Differential Regulation of Neurotrophin and *trk* Receptor mRNAs in Catecholaminergic Nuclei during Chronic Opiate Treatment and Withdrawal

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The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) and their receptors *trkB* and *trkC*, respectively, are expressed in the locus coeruleus (LC) and ventral tegmental area (VTA), brain regions known to be involved in opiate addiction. Previously, administration of exogenous neurotrophins has been shown to oppose effects of chronic morphine treatment on LC and VTA neurons. However, the response of endogenous neurotrophins in LC and VTA to opiate treatment is unknown. In this study, BDNF, NT-3, *trkB*, and *trkC* mRNAs were analyzed in these regions after chronic morphine treatment and during antagonist precipitated withdrawal. Although chronic morphine exposure resulted in only modest increases in BDNF and NT-3 mRNA expression in LC, precipitated withdrawal led to a marked, rapid, and prolonged increase in BDNF mRNA and a delayed decrease in NT-3

mRNA. Levels of *trkB* and *trkC* mRNAs, which were unchanged by chronic morphine treatment, were elevated in LC at 2 and 6 hr of withdrawal. By 20 hr, *trkB* mRNA levels in LC had returned to control, whereas *trkC* mRNA levels fell below control values. In contrast to the substantial alterations observed in LC, there was no regulation of the neurotrophins or *trk* mRNAs within the VTA during chronic opiate treatment or withdrawal, with the exception of an increase in *trkB* mRNA at 6 hr of withdrawal. These results suggest that neurotrophins and their receptors per se may be involved in opiate-induced plasticity of the LC, whereas other mechanisms would appear to be involved in the VTA.

Key words: locus coeruleus; ventral tegmental area; opiates; neurotrophins; neural plasticity; morphine

The locus coeruleus (LC), the major noradrenergic cell group in the brain (for review, see Aston-Jones et al., 1995), has been implicated in opiate physical dependence and withdrawal (Nestler and Aghajanian, 1997). Acutely, opiates inhibit LC neuronal activity (Korf et al., 1974; Bird and Kuhar, 1977; Valentino and Wehby, 1988). In response to continued opiate treatment, several long-term biochemical alterations occur in LC, including increases in tyrosine hydroxylase, adenylyl cyclase, and protein kinase A (Duman et al., 1988; Nestler and Tallman, 1988; Guitart et al., 1990; Nestler et al., 1993). In addition, tolerance develops such that LC neuronal firing rates return toward control levels, and the neurons show dependence such that abrupt opiate removal causes marked increases in LC neuronal activity (Aghajanian, 1978; Valentino and Wehby, 1989; Rasmussen et al., 1990). This increase in LC activity appears both necessary and sufficient to induce many opiate withdrawal behaviors (Grant et al., 1988; Maldonado and Koob, 1993; Nestler and Aghajanian, 1997).

Another catecholaminergic nucleus implicated in opiate addiction is the ventral tegmental area (VTA) (Wise, 1996; Koob and Le Moal, 1997; Koob and Nestler, 1997). This nucleus, which

contains dopaminergic neurons that project to the nucleus accumbens and other forebrain regions (for review, see Lindvall and Björklund, 1983), is thought to be involved in rewarding aspects of opiate administration. Like the LC, the VTA undergoes long-term biochemical changes after chronic opiate exposure, including increases in tyrosine hydroxylase and certain glutamate receptor subunits and decreases in neurofilaments (Nestler et al., 1993, 1996). Although opiates acutely activate VTA dopamine neurons by inhibiting inhibitory interneurons (Johnson and North, 1992), the chronic electrophysiological effects of opiates on these cells have only recently been investigated (Bonci and Williams, 1997).

Recent evidence suggests that neurotrophins, in addition to their well known roles in neuronal development and survival, may be involved in maintenance of neuronal phenotype, neuronal plasticity, and neuroprotection for adult neurons (Gall, 1993; Korsching, 1993; Lindsay et al., 1994). Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are two neurotrophins that increase survival of LC neurons (Friedman et al., 1993; Sklair-Tavron and Nestler, 1995) and ventral midbrain dopamine neurons (Hyman et al., 1991, 1994; Spenger et al., 1995) *in vitro*. Both BDNF and NT-3 mRNAs and their cognate receptor mRNAs *trkB* and *trkC*, respectively, are expressed in adult LC (Seroogy, 1994; Smith et al., 1995; Zhang et al., 1998) and VTA (Gall et al., 1992; Seroogy and Gall, 1993; Altar et al., 1994; Seroogy et al., 1994; Numan and Seroogy, 1998). Furthermore, neurotrophin mRNA expression can be regulated in these

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nuclei by specific pharmacological treatments (Numan and Seroogy, 1994; Seroogy, 1994; Hung and Lee, 1996).

Recent studies suggest a role for neurotrophins in opiate addiction. Administration of exogenous BDNF or NT-3 opposes effects of chronic opiate treatment on LC neurons *in vitro* (Sklair-Tavron and Nestler, 1995) and on VTA neurons *in vivo* (Berhow et al., 1995; Sklair-Tavron et al., 1996). However, the response of endogenous neurotrophin systems in the LC and VTA to opiate treatment is unknown. In the present study, expression of mRNAs for BDNF and NT-3 and for their high-affinity receptors *trkB* and *trkC*, respectively, was examined in the LC and VTA after chronic morphine treatment and during opiate withdrawal to study possible plasticity of these neurotrophin systems in the context of opiate addiction.

MATERIALS AND METHODS

Animals and treatments

Adult male Sprague Dawley rats (150–200 gm; $n = 32$; CAMM, Wayne, NJ) were used for this study. The opiate treatment paradigms used in these experiments followed protocols described previously by Rasmussen and colleagues (1990).

Chronic morphine. For 5 d, once a day, rats ($n = 4$) were lightly anesthetized with fluothane and implanted with a morphine pellet (75 mg of morphine base; National Institute on Drug Abuse) subcutaneously. Control rats ($n = 4$) were lightly anesthetized but not implanted with pellets. On day six all rats were decapitated.

Morphine withdrawal (short-term survival). Rats were treated as above; however, on day six, sham-treated rats ($n = 4$) were injected with 0.9% saline subcutaneously and decapitated 2 hr later. Morphine-treated rats were injected with naltrexone (100 mg/kg in 0.9% saline, s.c.), housed individually, and decapitated 2 hr ($n = 4$) or 6 hr ($n = 4$) after the injection.

Morphine withdrawal (long-term survival). Rats were treated as in the chronic morphine paradigm; however, on day six, sham-treated rats ($n = 4$) were killed by rapid decapitation. Morphine-treated rats had their last two implanted morphine pellets removed under light fluothane anesthesia and then were injected with naltrexone (100 mg/kg in 0.9% saline, s.c.) and housed separately. After 6 hr of withdrawal, rats received another injection of naltrexone. Some of the rats ($n = 4$) were decapitated after 20 hr of withdrawal. Finally, the remaining group ($n = 4$) received a third injection of naltrexone after 24 hr of withdrawal and were decapitated at 70 hr. For the long withdrawal periods, rats were supplied with mashed food and water in the cage with them as well as with a local heat source.

Tissue collection

After decapitation, brains were quickly removed and frozen with dry ice. Coronal sections (10 μ m) throughout the rostrocaudal extent of the LC and the VTA were cut in a cryostat, thaw-mounted onto Superfrost Plus (Fisher Scientific, Pittsburgh, PA) glass slides, and stored at -20°C until hybridization.

In situ hybridization

Coronal sections through the rostrocaudal extent of the LC and VTA were processed for the *in situ* hybridization localization of BDNF, NT-3, *trkB*, and *trkC* mRNAs by using ^{35}S -labeled cRNA probes as described previously (Seroogy et al., 1994; Numan and Seroogy, 1997; Seroogy and Herman, 1997). These nuclei were identified by standard anatomical landmarks (Paxinos and Watson, 1986). Briefly, the slide-mounted sections were brought to room temperature and then placed in 4% paraformaldehyde for 10 min. This was followed by washes in 0.1 M phosphate buffer (PB), 0.1 M PB/0.2% glycine, and 0.25% acetic anhydride in 0.1 M triethanolamine. The sections were then dehydrated with increasing concentrations of ethanol, delipidated in chloroform, and air-dried. Sections were hybridized overnight at 60°C in a hybridization mixture consisting of 10% dextran sulfate, 50% formamide, $1\times$ Denhardt's solution, 0.15 mg/ml yeast tRNA, 40 mM dithiothreitol, 0.33 mg/ml denatured salmon sperm DNA, 1 mM EDTA, 20 mM Tris-HCl, and the ^{35}S -labeled cRNA probe at a concentration of 1.0×10^6 cpm/50 μ l per slide. Sense and antisense cRNA probes were prepared by *in vitro*

transcription using linearized DNA constructs in the presence of RNA polymerase (T3, T7, or SP6) and ^{35}S -UTP (New England Nuclear, Boston, MA). BDNF and NT-3 cDNA constructs (kindly supplied by J. Lauterborn and C. Gall, University of California at Irvine) resulted in antisense transcripts that were 540 and 550 bases long, respectively. The cDNA constructs for *trkB* and *trkC* (generous gifts from D. McKinnon, State University of New York at Stony Brook) resulted in antisense RNA transcripts that were 196 and 300 bases long, respectively. The *trkB* cRNA probe used in this study detected only the kinase-specific, full-length catalytic form of the receptor mRNA (Sternini et al., 1996). In contrast, the probe used to detect *trkC* mRNA recognized mRNA transcripts for both the full-length catalytic and truncated noncatalytic forms of the receptor (Dixon and McKinnon, 1994; Albers et al., 1996).

For treatment after hybridization, sections were washed several times in $4\times$ SSC ($1\times$ SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) containing 10 mM sodium thiosulfate, at 37°C . The sections were then incubated in ribonuclease A (0.05 mg/ml) for 30 min at 45°C . This was followed by several washes in decreasing concentrations of SSC (2, 0.5, and $0.1\times$) at 37°C . All but the final wash also contained 10 mM sodium thiosulfate. The sections were briefly rinsed in dH_2O , dipped in 95% ethanol, and finally air-dried. To generate film autoradiograms the sections were exposed to β -Max Hyperfilm (Amersham, Arlington Heights, IL) for 7–15, 7–18, 14–22, and 21–25 d for *trkC*, *trkB*, BDNF, and NT-3 mRNAs, respectively. It should be noted that the variability in film exposure for a single probe comes from the different experiments (chronic morphine, 2 and 6 hr morphine withdrawal, or 20 and 70 hr withdrawal in LC or VTA) and not from within a single experiment. Therefore, for each probe all of the groups for one experiment (e.g., control, 2 and 6 hr withdrawal in LC) were processed at the same time and were on film for the same amount of time. As controls for specificity, some sections were pretreated with ribonuclease A (0.05 mg/ml) for 30 min at 45°C before hybridization with the ^{35}S -labeled cRNA probes. Some sections were also hybridized with BDNF, NT-3, *trkB*, or *trkC* sense strand ^{35}S -labeled riboprobes. No specific labeling was observed under any of these control conditions.

Film autoradiograms were analyzed by densitometry using NIH Image software to compare densities of hybridization of each probe in the LC and VTA between control rats and treated rats. From each animal, at least six measurements of hybridization density, also referred to as gray level, were taken for each probe from both LC and VTA. A paired background measurement was subtracted from each gray level, leading to a corrected gray level. Once a mean corrected gray level for LC and VTA was attained for each animal, statistical analysis was performed. Statistical analysis included Student's unpaired *t* test and ANOVA followed by Student Newman-Keuls *post hoc* analyses where appropriate. The NIH image software was also used to acquire images of representative sections from film autoradiograms.

RESULTS

Locus coeruleus

Chronic morphine

Coronal sections throughout the LC of control and morphine-treated rats were processed for the *in situ* hybridization localization of BDNF, NT-3, *trkB*, and *trkC* mRNAs. Densitometric analysis of film autoradiograms revealed a small but significant increase ($+10 \pm 1.8\%$) in BDNF mRNA expression in the LC after chronic morphine treatment as compared with control rats ($p < 0.05$) (Fig. 1A, Table 1). Similarly, chronic morphine administration induced a slight increase ($+8 \pm 1.9\%$) in NT-3 mRNA expression in this brain region ($p < 0.05$) (Fig. 1B, Table 1). In contrast to the small increases in neurotrophin mRNA expression in the LC after chronic opiate treatment, *trkB* and *trkC* cRNA hybridization densities were not significantly altered ($p > 0.05$) (Fig. 1C,D, Table 1). However, it should be noted that there did appear to be a trend toward a decrease ($-9 \pm 2.1\%$) in *trkC* mRNA levels in morphine-treated versus control rats ($p = 0.062$) (Fig. 1D, Table 1).

Figure 1. Analysis of BDNF (A), NT-3 (B), *trkB* (C), and *trkC* (D) mRNA expression in LC after chronic opiate treatment. Note the significant increase in BDNF (A) and NT-3 (B) mRNA levels (asterisks) in LC after chronic opiate exposure. Although there was a trend toward a decrease in *trkC* mRNA levels (D; $p = 0.062$), no significant alterations in *trkB* (C) or *trkC* (D) mRNA expression were observed in LC with chronic morphine treatment. Results are expressed as a percentage of control values. Values are the mean $\pm$ SEM.

Morphine withdrawal

Visual inspection of film autoradiograms revealed a substantial increase in BDNF mRNA levels in the LC after 2 hr of morphine withdrawal as compared with control animals (Fig. 2A). There also appeared to be a further elevation in the BDNF cRNA hybridization density after 6 hr of withdrawal (Fig. 2A). Densitometric analysis of film autoradiograms confirmed that there were significant alterations in BDNF mRNA levels during precipitated opiate withdrawal [$F_{(2,8)} = 223.451$; $p < 0.001$]. *Post hoc* analyses revealed that BDNF mRNA levels were significantly raised at 2 hr ($+100 \pm 5\%$) and 6 hr ($+147 \pm 1\%$) of morphine withdrawal as compared with control levels ($p < 0.05$) (Fig. 2C, Table 1). Furthermore, BDNF mRNA levels in the LC were higher at 6 hr of morphine withdrawal than at 2 hr ($p < 0.05$) (Fig. 2C, Table 1).

To study the duration of BDNF mRNA induction during withdrawal, another set of animals was processed for the *in situ* hybridization localization of the neurotrophins and their receptors in the LC after longer withdrawal periods. As with the earlier time points, robust increases in BDNF mRNA levels occurred after longer periods of withdrawal [$F_{(2,9)} = 69.717$; $p < 0.001$]. BDNF mRNA levels were significantly increased at both 20 hr ($+294 \pm 7\%$) and 70 hr ($+192 \pm 29\%$) of opiate withdrawal as

compared with controls ($p < 0.05$) (Fig. 2B,C, Table 1). It should be noted that there was a significant reduction in BDNF mRNA levels at 70 hr of withdrawal as compared with 20 hr ($p < 0.05$) (Fig. 2B,C, Table 1), indicating a partial return toward control levels.

In contrast to the increase in BDNF mRNA expression in the LC during morphine withdrawal, densitometric analysis of film autoradiograms revealed no significant alterations in NT-3 mRNA levels at 2 or 6 hr of withdrawal as compared with controls [$F_{(2,8)} = 1.029$; $p > 0.1$] (Fig. 3A,C, Table 1). However, significant reductions in NT-3 mRNA levels were found after the longer opiate withdrawal periods [$F_{(2,9)} = 70.719$; $p < 0.001$] (Fig. 3B,C, Table 1). *Post hoc* analyses indicated that there was a significant decrease in NT-3 cRNA hybridization in the LC after 20 hr ($-49 \pm 2.9\%$) and 70 hr ($-19 \pm 1.3\%$) of morphine withdrawal ($p < 0.05$) (Fig. 3C, Table 1). The magnitude of this reduction was significantly smaller at 70 hr as compared with 20 hr ($p < 0.05$) (Fig. 3C, Table 1).

In film autoradiograms, *trkB* mRNA expression appeared to be increased in the LC at both 2 and 6 hr of morphine withdrawal as compared with controls (Fig. 4A). Densitometric analysis demonstrated a significant increase in the hybridization density for *trkB* mRNA after withdrawal [$F_{(2,8)} = 6.71$; $p < 0.02$]. *Post hoc* analyses revealed small but significant increases in *trkB* mRNA levels at 2 hr ($+11 \pm 2.9\%$) and 6 hr ($+9 \pm 2.3\%$) of opiate withdrawal ($p < 0.05$) (Fig. 4C, Table 1). In contrast, no changes in *trkB* mRNA levels were observed at 20 and 70 hr of withdrawal as compared with controls ($p > 0.05$) (Fig. 4B,C, Table 1).

As with *trkB*, there appeared to be increased *trkC* mRNA levels in the LC at 2 and 6 hr of morphine withdrawal as compared with controls (Fig. 5A). Densitometric analysis of film autoradiograms confirmed that there were significant changes in the hybridization density for *trkC* mRNA during withdrawal from morphine [$F_{(2,8)} = 17.09$; $p < 0.01$]. *Post hoc* analyses showed that *trkC* mRNA levels were significantly elevated at 2 hr ($+24 \pm 3.8\%$) and 6 hr ($+23 \pm 3.7\%$) of morphine withdrawal as compared with controls ($p < 0.05$) (Fig. 5C, Table 1). In striking contrast to the effects of early withdrawal, significant reductions in *trkC* mRNA levels were observed in the LC after longer withdrawal periods [$F_{(2,9)} = 11.25$; $p < 0.05$] (Fig. 5B,C, Table 1). Levels of *trkC* mRNA were significantly decreased at 20 hr ($-22 \pm 1\%$) and 70 hr ($-12 \pm 4.2\%$) hr of withdrawal as compared with controls ($p < 0.05$) (Fig. 5C, Table 1).

Ventral tegmental area

Chronic morphine

Coronal sections throughout the VTA of control and morphine-treated rats were processed for the *in situ* hybridization localization of BDNF, NT-3, *trkB*, and *trkC* mRNAs. No changes in neurotrophin or neurotrophin receptor mRNA levels could be detected in the VTAs of opiate-treated rats as compared with controls by visual inspection of film autoradiograms (Fig. 6). Densitometric analysis of film autoradiograms confirmed that there were no alterations in the expression of BDNF, NT-3, *trkB*, or *trkC* mRNAs in the VTA after chronic morphine treatment ($p > 0.05$) (Fig. 7). Similarly, there did not appear to be any regulation of the neurotrophins or their receptor mRNAs in the nearby substantia nigra (Fig. 6).

Morphine withdrawal

At 2 and 6 hr of morphine withdrawal, there were no significant differences in the VTA for BDNF, NT-3, or *trkC* mRNAs as

Table 1. Neurotrophin and *trk* receptor mRNA levels in LC after opiate treatment and withdrawal

	BDNF mRNA (%)	NT-3 mRNA (%)	<i>trkB</i> mRNA (%)	<i>trkC</i> mRNA (%)
Chronic morphine				
C	100 ± 2.4	100 ± 2.3	100 ± 1	100 ± 3.1
M	110 ± 1.8 ^a	108 ± 1.9 ^a	101 ± 1	91 ± 2.1
Morphine withdrawal				
C	100 ± 6	100 ± 4.4	100 ± 1.1	100 ± 2.7
2 hr	200 ± 5 ^a	107 ± 3.9	111 ± 2.9 ^a	124 ± 3.8 ^a
6 hr	247 ± 1 ^b	100 ± 3.4	109 ± 2.3 ^a	123 ± 3.7 ^a
Morphine withdrawal				
C	100 ± 6.5	100 ± 4	100 ± 1.7	100 ± 3.7
20 hr	394 ± 7 ^c	51 ± 2.9 ^d	96 ± 2.1	78 ± 1 ^e
70 hr	292 ± 29 ^a	81 ± 1.3 ^e	95 ± 2.3	88 ± 4.2 ^e

Summary of the effects of opiate treatment and withdrawal on neurotrophin and *trk* receptor mRNA levels in LC. Data are presented as percentage of control. Values are the mean ± SEM. C, Control; M, morphine. *n* = 4 for all groups, except *n* = 3 for the 6 hr withdrawal group. The data given in this table are shown in graphic form in Figures 1–5.

^aSignificantly increased from control.

^bSignificantly increased from control and 2 hr.

^cSignificantly increased from control and 70 hr.

^dSignificantly decreased from control and 70 hr.

^eSignificantly decreased from control.

Figure 2. Effect of precipitated opiate withdrawal on BDNF mRNA expression in LC. *A*, Film autoradiograms demonstrating BDNF cRNA hybridization in LC of control rats and rats exposed to 2 or 6 hr of opiate withdrawal. *B*, Film autoradiograms of BDNF mRNA expression in LC in control rats and rats exposed to 20 or 70 hr of opiate withdrawal. *C*, Densitometric analysis of film autoradiograms reveals robust increases in BDNF mRNA levels at 2, 6, 20, and 70 hr of opiate withdrawal as compared with control ($p < 0.05$). Results are expressed as a percentage of control values. Values are the mean ± SEM (SEM for 2 and 6 hr control group = ±6; SEM for 20 and 70 hr control group = ±6.5.) *Significantly increased from control. ** Significantly increased from control and 2 hr. †Significantly different from control and 20 hr. ††Significantly different from control and 70 hr. Scale bar, 500 μ m.

compared with controls ($p > 0.05$) (Fig. 8*A,B,D*). However, a small but significant effect of morphine withdrawal on *trkB* mRNA expression was detected in this brain region [$F_{(2,9)} = 5.094$; $p < 0.05$]. *Post hoc* analyses showed a significant increase in *trkB* mRNA hybridization in the VTA at 6 hr ($+23 \pm 3.9\%$) of morphine withdrawal as compared with controls ($p < 0.05$) (Fig. 8*C*). Withdrawal had no apparent effect on neurotrophin or *trk* receptor mRNA levels in the substantia nigra (data not shown).

DISCUSSION

The results of the present study demonstrate that chronic opiate treatment and withdrawal differentially regulate neurotrophin and their receptor mRNAs in the LC. Chronic morphine treatment led to small, but significant, increases in BDNF and NT-3 mRNA levels and no changes in *trkB* or *trkC* mRNAs in this brain region. Precipitated opiate withdrawal resulted in a robust in-

crease in BDNF mRNA expression at all time points examined. In contrast to BDNF, there was a decrease in NT-3 mRNA levels at the more prolonged time points (20 and 70 hr) of withdrawal. Although both *trkB* and *trkC* mRNAs were increased at 2 and 6 hr of withdrawal, *trkB* mRNA had returned to control levels by 20 hr, whereas *trkC* mRNA levels were reduced to below control values at the 20 and 70 hr withdrawal time points.

It is interesting to note that the modulation of NT-3 mRNA expression in the LC during withdrawal was not immediate. Although BDNF, *trkB*, and *trkC* mRNA levels were increased at 2 hr of morphine withdrawal, decreased NT-3 mRNA expression was not observed until 20 hr of withdrawal. There are precedents for a delayed decrease in NT-3 mRNA levels in the brain. For example, experimental brain trauma resulted in a significant increase in BDNF mRNA in the hippocampal dentate gyrus by 1 hr after injury, whereas NT-3 mRNA levels were not signifi-

Figure 3. Effect of precipitated opiate withdrawal on NT-3 mRNA expression in LC. *A*, Film autoradiograms of NT-3 mRNA expression in LC of control rats and of rats after 2 or 6 hr of opiate withdrawal. *B*, Film autoradiograms demonstrating the expression of NT-3 mRNA in LC of control rats and rats exposed to 20 or 70 hr of opiate withdrawal. *C*, Results from analysis of film autoradiograms show that there are no alterations in NT-3 mRNA levels at 2 and 6 hr of opiate withdrawal as compared with control. In contrast, there was a decrease in NT-3 mRNA levels at 20 and 70 hr of opiate withdrawal as compared with control ($p < 0.05$). Results are expressed as a percentage of control values. Values are the mean $\pm$ SEM (SEM for 2 and 6 hr control group = ± 4.4 ; SEM for 20 and 70 hr control group = ± 4 .) *Significantly decreased from control. **Significantly different from control and 70 hr. Scale bar, 500 μ m.

Figure 4. Effect of precipitated opiate withdrawal on *trkB* mRNA expression in LC. *A*, Film autoradiograms demonstrating *trkB* mRNA expression in LC of control rats and rats exposed to 2 or 6 hr of opiate withdrawal. *B*, Film autoradiograms of *trkB* cRNA hybridization in LC in control rats and rats exposed to 20 or 70 hr of opiate withdrawal. *C*, Densitometric analysis of film autoradiograms reveals an increase in *trkB* mRNA levels at 2 and 6 hr of opiate withdrawal as compared with control ($p < 0.05$), and no changes in *trkB* mRNA expression at 20 and 70 hr of opiate withdrawal as compared with control. Results are expressed as a percentage of control values. Values are the mean $\pm$ SEM (SEM for 2 and 6 hr control group = ± 1.1 ; SEM for 20 and 70 hr control group = ± 1.7 .) *Significantly increased from control. Scale bar, 500 μ m.

cantly decreased from controls until the 6 hr time point (Hicks et al., 1997).

The early increase in *trkC* mRNA expression followed by a decrease to below control levels in the LC during opiate withdrawal is difficult to interpret functionally because of the fact that the probe used recognized mRNA transcripts for both the catalytic and noncatalytic isoforms of the receptor (Dixon and McKinnon, 1994; Albers et al., 1996). Therefore, it is unclear whether the changes in *trkC* mRNA detected in this study are caused by alterations in the levels of the catalytic or noncatalytic transcripts, or both.

Chronic opiate exposure led to a small but significant increase in BDNF mRNA in the LC, with a robust increase observed during opiate withdrawal. It has been hypothesized that BDNF and other neurotrophins may in some cases be responsible for maintenance of neuronal phenotype (Korsching, 1993; Lindsay et al., 1994). Within this context, one possibility is that BDNF acts

in a paracrine or autocrine manner in the LC, which is plausible because LC neurons express both BDNF and *trkB* mRNAs (Se-roogy, 1994; Zhang et al., 1998). If so, the increase in BDNF expression could serve to counter the effects of chronic morphine and morphine withdrawal on the biochemistry of these neurons. Future studies with antagonists to BDNF will be needed to examine the validity of this hypothesis. Besides a potential paracrine role for BDNF in the LC, recent evidence suggests that LC neurons can anterogradely transport BDNF to their projection regions (Fawcett et al., 1998). Because the LC has widespread projections throughout the CNS (Lindvall and Björklund, 1983), this anterogradely transported BDNF may be a trophic factor for many neurotrophin-responsive neuronal populations. Increased expression of BDNF in the LC during morphine treatment and withdrawal could then contribute to adaptations and homeostatic mechanisms that occur in these other brain regions as well.

Neurotrophins and their receptors have been observed to ex-

Figure 5. Effect of precipitated opiate withdrawal on *trkC* mRNA expression in LC. *A*, Film autoradiograms showing the expression of *trkC* mRNA in LC of control rats and rats exposed to 2 or 6 hr of opiate withdrawal. *B*, Film autoradiograms of *trkC* mRNA expression in LC in control rats and rats exposed to 20 or 70 hr of opiate withdrawal. *C*, Densitometric analysis of film autoradiograms demonstrates an increase in *trkC* mRNA levels at 2 and 6 hr of opiate withdrawal as compared with control ($p < 0.05$), followed by a decrease in *trkC* mRNA levels at 20 and 70 hr of opiate withdrawal as compared with control ($p < 0.05$). Results are expressed as a percentage of control values. Values are the mean $\pm$ SEM (SEM for 2 and 6 hr control group = ± 2.7 ; SEM for 20 and 70 hr control group = ± 3.7). *Significantly increased from control. †Significantly decreased from control. Scale bar, 500 μ m.

Figure 6. Film autoradiograms of BDNF (*A*, *B*), NT-3 (*C*, *D*), *trkB* (*E*, *F*), and *trkC* (*G*, *H*) cRNA hybridization in the ventral midbrain of control (*A*, *C*, *E*, *G*) and morphine-treated (*B*, *D*, *F*, *H*) rats. Note that no significant alterations in neurotrophin or neurotrophin receptor mRNA levels were observed in VTA after chronic opiate exposure. *SNpc*, Substantia nigra pars compacta. Scale bar, 1710 μ m.

hibit plasticity after several manipulations and seem to be especially sensitive to alterations in neuronal activity. The increased neuronal activity observed with seizures leads to increases in BDNF (Ernfors et al., 1991; Isackson et al., 1991; Rocamora et al., 1992; Lindefors et al., 1995; Nibuya et al., 1995; Suzuki et al., 1995) and *trkB* (Merlio et al., 1993; Lindefors et al., 1995; Nibuya et al., 1995) mRNA levels in the hippocampus (for review, see Gall, 1993). It has been suggested that BDNF and its high-affinity receptor *trkB* may play a role in neuroprotection (Lindvall et al.,

1992; Merlio et al., 1993) or in the long-term biochemical and morphological changes associated with seizure (Gall, 1993). The early increase in BDNF, *trkB*, and *trkC* mRNAs and the delayed decrease in NT-3 and *trkC* mRNAs in the LC after precipitated opiate withdrawal may be in response to the increase in LC neuronal activity that occurs during withdrawal (Valentino and Wehby, 1989; Rasmussen et al., 1990). LC firing rate is increased within 3 min of precipitated opiate withdrawal, peaks by 15–30 min, and returns to control levels by 3 d (Rasmussen et al., 1990). Of particular interest are the findings that expression of certain

Figure 7. Analysis of BDNF (*A*), NT-3 (*B*), *trkB* (*C*), and *trkC* (*D*) mRNA expression in VTA after chronic opiate treatment. No significant alterations in neurotrophin (*A*, *B*) or neurotrophin receptor (*C*, *D*) mRNA levels were observed in VTA with chronic morphine treatment. Results are expressed as a percentage of control values. Values are the mean $\pm$ SEM.

neurotrophins and their receptors are still significantly altered at 70 hr of withdrawal, a time at which all detectable withdrawal behaviors have dissipated and the neuronal activity of the LC has returned to normal levels (Rasmussen et al., 1990). The persisting adaptations in neurotrophin systems could be involved, therefore, in more protracted aspects of opiate abstinence, phenomena that are well described clinically but have been difficult to detect in laboratory animals (Koob and Nestler, 1997). The hypothesis that BDNF induction in LC neurons during opiate withdrawal is secondary to the increased firing of these neurons is supported by the previous finding that administration of reserpine, which also increases the firing rate of LC neurons (Melia et al., 1992), induces a transient upregulation of BDNF mRNA within LC neurons (Seroogy, 1994).

In contrast to the alterations observed in LC after chronic morphine treatment or withdrawal, there was little regulation of the neurotrophins or their receptor mRNAs within the VTA. In fact, the only change observed was a small but significant increase in *trkB* mRNA in the VTA at 6 hr of opiate withdrawal. Neurotrophin expression within the VTA has previously been determined to be malleable in adult rodents. BDNF mRNA expression has been observed to be transiently increased in the VTA after 6-hydroxydopamine-induced (Numan and Seroogy, 1994) and

Figure 8. Densitometric analysis of neurotrophin (*A*, *B*) and neurotrophin receptor (*C*, *D*) mRNA expression in VTA during precipitated opiate withdrawal. No significant alterations in BDNF (*A*), NT-3 (*B*), or *trkC* (*D*) mRNA levels were observed in VTA during withdrawal from opiates. *C*, *trkB* mRNA levels were significantly increased as compared with control at 6 hr of morphine withdrawal ($p < 0.05$). Results are expressed as a percentage of control values. Values are the mean $\pm$ SEM. *Significantly increased from control.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced (Hung and Lee, 1996) lesions. This evidence suggests that the lack of changes in the VTA in the present study are not caused by an inability of the neurons to alter neurotrophin expression. It may not be surprising that little regulation of the neurotrophins or their receptor mRNAs was observed in the VTA during opiate withdrawal. Opiates have been shown to acutely inhibit the inhibitory GABAergic neurons in the VTA, thereby activating the dopaminergic neurons (Johnson and North, 1992). Although the electrophysiological activity of dopaminergic neurons during opiate withdrawal is not known, there is indirect evidence that the opiate-induced inhibition of the GABAergic interneurons is reversed during withdrawal, leading to a pronounced inactivation of the VTA dopaminergic neurons (Bonci and Williams, 1997). Despite the lack of regulation of the neurotrophins and receptors themselves, however, previous work has shown a clear modulation of postreceptor signaling proteins in the VTA after chronic morphine treatment (Russell et al., 1994; Berhow et al., 1996).

The results of the present study provide further evidence for the involvement of neurotrophin systems in opiate addiction, particularly in the LC. Although the exact physiological role played by these changes in the neurotrophins and their receptor mRNAs during the course of chronic morphine treatment and withdrawal will require further research, these findings highlight the complex array of adaptations that contribute to the drug-addicted state.

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