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Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor

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Abstract

Mounting evidence suggests that chronic stress may have a detrimental effect on dopaminergic function and, in certain individuals, could contribute to the pathophysiology of central nervous system disorders like depression, schizophrenia, and Parkinson's disease. Therefore, the effects of chronic elevated brain levels of corticotropin-releasing factor (CRF), a crucial mediator of the behavioral stress response, on dopaminergic function were investigated. Rats treated intracerebroventricularly (i.c.v.) with 1 μg of CRF per day for 13 days displayed a decreased stereotyped response to D-amphetamine 1 day after chronic CRF and 1 month post-CRF. These rats also displayed an increased cataleptic response to eticlopride at 2 days post-CRF, consistent with decreased functional activity in the dopaminergic systems. CRF treatment induced a transient decrease of dopamine tissue levels in the prefrontal cortex at 1 day and 1 week post-CRF, an increase in the nucleus accumbens 1 week post-CRF and no change in the striatum. An increase of the dihydroxyphenylacetic acid/dopamine (DOPAC/DA) ratio, an indicator of dopamine turnover, also was seen in the prefrontal cortex and striatum in CRF-treated animals at 1 week post-CRF. The dopaminergic system is very sensitive to oxidative insults. Levels of malondialdehyde, a membrane lipid peroxidation marker, also were measured in the same brain areas. In the prefrontal cortex, we observed a decrease of malondialdehyde at 1 week after chronic CRF treatment. This result may indicate an activation of the antioxidant system in response to chronic stress. These results show that chronic hyperactivity of the CRF system leads to a transient dysfunction of the dopaminergic systems, possibly through oxidative mechanisms, and suggest that stress could be a cofactor in the pathogenesis and/or progression of disorders of the dopaminergic systems.

Keywords: Chronic stress; CRF; Dopamine; Amphetamine stereotypy; Catalepsy; Oxidative stress

1. Introduction

Stress can be defined as any condition that seriously perturbs physiological and psychological homeostasis. While the physiological and behavioral responses to stress are necessary survival mechanisms, prolonged stress can have severe repercussions, varying from anxiety to impairments in learning and memory and post-traumatic stress disorder (Bremner, 1999). Recent studies suggest that chronic stress may contribute to the pathophysiology of psychiatric disorders (e.g., depression, schizophrenia) as well as neurodegenerative diseases, such as Parkinson's disease, by affecting the dopaminergic systems (reviewed in Pani et al., 2000).

Corticotropin-releasing factor (CRF) plays a critical role in integrating stress responses, both by mediating the activation of the hypothalamic pituitary adrenal (HPA) axis and the extrahypothalamic behavioral stress response (Dunn and Berridge, 1990). In the brain, activation by stress leads to a stimulation of dopaminergic neurons (Finlay and Zigmond, 1997). It can be hypothesized that protracted activation of the brain CRF system can produce neuropathological damage in the brain dopamine system. Dopaminergic neurons are particularly susceptible to oxidative damage (Beal, 1996) and dopamine itself causes neurotoxicity through the formation of reactive oxygen species (Stokes et al., 1999). Chronic administration of a variety of

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stressors, including psychological stressors, physical stress, air pollutants, and inflammatory disorders, also have been shown to induce oxidative stress, both in peripheral organs and in the brain (Moller et al., 1996; Madrigal et al., 2001). Chronic stress could cause neuropathological damage to the brain dopamine system directly or indirectly through oxidative mechanisms.

To test this hypothesis, we investigated the effect of chronic intracerebroventricular (i.c.v.) CRF administration on D-amphetamine-induced stereotyped behavior and on catalepsy induced by the dopamine D_2 receptor antagonist eticlopride. These behavioral tests have been shown to be very sensitive to impairment of the function of the midbrain dopamine system (Amalric and Koob, 1987, 1993). In addition, we measured tissue levels of dopamine and dihydroxyphenylacetic acid (DOPAC) in the primary dopaminergic projection areas, such as the prefrontal cortex, striatum, and nucleus accumbens. Finally, malondialdehyde (MDA) levels (a product of membrane lipid peroxidation) were measured in the same brain areas as a marker of oxidative damage.

2. Methods

2.1. Subjects

Fifty-four male Wistar rats (Charles River, Kingston, NY), weighing 200–225 g at the start of the experiment, were used. Twenty-eight rats were used for behavioral experiments and 26 for biochemical assay. Rats were housed 3 per cage and provided with ad libitum access to food and water and maintained on a 12 hr light–dark cycle (lights on 7:00 am–7:00 pm).

2.2. Intracerebroventricular surgery

For stereotaxic surgery, rats were anesthetized under chronic halothane vapor (1.0–1.5%) and placed in a Kopf stereotaxic instrument (Kopf Instrument, Tujunga, CA). A 7-mm stainless steel guide cannula (23 gauge) was secured to the skull with three stainless steel screws and Silux dental cement. The coordinates were: AP, +0.6 mm from bregma; L, ±2.0 mm from the midline; DV, −3.2 mm from the skull surface, with the incisor bar 5 mm above the inter-aural line (Pellegrino et al., 1979). An 8.5 mm stylet was placed into the cannula, and the rats were allowed 7 days to recover from surgery before treatment.

2.3. Drugs and treatments

Rat/Human CRF (Rivier et al., 1983) was kindly provided by Dr. Jean Rivier (Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA). For i.c.v. injections, CRF was dissolved in isotonic saline and injected by gravity. The stylet was removed from the guide cannula, and an 8.5 mm (30 gauge) stainless steel injector connected to about 70 cm calibrated PE 10 tubing was inserted. The plastic tubing then was raised above the head of the rat until flow began. Five microliters of either saline or CRF (1 μg/5 ml solution) were infused over an approximately 60-s period. To prevent efflux, the injector was left in place for an additional 30 s before the stylet again was placed in the guide cannula. The rats were injected after 7 days recovery with 1 μg/day of CRF for 13 days. The control group received the same volume of saline. Rats were not restrained during i.c.v. injections. D-Amphetamine sulfate was obtained from Sigma Chemical Co. (St. Louis, MO) and was dissolved in isotonic saline. Eticlopride was obtained from Research Biochemicals International (Natick, MA) and was dissolved in saline. All drugs were injected subcutaneously (s.c.) in a volume of 1.0 ml/kg of body weight.

2.4. Apparatus

A bank of 16 photocell cages was used to measure locomotor activity and stereotyped behavior. Each cage measured $20 \times 25 \times 36$ cm, and was made of wire mesh. Two infrared photocell beams were situated across the long axis 2 cm above the floor. Interruption of a beam was registered by a computer situated in an adjoining room. Background noise was provided by a white noise generator.

2.5. Behavioral procedure

2.5.1. D-Amphetamine-induced stereotyped behavior

Rats (14 saline- and 14 CRF-treated) were habituated to the photocell cages for 3 h for two consecutive days prior to the experiment day to overcome the potentially stressful nature of a novel environment. At different time points–1 day, 1 week, and 1 month after chronic i.c.v. treatment–the rats were placed in the locomotor activity cages for 90 min and injected s.c. with 4.0 mg/kg D-amphetamine. Stereotyped behavior was rated for 180 min after the injection. Locomotor activity was recorded, but these data are not shown. During the 3 h test, each rat was observed every 10 min for about 10 s. Stereotyped behavior was rated according to the Creese and Iversen (1973) rating scale. This scale rates the intensity of stereotypy on a 7-point scale. The scores are defined as follows: 0 (asleep or stationary), 1 (active), 2 (predominantly active, bursts of stereotyped sniffing or rearing), 3 (stereotyped activity, sniffing along fixed path of cage), 4 (stereotyped sniffing or rearing maintained in one location), 5 (stereotyped behavior in one location with bursts of gnawing or licking), 6 (continual gnawing or licking of cage bars). Behavior was rated by one observer blind to the rats' experimental treatment.

2.5.2. Catalepsy test

Catalepsy was measured using the bar test according to Pulvirenti and Koob (1993). Rats were injected with the

dopamine D_2 antagonist eticlopride (0.05 mg/kg, s.c.). Four hours after the injection, both of the rat's forepaws were placed on a bar 9 cm from the floor. The time elapsed until the rat repositioned both forepaws on the floor was recorded by an experimenter blind to the treatment condition. A cutoff of 5 min per observation was used. Since repeated injection of D_2 antagonists induces sensitization to the cataleptic effect of the drug (Barnes et al., 1990), rats were tested only at one time point, 2 days after chronic CRF or saline treatment and 1 day after having been tested for D-amphetamine stereotypy. Preliminary experiments demonstrated that exposure to amphetamine 24 h earlier does not interfere with the cataleptic effect of eticlopride.

2.6. Biochemical measures

Three groups of animals were sacrificed at 1 day, 1 week, and 1 month after 13 days of treatment with CRF ($n=17$) or saline ($n=9$). Rats were anesthetized with CO_2, and the brain was quickly removed and dissected using a wire brain slicer. Prefrontal cortex, striatum, and nucleus accumbens were collected and promptly frozen on dry ice. For each rat, one side of the brain areas was used for dopamine and DOPAC level determination and the contralateral side for MDA assay. Brain tissue was stored at −80 °C until analysis.

2.6.1. Analysis of total tissue dopamine and DOPAC levels

Tissue samples of each region were placed into tared 1.5 ml eppendorf tubes, and the weight of tissue in each tube was determined. One ml of 0.1 N perchloric acid containing 50 nM methylserotonin as an internal standard was added to each tube. The tissue then was disrupted ultrasonically, and the tubes were centrifuged to pellet all particulate matter.

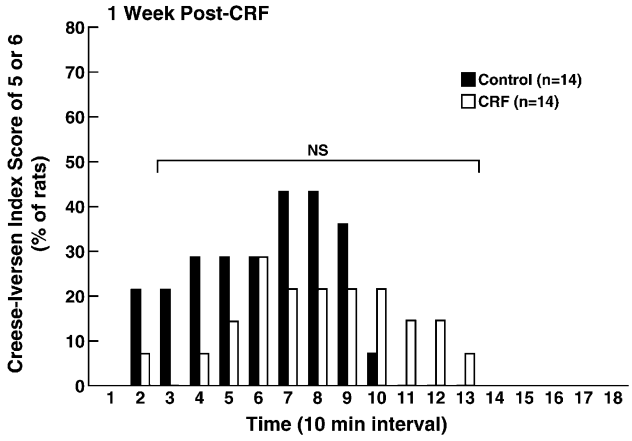

Fig. 2. Stereotyped behavior induced by 4 mg/kg D-amphetamine 1 week after chronic treatment with CRF (1 μg/day, i.c.v. for 13 days). The values represent the percentage of rats displaying stereotypy with a Creese-Iversen index score of 5 or 6 (see Methods for details) at each 10 min interval during a 180 min testing session. Statistical analysis by Information Statistic revealed a non-significant effect of treatment.

Aliquots of 100 μl of the supernatant were analyzed for dopamine and DOPAC. Concentrations of dopamine and DOPAC were determined with a microbore high-performance liquid chromatography system equipped with a Spherisorb C-18 column (100 × 1 mm, 3 μm spheres) using a mobile phase composed of a 54 mM dibasic sodium phosphate buffer with 12% methanol (v/v), 0.2 mM EDTA, 0.9 mM 1-decanesulfonic acid, and 4.9 mM triethylamine, at a final apparent pH of 4.8, pumped at 25 μl/min by an ISCO model 500D syringe pump. The monoamines were detected using a glassy carbon electrode set at +700 mV vs. Ag/AgCl by an amperometric detector (Model 400, Princeton Applied Research, Oak Ridge, TN). The detection limit for each monoamine was approximately 0.2 nM.

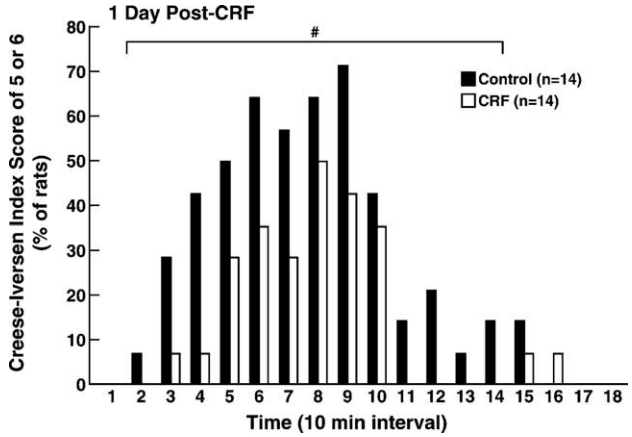

Fig. 1. Stereotyped behavior induced by subcutaneous administration of 4 mg/kg D-amphetamine 1 day after chronic treatment with CRF (1 μg/day, i.c.v. for 13 days). The values represent the percentage of rats displaying stereotypy with a Creese-Iversen index score of 5 or 6 (see Methods for details) at each 10 min interval during a 180 min testing session. Statistical analysis by Information Statistic revealed an overall effect of the treatment (#$p<0.05$ CRF vs. control group for 0–180 min).

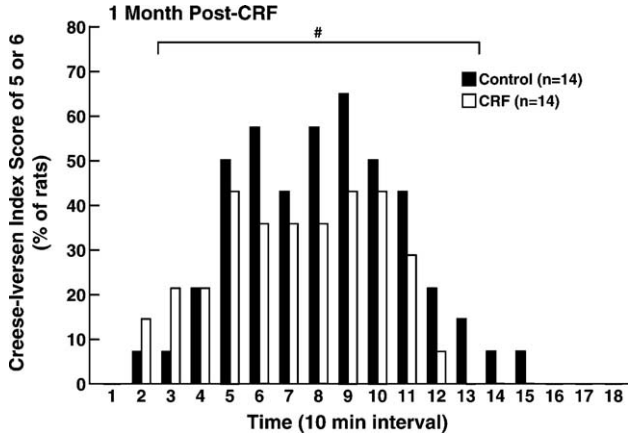

Fig. 3. Stereotyped behavior induced by 4 mg/kg D-amphetamine 1 month after chronic treatment with CRF (1 μg/day, i.c.v. for 13 days). The values represent the percentage of rats displaying stereotypy with a Creese-Iversen index score 5 or 6 (see Methods for details) at each 10 min interval during a 180 min testing session. Statistical analysis by Information Statistic revealed an overall effect of the treatment (#$p<0.05$ CRF vs. control group for 30–140 min).

Table 1
Stereotyped behavior 1 day after chronic treatment

Score	Group	Time (min)																	
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
1–2	Control	4	0	0	0	0	0	0	0	0	0	0	0	3	0	5	2	10	10
	CRF	7	1	0	0	0	0	0	0	0	0	1	1	2	3	6	7	11	11
3–4	Control	10	13	10	8	7	5	6	5	4	8	12	11	10	12	7	12	4	3
	CRF	7	13	13	13	10	9	10	7	8	9	13	13	12	11	7	6	3	3
5–6	Control*#	0	1	4	6	7	9	8	9	10	6	2	3	1	2	2	0	0	0
	CRF	0	0	1	1	4	5	4	7	6	5	0	0	0	0	1	1	0	0

The values represent the number of animals displaying a certain score (Creese-Iversen index 1–2, 3–4, and 5–6) for each time point for each group. $n=14$ per group. *$p<0.05$ control vs. CRF, Information Statistic 0–180 min. #$p<0.05$ control vs. CRF, Information Statistic 30–140 min.

2.6.2. Malondialdehyde assay

The tissue was homogenized in 10% w/v in phosphate-buffered saline containing butylated-hydroxytoluene (5 mM). MDA total levels were determined using a colorimetric assay (Bioxytech MDA-586, OXIS International, Portland, OR). With this method, *N*-methyl-2-phenylndole is allowed to react with MDA at 45 °C. One molecule of MDA reacts with 2 molecules of *N*-methyl-2-phenylndole to yield a stable chromophore with maximal absorbance at 586 nm. MDA measurements were performed on extracts of prefrontal cortex, striatum, and nucleus accumbens. MDA concentrations are expressed as μmoles of MDA per mg of total protein.

2.7. Statistical analysis

Stereotypy ratings were analyzed using the Information Statistic (Kullback, 1968; Robbins, 1977). This statistical analysis is analogous to χ^2 but is not constrained by small-cell frequencies. Stereotyped behavior was analyzed in two ways. First, at each of the 18 time intervals, the number of animals that displayed a score of 5 or 6 on the Creese and Iversen scale (1973) was processed, and these 18 2I values were added to give a total 2I. The same statistical analysis was applied for the score 3–4 and 1–2. A subsequent analysis of only 14 time intervals (30–140 min) was conducted to capture the active periods of intense stereotyped behavior (score of 5–6). Catalepsy was analyzed using the Mann–Whitney *U*-test for non-parametric measures. Biochemical data were analyzed using a one-way analysis of variance (ANOVA). Individual means comparisons for the main treatment effects of the biochemical data were analyzed using a Fisher's *post hoc* test.

3. Results

3.1. Effect of chronic CRF treatment on stereotyped behavior induced by D-amphetamine

Chronic CRF treatment (1 μg/day, i.c.v. for 13 days) reduced the stereotyped behavior induced by D-amphetamine at 1 day after the treatment. Fig. 1 shows the percentage of the rats displaying stereotyped behavior rated 5 or 6 over the 3 h test period at 1 day after the CRF chronic treatment. This measure reached a maximum level at 80–90 min after D-amphetamine injection (4 mg/kg, s.c.) and then decreased to a minimum 3 h later. The overall Information Statistic was significant ($2I=31.66$, $df=18$, $p<0.05$). The stereotyped response (score 5 and 6) to D-amphetamine at 1 week and 1 month did not differ significantly between groups over the full 18 time intervals, although the response did differ for the 30–140 min time points at 1 month ($2I=22.9$, $df=12$, $p<0.05$), and there was a trend to lower values in the CRF group at 1 week (Figs. 2 and 3). Tables 1–3 show the number of animals displaying scores 1–2, 3–4, or 5–6 for each time point at 1 day, 1 week, and 1 month after chronic treatment. No significant differences were found comparing

Table 2
Stereotypy behavior 1 week after chronic treatment

Score	Group	Time (min)																	
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
1–2	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	8	13
	CRF	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3	5	8	13
3–4	Control#	14	11	11	10	10	10	8	9	9	13	14	14	14	13	11	12	6	1
	CRF	14	13	14	13	11	10	11	10	11	11	12	12	11	13	11	9	6	1
5–6	Control	0	3	3	4	4	4	6	5	5	1	0	0	0	1	1	0	0	0
	CRF	0	1	0	1	3	4	3	4	3	3	2	2	1	0	0	0	0	0

The values represent the number of animals displaying a certain score (Creese-Iversen index 1–2, 3–4, and 5–6) for each time point for each group. $n=14$ per group. #$p<0.05$ control vs. CRF, Information Statistic 30–140 min.

Table 3
Stereotypy behavior 1 month after chronic treatment

Score	Group	Time (min)																	
		10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180
1–2	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	8	12	14
	CRF	0	0	0	0	0	0	0	0	0	0	0	0	0	3	7	11	14	13
3–4	Control*	14	12	10	10	6	5	7	5	4	6	8	11	12	12	9	6	2	0
	CRF	14	13	12	12	9	10	10	10	9	9	10	13	14	11	7	3	0	1
5–6	Control#	0	2	4	4	8	9	7	9	10	8	6	3	2	1	1	0	0	0
	CRF	0	1	2	2	5	4	4	4	5	5	4	1	0	0	0	0	0	0

The values represent the number of animals displaying a certain score (Creese-Iversen index 1–2, 3–4, and 5–6) for each time point for each group. $n=14$ per group. *$p<0.05$ control vs. CRF, Information Statistic 0–180 min. #$p<0.05$ control vs. CRF, Information Statistic 30–140 min.

the number of the rats displaying stereotyped behavior rated 3 or 4 over the 3 h test for a full 18 time point period at 1 day and 1 week after the treatment, while at 1 month the CRF group showed an increased 3–4 score compared to control ($2I=28.53$, $df=18$, $p=0.054$) (Table 3). A similar significant increase of the 3–4 score for the CRF group vs. control at 1 week was revealed by statistical analysis taking into account the 30–140 min period ($2I=21.0$, $df=12$, $p=0.05$) (Table 2). Finally, no significant differences were observed between the control and CRF groups for the stereotypy score 1 or 2 at all time points (Tables 1–3). It should be noted that the dose of D-amphetamine used in this experiment (4 mg/kg) induced a stereotyped behavior score of 3–4 in the majority of the animals and for most of the observation period (180 min), while few animals showed more intense stereotyped behavior, such as licking and gnawing of the cage (score 5–6). The chronic treatment with CRF preferentially affected this intense stereotyped behavior. Significant differences between the two groups at 1 day were evident only for high stereotypy scores of 5–6. Moreover, the significant increase of the 3–4 score in the CRF group at 1 month (Table 3) reflects increased locomotion due to less severe stereotyped behavior, such as sniffing along a fixed path of the cage (score 3), and the same effect is present also at 1 week for the 3–4 score (Table 2).

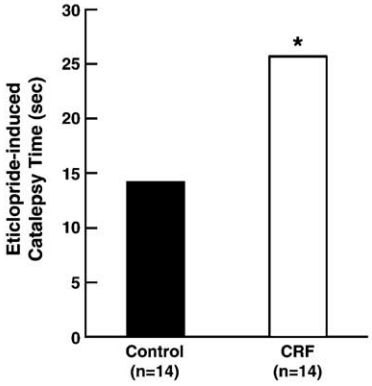

Fig. 4. Catalepsy induced by subcutaneous administration of 0.05 mg/kg eticlopride 2 days after chronic treatment with CRF (1 μg/day, i.c.v. for 13 days). The values represent the median of $n=14$ animals of the time spent by rats to reposition both forepaws from the bar to the floor (see Methods for details). The test was performed 4 h after the eticlopride injection. Statistical analysis for nonparametric measures revealed a significant effect of chronic CRF treatment on the cataleptic efficiency of eticlopride (*$p<0.05$ CRF vs. control, Mann–Whitney U-test).

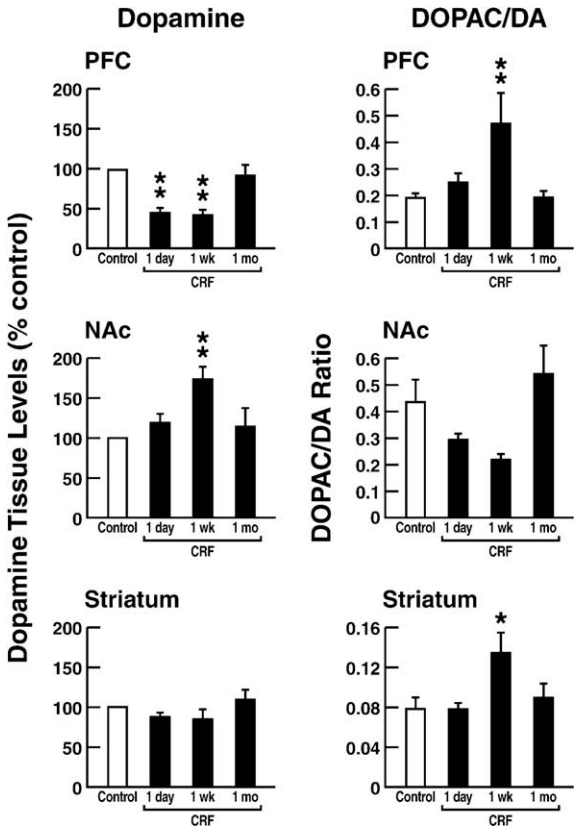

Fig. 5. (Left) Dopamine tissue levels in prefrontal cortex, nucleus accumbens, and striatum after chronic CRF treatment (1 μg/day, i.c.v. for 13 days). Values are expressed as percentage of the control average and represent the mean ± SEM of $n=5–9$ animals. Statistical analysis by ANOVA revealed an overall effect of CRF treatment in prefrontal cortex and nucleus accumbens (**$p<0.005$ vs. control, Fisher's *post hoc* test). (Right) DOPAC/DA ratios in prefrontal cortex, nucleus accumbens, and striatum after chronic CRF treatment (1 μg/day, i.c.v. for 13 days). The values represent the mean ± SEM of $n=5–9$ animals. Statistical analysis by ANOVA revealed an overall effect of CRF treatment in the prefrontal cortex, nucleus accumbens, and striatum. Fisher's *post hoc* test was significant for prefrontal cortex and striatum (*$p<0.01$; **$p<0.005$ vs. control).

3.2. Effect of chronic CRF treatment on catalepsy induced by eticlopride

Chronic CRF treatment (1 μg/day, i.c.v. for 13 days) increased the cataleptic effect of 0.05 mg/kg eticlopride, a D_2 antagonist, 2 days after the treatment (Fig. 4) ($p<0.05$, Mann–Whitney *U*-test for nonparametric measure treated vs. control rats). Because repeated injection of D_2 antagonists induces sensitization to the cataleptic effect of the drug (Barnes et al., 1990) rats were tested only at one time point, 2 days after the chronic i.c.v. CRF or saline treatment and 1 day after having been tested for D-amphetamine stereotypy. Preliminary experiments showed that a previous (24 h) exposure to D-amphetamine does not interfere with the cataleptic effect of eticlopride (data not shown).

3.3. Effect of chronic CRF on dopamine and DOPAC levels

As shown in Fig. 5, tissue dopamine levels in the prefrontal cortex were significantly lower at 1 day and 1 week after the chronic CRF treatment compared to control animals (overall one-way ANOVA $F_{3,21}=7.3$, $p<0.005$; Fisher's *post hoc* test, $p<0.005$, 1 day and 1 week vs. control). Dopamine levels returned to baseline 1 month after CRF treatment. In the nucleus accumbens, dopamine levels were significantly higher at 1 week after the treatment (overall one-way ANOVA $F_{3,22}=4.6$, $p<0.05$; Fisher's *post hoc* test, $p<0.005$, 1 week vs. control). No significant differences in dopamine levels were found in the striatum at all time points. The DOPAC/DA ratios were significantly increased at 1 week after the treatment, both in prefrontal cortex (overall one-way ANOVA $F_{3,21}=5.8$, $p<0.005$; Fisher's *post hoc* test, $p<0.005$, 1 week vs. control) and striatum (overall one-way ANOVA $F_{3,22}=3.7$, $p<0.05$; Fisher's *post hoc* test, $p<0.01$, 1 week vs. control). One-way ANOVA revealed an overall effect of the treatment in the nucleus accumbens on the DOPAC/DA ratio ($F_{3,23}=3.12$, $p<0.05$) but no significant effect in *post hoc* analysis. All of these changes were transient, and values returned to baseline levels one month after chronic CRF treatment and were in agreement with the behavioral data.

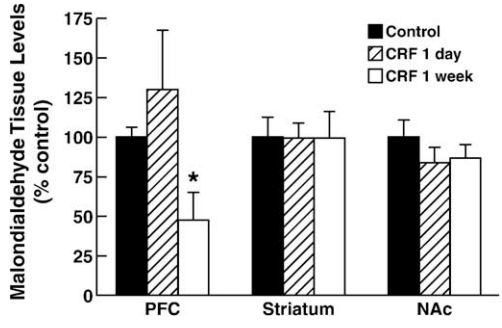

Fig. 6. Malondialdehyde tissue levels in the prefrontal cortex, nucleus accumbens, and striatum 1 day and 1 week after chronic CRF treatment (1 μg/day, i.c.v. for 13 days). The values are expressed as percentage of the control average and represent the mean±SEM of $n=3-8$ animals. Statistical analysis by ANOVA revealed an overall effect of the treatment only in the prefrontal cortex (*$p<0.05$, 1 week vs. control, Fisher's *post hoc* test).

3.4. Effect of chronic CRF on malondialdehyde levels

A significant decrease of MDA levels was observed in the prefrontal cortex (Fig. 6) at 1 week after chronic CRF treatment compared to the control group (one-way ANOVA $F_{2,12}=5.4$, $p<0.01$; Fisher's *post hoc* test, $p<0.05$, 1 week vs. control). Such a decrease was preceded by a non-significant increase at 1 day (Fig. 6). There was no significant effect of chronic CRF treatment on MDA levels in the nucleus accumbens or striatum at all time points.

4. Discussion

The results of the present study show that chronic CRF treatment (1 μg/day for 13 days) produces a lower stereotyped response to D-amphetamine and increased eticlopride-induced catalepsy at day 1 and day 2, respectively, after the treatment. These data are consistent with impaired striatal dopaminergic function. The biochemical data showed that dopamine levels were decreased in the prefrontal cortex at 1 day and 1 week after the treatment, increased in the nucleus accumbens at 1 week, and unchanged in the striatum. Increased dopamine turnover both in the striatum and in the prefrontal cortex was observed only at 1 week after the CRF treatment.

The behavioral data showed decreased intense stereotyped behavior (score 5–6) in the CRF-treated rats that was more evident early after the treatment (1 day). Nevertheless, this trend was present even later after the chronic CRF treatment and was significant at 1 month after CRF treatment. In fact, the significant increase of the 3–4 score in the CRF group compared to control at 1 week and 1 month (Tables 2 and 3) presumably reflects increased locomotion due to less severe stereotyped behavior, such as sniffing along a fixed path of the cage (score 3) and decreased acute stereotypy signs, such as licking and gnawing the cage bars (score 5–6).

Acutely, low doses of CRF (0.02–0.1 μg) have been shown to potentiate D-amphetamine-induced stereotyped behavior (Cole and Koob, 1989), and acute stress induces increased dopamine release in dopaminergic projection areas (Castro and Zigmond, 2001; Finlay and Zigmond, 1997; Gresch et al., 1994; Mizoguchi et al., 2000). It is possible that chronic administration of CRF could lead to a persistent activation of the dopaminergic system. Our behavioral data suggest that this protracted activation of the CRF system causes a prolonged impairment of dopaminergic function after chronic stimulation.

The mesostriatal dopaminergic pathway is hypothesized to be primarily responsible for the stereotyped behavior elicited by D-amphetamine administration and eticlopride-

induced catalepsy (Dickson et al., 1994; Amalric and Koob, 1993). The fact that the striatal dopamine levels are not changed suggests that the lower stereotyped response to D-amphetamine and the increased sensitivity to the cataleptic action of a D_2 antagonist may be due to either a more subtle neurodegenerative change not sensitive to the measures used in the present study, or to a rearrangement of the dopaminergic receptors in this area, such as a concomitant decrease of D_1 and D_2 receptors. In agreement with the receptor downregulation hypothesis, previous studies showed that chronic mild stress causes a decrease in D_2 receptor binding in the nucleus accumbens (Willner et al., 1991; Papp et al., 1994) accompanied by a pronounced functional subsensitivity to the rewarding and locomotor stimulant effects of the D_2/D_3 agonist quinpirole administered systemically or within the nucleus accumbens (Papp et al., 1993). The increased striatal dopamine turnover observed at 1 week after CRF treatment, when behavioral signs had subsided, could therefore represent a compensatory response to cope with an increased dopaminergic demand. The biochemical results, in particular the decrease of dopamine levels, suggest that some brain areas, such as the prefrontal cortex, are more vulnerable than others, such as the striatum, to chronic CRF.

These results coincide with previous studies demonstrating that dopaminergic projection areas display differential sensitivity to the effects of stress (Horger and Roth, 1996; Finlay and Zigmond, 1997; Gresch et al., 1994; Mizoguchi et al., 2000). An acute stressor (e.g., tail shock) induces higher dopamine efflux in the prefrontal cortex than in other dopamine projection areas, and chronic stress (e.g., exposure to cold) induces a sensitization of dopamine efflux in the prefrontal cortex in response to tail shock (Gresch et al., 1994). Decreased prefrontal cortex dopamine content also has been observed after exposure to cold water (Mizoguchi et al., 2000). As in the present study, the increased dopamine content in the nucleus accumbens has been shown to accompany prefrontal cortex dopamine depletions (Simon et al., 1988; Espejo and Minano, 2001). Nevertheless, it should be noted that the increase in nucleus accumbens dopamine levels is present only at 1 week and not at 1 day. This temporal discrepancy is not easily explained. However, it is well documented that a decrease in prefrontal cortex dopamine causes subtle changes to functional dopamine tone in other dopamine projection areas (Simon et al., 1988).

Consistent with the notion that the prefrontal cortex is more sensitive to chronic stimulation of the CRF system than other areas, a significant decrease in the level of MDA, a marker of lipid peroxidation (Madrigal et al., 2001; Liu et al., 2000), was detected at 1 week after chronic CRF treatment in the prefrontal cortex following a non-statistically significant increase 1 day after CRF treatment. Such a pattern of change in MDA level has been interpreted previously as a sign of the induction of the antioxidant system in response to a chronic mild oxidative insult (Liu et al., 2000). Dopaminergic transmission in the prefrontal cortex has been implicated in the working memory deficit in Parkinson's disease, depression, schizophrenia, and other disorders (Mattay et al., 2002; Fibiger, 1995), and both in animal models of Parkinson's disease as well as in humans, the onset of cognitive deficit precedes motor impairments (Schneider and Pope-Coleman, 1995; Dubois and Pillon, 1997; Kulisevsky, 2000). In the present study, no cognitive tests were employed to investigate whether the depletion of dopamine observed in the prefrontal cortex was accompanied by functional behavioral deficits. Future studies will be necessary to address this point.

Taken together, the current data suggest that chronic exposure to high CRF levels induces region-specific responses in dopamine projection areas. While these changes proved to be reversible, it is possible that a stronger or more protracted CRF activation could contribute to the pathogenesis and/or progression of disorders of the dopaminergic systems, particularly in vulnerable individuals.

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