berhow et al. 1995). Further work is needed to evaluate the involvement of these and many other mechanisms in stress regulation of the mesolimbic dopamine system.

The common biochemical adaptations observed in response to stress and drugs of abuse presumably underlie some common functional adaptations induced by these treatments in the mesolimbic dopamine system. In addition, the similar biochemical actions would suggest forms of cross-tolerance or cross-sensitization among the treatments. As discussed in the Introduction, such interactions have been documented increasingly with stress, cocaine, and other stimulants, and opiates with respect to their regulation of drug reinforcement and locomotor activity mechanisms at the level of the mesolimbic dopamine system, and studies of similar phenomena with ethanol are beginning to appear.

Although the precise functional significance of the various stress- and drug-induced biochemical adaptations observed in the VTA and NAc remains unclear, progress has been made in recent years in relating certain adaptations to specific behavioral phenomena. The stress- and drug-induced increase in TH would be expected to enhance the maximal capacity of VTA dopaminergic neurons to synthesize dopamine, which could have important consequences on several behaviors given the important role of dopaminergic neurotransmission in the VTA-NAc pathway in the regulation of drug reinforcement and locomotor activity. Indeed, increased levels of TH in the VTA have been correlated temporally with the onset of locomotor sensitization to cocaine (Sorg et al. 1993). Drug-induced changes in levels of NFs might be expected to be associated with reductions in axonal caliber, axoplasmic transport, and the size and degree of dendritic arborizations, based on the putative role of NFs in neuronal function (Nestler 1992; Nestler et al. 1993). Direct evidence for this comes from a recent study, where chronic morphine administration was found to produce a ~50% reduction in axoplasmic transport from the VTA to the NAc (Beitner-Johnson and Nestler 1993). Such a reduction in axoplasmic

transport is consistent with the observation that increased TH levels in the VTA are not associated with increased enzyme levels in the NAc; in fact, levels of TH tend to be reduced in the NAc of drug-treated animals (Beitner-Johnson and Nestler 1991; Ortiz et al. 1995b; Self et al. 1995). Finally, stress- and drug-induced increases in levels of GFAP might suggest a reactive gliosis, or even frank neural injury, in the chronic stress- or drug-treated state (Nestler 1992; Nestler et al. 1993). This view is supported by recent findings that chronic infusion of certain neurotrophins directly into the VTA can both prevent and reverse the ability of morphine and of cocaine to alter levels of TH and GFAP in this brain region (Berhow et al. 1995). It would now be interesting to study these various parameters of VTA structure and function in chronic stress-treated animals.

The biochemical adaptations induced in the NAc by chronic exposure to stress or drugs of abuse have also been related to specific functional end points. For example, administration of agents that inhibit Giα (e.g., pertussis toxin) or activate protein kinase A (e.g., excitatory cyclic AMP analogs) directly into the NAc increases cocaine and heroin self-administration, whereas agents that produce the opposite effect reduce drug self-administration (Self et al. 1994; Self and Nestler 1995). Administration of these agents into the caudate putamen exert no effect on self-administration behavior. Analogous studies have supported a role for Giα and the cyclic AMP pathway in the locomotor activation and sensitization seen in response to drug exposure (Cunningham and Kelley 1993; Striplin and Kalivas 1993; Miserendino and Nestler 1995). Furthermore, the upregulation of the cyclic AMP pathway could account for the functional supersensitivity of D_1 dopamine receptors demonstrated electrophysiologically in NAc neurons following chronic cocaine treatment (Henry and White 1991), because this functional supersensitivity occurs in the absence of detectable changes in D_1 receptors themselves and because D_1 receptors are generally believed to produce their effects via activation of the cyclic AMP pathway. In addition, the time courses of the reduction in Giα levels and of the D_1 receptor supersensitivity have been correlated temporally with the onset and persistence of locomotor sensitization (Henry and White 1991; Striplin et al. 1993).

In summary, the results of the present study provide additional information concerning specific proteins in the brain that exhibit long-term adaptations in response to chronic stress. Although the functional significance of these adaptations clearly requires much further investigation, most of the adaptations seen with stress also occur following chronic exposure to morphine, cocaine, or ethanol, but not to drugs without reinforcing properties. The studies of Fischer and Lewis rats demonstrate further that the biochemical responses to stress and drug treatments can be influenced by genetic factors. These rat strains could, therefore, provide a useful model system in which to investigate the molecular mechanisms by which specific environmental factors (e.g., stress, drugs) and genetic factors combine to control mesolimbic dopamine function and the behaviors subserved by this neural pathway.

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REFERENCES

Beitner-Johnson D, Nestler EJ (1991): Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J. Neurochem 57:344–347

Beitner-Johnson D, Nestler EJ (1993): Chronic morphine impairs axoplasmic transport in the mesolimbic dopamine system of the rat brain. NeuroReport 5:57–60

Beitner-Johnson D, Guitart X, Nestler EJ (1991): Dopaminergic brain reward regions of Lewis and Fischer rats display different levels of tyrosine hydroxylase and other morphine- and cocaine-regulated phosphoproteins. Brain Res 561:146–149

Beitner-Johnson D, Guitart X, Nestler EJ (1992): Neuofilaments and the mesolimbic dopamine system: Common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. J Neurosci 12:2165–2176

Beitner-Johnson D, Guitart X, Nestler EJ (1993): Glial fibrillary acidic protein and the mesolimbic dopamine system: Regulation by chronic morphine and Lewis-Fischer strain differences in the rat ventral tegmental area. J Neurochem 61:1766–1773

Berhow MT, Russell DS, Terwilliger RZ, Beitner-Johnson D, Self DW, Lindsay RM, Nestler EJ (1995): Influence of neurotrophic factors on morphine- and cocaine-induced biochemical changes in the mesolimbic dopamine system. Neuroscience 68:969–979

Campeau S, Hayward MD, Hope BT, Rosen JB, Nestler EJ, Davis M (1991): Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear. Brain Res 565:349–352

Cole BJ, Cador M, Stinus L, Rivier C, Rivier J, Vale W, Le Moal M, Koob GF (1990): Critical role of the hypothalamic pituitary adrenal axis in amphetamine-induced sensitization of behavior. Life Sci 47:1715–1720

Cunningham ST, Kelley AE (1992): Evidence for opiate-dopamine cross-sensitization in nucleus accumbens: Studies of conditioned reward. Brain Res Bull, 29:675–680

Cunningham ST, Kelley AE (1993): Hyperactivity and sensitization to psychostimulants following cholera toxin

infusion into the nucleus accumbens. J Neurosci 13: 2342–2350

Dhabbar FS, McEwen BS, Spencer RL (1993): Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels—a comparison between Sprague-Dawley, Fischer 344 and Lewis rats. Brain Res 616:89–98

Deutch AY, Roth RH (1990): The determinants of stress-induced activation of the prefrontal cortical dopamine system. Prog Brain Res 85:357–393

Dworkin SI, Smith JE (1993): Opiates/opioids and reinforcement. In Korenman SG and Barchas JD (eds), Biological Basis of Substance Abuse, New York, Oxford University Press, pp 327–338

Fahlke C, Hansen S, Engel JA, Hard E (1994): Effects of ventral striatal 6-OHDA lesions or amphetamine sensitization on ethanol consumption in the rat. Pharmacol Biochem Behav 47:345–349

Fibiger HC, Phillips AG, Brown EE (1992): The neurobiology of cocaine-induced reinforcement. Ciba Foundation Symp 166:96–124

Glowa JR, Geyer MA, Gold PW, Sternberg EM (1992): Differential startle amplitude and corticosterone response in rats. Neuroendocrinology 56:719–723

Griffin AC, Whitacre CC (1991): Sex and strain differences in circadian rhythm fluctuation of endocrine and immune function in the rat: Implications for rodent models of autoimmune disease. J Neuroimmunol 35:53–64

Goeders NE (1992): Potential involvement of anxiety in the neurobiology of cocaine. Ann N Y Acad Sci 654:357–367

Goeders NE, Guerin GF (1994): Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology 114:63–70

Guitart X, Beitner-Johnson DB, Marby D, Kosten TA, Nestler EJ (1992): Fischer and Lewis rats strains differ in basal levels of neurofilament proteins and their regulation by chronic morphine in the mesolimbic dopamine system. Synapse 12:242–253

Guitart X, Kogan JH, Berhow MT, Terwilliger RZ, Aghajanian GK, Nestler EJ (1993): Lewis and Fischer rat strains display differences in biochemical, electrophysiological, and behavioral parameters: Studies in the nucleus accumbens and locus coeruleus of drug-naive and morphine-treated animals. Brain Res 611:7–17

Hamamura T, Fibiger HC (1993) Enhanced stress-induced dopamine release in the prefrontal cortex of amphetamine-sensitized rats. Eur J Pharmacol 237:65–71

Henry DJ, White FJ (1991): Repeated cocaine administration causes persistent enhancement of D_1 dopamine receptor sensitivity within the rat nucleus accumbens. J Pharmacol Exp Ther 258:882–890

Imperato A, Angelucci L, Casolini P, Zocchi A, Puglisi-Allegra S (1992): Repeated stressful experiences differently affect limbic dopamine release during and following stress. Brain Res 577:194–199

Kalivas PW, Samson HH (eds) (1992): The neurobiology of drug and alcohol addiction. Ann N Y Acad Sci Volume 654

Kalivas PW, Stewart J (1991): Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Research Rev 16:223–244

Koob (1992) Drugs of abuse: Anatomy, pharmacology, and function of reward pathways. Trends Pharmacol Sci 13:177–184

Kuhar MJ, Ritz MC, Boja JW (1991): The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci 14:299–302

Marinelli M, Piazza PV, Deroche V, Maccari S, Le Moal M, Simon H (1994): Corticosterone circadian secretion differentially facilitates dopamine-mediated psychomotor effect of cocaine and morphine. J Neurosci 14:2724–2731

Melia KR, Ryabinin AE, Schroeder R, Bloom FE, Wilson MC (1994): Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. J Neurosci 14:5929–5938

Miserendino MJD, Nestler EJ (1995): Behavioral sensitization to cocaine: Modulation by the cyclic AMP system in the nucleus accumbens. Brain Res 674:299–306

Morgan JI, Curran T (1991): Stimulus-transcription coupling in the nervous system. Annu Rev Neurosci 14:421–452

Morinobu S, Nibuya M, Duman RS (1995): Chronic antidepressant treatment down-regulates the induction of c-fos mRNA in response to acute stress in rat cortex. Neuropsychopharmacology 12:221–228

Nestler EJ (1992): Molecular mechanisms of drug addiction. J Neurosci 12:2439–2450

Nestler EJ, Hope BT, Widnell KL (1993): Drug addiction: A model for the molecular basis of neural plasticity. Neuron 11:995–1006

Ortiz J, DeCaprio JL, Kosten TA, Nestler EJ (1995a): Strain-selective effects of corticosterone on locomotor sensitization to cocaine and on levels of tyrosine hydroxylase and glucocorticoid receptor in the ventral tegmental area. Neuroscience 67:383–397

Ortiz J, Fitzgerald LW, Charlton M, Lane SB, Trevisan L, Guitart X, Shoemaker W, Duman RS, Nestler EJ (1995b): Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. Synapse 21:289–298

Piazza PV, Maccari S, Deminiere JM, Le Moal M, Mormede P, Simon H (1991): Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc Natl Acad Sci U S A 88:2088–2092

Ramsey NF, Van Ree JM (1993): Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naive rats. Brain Res 608:216–222

Robinson TE, Berridge KC (1993): The neural basis of drug craving: An incentive-sensitization theory of addiction. Brain Res Rev 18:247–291

Rosecrans JA, Robinson SE, Johnson JH, Mokler DJ, Hong JS (1986): Neuroendocrine, biogenic amine and behavioral responsiveness to a repeated foot-shock-induced analgesia (FSIA) stressor in Sprague-Dawley (CD) and Fischer-344 (CDF) rats. Brain Res 382:71–80

Sapolsky RM, Krey LC, McEwen BS (1984): Stress down-regulates corticosterone receptors in a site-specific manner in the brain. Endocrinology 114:287–292

Self DW, Nestler EJ (1995): Molecular mechanisms of drug reinforcement and addiction. Annu Rev Neurosci 18:463–495

Self DW, Terwilliger RZ, Nestler EJ, Stein L (1994): Inactivation of Gi and Go proteins in nucleus accumbens

reduces both cocaine and heroin reinforcement. J Neurosci 14:6239–6247

Self DW, McClenahan A, Terwilliger RZ, Nestler EJ (1995): Biochemical adaptations in the rat mesolimbic dopamine system in response to heroin self-administration. Synapse 21:312–318

Shaham Y, Stewart J (1994): Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration. Psychopharmacology 114:523–527

Shaham Y, Alvares K, Nespor SM, Grunberg NE (1994): Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacol Biochem Behav 41:615–619

Shintani F, Nakaki T, Kanba S, Sato K, Yagi G, Shiozawa M, Aiso S, Kato R, Asai M (1995): Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in release of hypothalamic monoamines in the rat. J Neurosci 15:1961–1970

Sorg BA, Kalivas PW (1993): Behavioral sensitization to stress and psychostimulants: Role of dopamine and excitatory amino acids in the mesocorticolimbic system. Sem Neurosci 5:343–350

Sorg BA, Chen SY, Kalivas PW (1993): Time course of tyrosine hydroxylase expression after behavioral sensitization to cocaine. J Pharmacol Exp Ther 266:424–430

Sternberg EM, Young SW, Bernardini R, Calogero AE, Ghrousos GP, Gold PW, Wilder RL (1989): A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats. Proc Natl Acad Sci U S A 86:4771–4775

Striplin CD, Kalivas PW (1993): Robustness of G protein changes in cocaine sensitization shown with immunoblotting. Synapse 14:10–15

Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM, Nestler EJ (1991): A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. Brain Res 548:100–110

Vezina P, Stewart J (1990): Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: Lack of conditioned effects. Brain Res 516:99–106

Vrana SL, Vrana KE, Koves TR, Smith JE, Dworkin SI (1993): Chronic cocaine administration increases CNS tyrosine hydroxylase enzyme activity and mRNA levels and tryptophan hydroxylase enzyme activity levels. J Neurochem 61:2262–2268

Willner P (1984): The validity of animal models of depression. Psychopharmacology 83:1–16

Willner P (1991): Animal models as simulations of depression. Trends Pharmacol Sci 12:131–136

Wise RA (1990): The role of reward pathways in the development of drug dependence. In Balfour DJK (ed), Psychotropic Drugs of Abuse. Oxford, Pergamon, Press pp 23–57

Wolfgang D, Chen I, Wand GS (1994): Effects of restraint stress on components of adenylyl cyclase signal transduction in the rat hippocampus. Neuropsychopharmacology 11:187–193