

# Attenuation of Acute Morphine Withdrawal in the Neonatal Rat by the Competitive NMDA Receptor Antagonist LY235959

Kathy L. Jones, Ph.D., Hongbo Zhu, M.S., Shirzad Jenab, Ph.D., Ted Du, B.S., Charles E. Inturrisi, Ph.D., and Gordon A. Barr, Ph.D.

*The present study examined the ability of LY235959, a competitive N-methyl-D-aspartate (NMDA) receptor antagonist, to attenuate behaviors and c-fos mRNA expression associated with acute morphine withdrawal in the infant rat. Rat pups were given a single dose of morphine (10.0 mg/kg, s.c.) or saline. Two hours later, pups were removed from the dam and injected with either LY235959 (10.0 mg/kg, s.c.) or saline. Fifteen minutes later acute morphine withdrawal was precipitated with naltrexone (10.0 mg/kg, s.c.) and behaviors were recorded every 15 s for the next 60 min. Immediately after behavioral testing, brain and spinal cord were assayed for c-fos mRNA analysis by solution hybridization. The intensity of the*

*morphine withdrawal syndrome was reduced in pups pre-treated with LY235959. Withdrawal behaviors such as head moves, moving paws, rolling, and walking were decreased, and vocalizations were completely eliminated in pups pre-treated with LY235959. Acute morphine withdrawal increased c-fos mRNA expression in the brain and the spinal cord, which was attenuated by pre-treatment of LY235959. Thus, in the 7-day-old rat, as in the adult, NMDA receptors play a role in the behavioral and molecular manifestations of acute morphine withdrawal.*

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In humans, exposure to opiates for either medical or nonmedical reasons leads to dramatic behavioral and neural changes resulting in physical dependence

(Trujillo and Akil 1991b). Although physical dependence is classically thought to develop after chronic exposure to opiates (chronic opiate dependence), the adaptational changes underlying physical dependence begin with the first exposure to an opiate (Heishman et al. 1989a,b; Kirby et al. 1990). Thus, acute opiate withdrawal refers to the withdrawal syndrome precipitated by administration of an opioid antagonist after either a single dose or a short-term infusion of opioid agonist (Martin and Eades 1964; Bickel et al. 1988).

Despite decades of research, our understanding of the mechanisms underlying opiate physical dependence and withdrawal is still very limited (Mayer et al. 1999; Trujillo 1999) and multiple mechanisms may operate in these processes (Thorat et al. 1994). There is increasing evidence indicating that N-methyl-D-aspartate (NMDA) receptor antagonists inhibit the development of *mu* receptor mediated opiate dependence in the adult

From the Department of Pharmacology, Weill Medical College of Cornell University, New York, NY (KLJ, SJ, TD, CEI), Biopsychology Doctoral Program, Hunter College, City University of New York, 695 Park Ave., New York, NY (KLJ, HZ, GAB), and New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY (GAB)

Address correspondence to: Gordon A. Barr, Department of Psychology, Hunter College, City University of New York 695 Park Avenue, New York, NY 10021, Tel.: (212) 772-5610, Fax: (212) 772-4477, E-mail: gbarr@hunter.cuny.edu

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rodent, and that the NMDA receptor and its second messenger systems play pivotal roles in opiate tolerance, dependence and withdrawal (Trujillo and Akil 1991a; Inturrisi 1997; Mao 1999; Mayer et al. 1999).

It is not known whether these mechanisms in adults apply to infants. On the one hand, opiate dependence can be established in fetuses or infant rat pups if the dams are exposed to opiates during their pregnancy or the pups are treated directly with opiates (Jones and Barr 1995, 2000; Windh et al. 1995; Thornton et al. 1997; Barr et al. 1998). On the other hand, the neonatal CNS is both structurally and functionally different from that of the adult, and significant changes in opioid actions occur both prenatally and postnatally (Barr 1992, 1993; Fitzgerald 1995; Marsh et al. 1997; Fitzgerald and Jennings 1999). More importantly, the NMDA receptor, which is believed to play a crucial role in the establishment of opiate dependence, undergoes qualitative and quantitative changes during development (Kalb et al. 1992; Hori and Kanda 1994; Kalb and Fox 1997). These include significant developmental alterations both in the density of the receptor (Tremblay et al. 1988; Morin et al. 1989; Represa et al. 1989), the sensitivity of the receptor complex to magnesium (Ben-Ari et al. 1988; Bowe and Nadler 1990; Morrisett et al. 1990), and the rise and decay times of the NMDA receptor-mediated excitatory post-synaptic currents (EPSCs) (Akaike and Rhee 1997; Bardoni et al. 1998). Recently, due to the advancement in cloning technology and gene knockout technology, the molecular structure of the NMDA receptor complex has been characterized (Ozawa et al. 1998; Dingledine et al. 1999). The accumulating evidence indicates that the various subunits (NR1, NR2A-D, NR3A) composing the native NMDA receptor channel undergo dramatic differential change during the first few weeks of the postnatal life in the rat (Dingledine et al. 1999). It has been suggested that the NMDA receptor is not present in fully mature form until the second postnatal week in the rat (Sircar 2000). Thus, the pharmacological effects of NMDA antagonism in the infant may not necessarily be comparable to those in the adult. In fact, the acute or chronic administration of NMDA receptor antagonists is ineffective in suppressing the expression of the behavioral signs of withdrawal from chronic morphine treatment followed by naltrexone challenge, in the 4 to 7-day-old rat (Bell and Beglan 1995; Zhu and Barr 2000, 2001), which is in contrast to reports in the adult (Trujillo and Akil 1991a).

Although several laboratories, including ours, have examined the behavioral, biochemical and pharmacological aspects of opiate withdrawal induced by chronic opiate administration in the infant animals (Jones and Barr 1995, 2000; Windh et al. 1995; Thornton and Smith 1997; Thornton et al. 1997; Ceger and Kuhn 2000; Zhu and Barr 2000, 2001), little is known about the acute morphine withdrawal syndrome in the infant animal

model (Ceger and Kuhn 2000). Thus, the present study investigated the behavioral and biochemical parameters of acute morphine withdrawal in the 7-day-old rat. In addition, since NMDA receptor antagonists were reported to inhibit both behaviors (Herman et al. 1995) and *c-fos* expression (Rasmussen et al. 1995) associated with opiate withdrawal, we also examined the pharmacological effect of the pre-treatment of the NMDA receptor antagonist, LY235959 on the acute withdrawal behavior and withdrawal associated *c-fos* expression.

## MATERIALS AND METHODS

### Animals

All animal procedures were in accordance with the "Principles of laboratory animal care" (NIH publication 1996) and approved by the institutional animal care and use committee at Weill Medical College of Cornell University. The subjects were the offspring of Sprague-Dawley rats. Pregnant dams were purchased on gestational day 19 or 20 (Charles River). Dams were individually housed in plastic tubs with wood chips in a colony room maintained at 22–24°C on a 12-h light/12-h dark photocycle with light onset at 7 A.M. The breeding colony existed in a separate room with minimal disturbances except for normal cleaning, feeding and record keeping. Dams had Purina Lab Chow (Purina 5012) and water available ad libitum. Pups were termed 0 days of age upon birth. After parturition, litters were culled to six male pups. Pups were tattooed with India ink (Geller and Geller 1966), which was injected into one or two paws to label permanently individual pups in each litter.

### Drug Treatment and Behavior Testing

Morphine sulfate was obtained from the National Institute on Drug Abuse (Rockville, MD). Naltrexone and NMDA was purchased from Sigma Chemical (St. Louis, MO). LY235959 was obtained from Eli Lilly and Company (Indianapolis, IN).

In the afternoon of the seventh day, animals were transported from the animal facility to our laboratory in plastic tubs with wood chip bedding and placed in an observation chamber maintained at approximately 33°C. Pups were weighed and assigned to one of four treatment groups ( $n = 25$  for each group). Two groups received 10 mg/kg morphine (s.c.) and two groups received saline injection. Two hours later pups were injected with either LY235959 (10.0 mg/kg, s.c.) or saline. The order of treatment conditions was assigned randomly within each experiment. After the drug treatment, the pup was then placed back into the observation chamber with the remainder of the litter (without the dam). Fifteen minutes later, naltrexone (10.0 mg/kg, s.c.) was injected to precipitate withdrawal. The be-

havior of the pups was then observed for 60 min after the injection of naltrexone. Every 15 s, the behavior of the pups was identified and recorded on a checklist (see Table 1 for definitions of behavior included in the checklist) by an observer who was blind to the drug and dose. The next pup was then tested until all treatment groups were completed.

High doses of NMDA receptor antagonists are reported to have motor effects on the rat (Trujillo and Akil 1995). Unfortunately, all of the data are from the adult and there is a lack of data in the pharmacological effect of these compounds in the infant rat. Thus, in order to determine a suitable dose of LY235959 for our morphine withdrawal test, a separate group of rats ( $n = 15$ ) was tested for motor behavior impairment. Cumulative doses (0, 1.0, 4.0, 14.0, 34.0 and 60.0 mg/kg, increasing every 15 min) of LY235959 were tested for their motor impairment liability using the 30° incline test, grasping and righting reflex. In the 30° incline test, pups were placed facing downward on a screen positioned at a 30° angle and allowed to position themselves upright. In the grasping test, the forepaws of the pup were placed on a rod and tested for their ability to grasp. In the righting test, pups were placed on their back and observed for their ability to return to the upright position. Pups were scored on a scale from 0 to 2: with 0 indicating no motor impairment, 1 indicating slight motor impairment and 2 indicating severe motor impairment. Specifically, in all three tests, each pup was given five trials for any specific drug dose, if the pup successfully completed four or five trials, it was scored as 0. If the pup completed two or three trials, it was scored as 1. If the pup completed fewer than two trials, it was scored as 2. In addition, using the same paradigm, we tested a separate group of rats ( $n = 8$ ) for motor behavior impairment at the specific dose of LY235959 (10 mg/kg) and with a similar time course (up to 75 min post injection of LY235959) as we employed in the morphine withdrawal experiment.

To determine the effectiveness of the dose of LY235959 (10 mg/kg) in inhibiting NMDA receptor re-

lated *c-fos* expression, in a separate experiment, rats were pre-treated either with LY235959 (10 mg/kg, s.c.) or saline ( $n = 5$  for each group). Then 15 min later, half of the LY235959 pre-treated and half of the saline pre-treated pups were treated with NMDA (20 mg/kg, i.p.); the remaining pups in each pre-treatment group were treated with saline. In this experiment, *c-fos* mRNA expression was measured and no behavior tests were involved.

### Tissue Collection and RNA Extraction

Immediately after withdrawal behavior testing, animals were sacrificed by decapitation. Since *c-fos* mRNA production typically peaks approximately 15–45 min following a behavioral event (Zhu and Inturrisi 1993), the 1 h exposure of the rats to morphine withdrawal helped to insure a representative sampling of the withdrawal period.

Following CO<sub>2</sub> narcosis and decapitation, the brain (minus cerebellum) and spinal cord were removed. Each tissue was immediately homogenized and the RNA recovered using the Trizol reagent (Life Sciences, Gaithersburg, MD). Each value represents the mean of five RNA extracts for each tissue ( $n = 5$ ).

### Solution Hybridization for *c-fos* mRNA

*c-Fos* mRNA levels were determined in tissue extracts of total cellular RNA using a previously described solution hybridization assay for *c-fos* mRNA (Zhu et al. 1993). A <sup>32</sup>P-labeled antisense riboprobe was prepared by in vitro transcription. The plasmid for the rat *c-fos* riboprobe contained a 970 bp BglIII-ScaI fragment (Zhu et al. 1993) obtained from a full length cDNA (Curran et al. 1987). *c-Fos* riboprobe transcripts (specific activity =  $6.5 \times 10^8$  dpm/μg) were applied to a CF11 column (Zhu et al. 1992, 1993), washed to remove unincorporated label, and eluted with TSE: ethanol 78:22 (v/v) (TSE is 0.05 M Tris-HCl, 0.1 M sodium chloride and 0.001 M EDTA, pH 7.0) to obtain a single stranded riboprobe fraction free of "snap back regions" which contribute to background in the solution hybridization assay (Zhu et al. 1992). Non-radiolabeled mRNA "sense" standards were prepared by in vitro transcription (Zhu et al. 1992) using the full length cDNA described above (Curran et al. 1987).

Total cellular RNA was determined by UV absorbency at 260 nm. Duplicate aliquots of each RNA sample were dried under a vacuum in 1.5 ml eppendorf tubes and then resuspended in 30 μg of hybridization buffer (10 mM N-tris [hydroxymethyl]methyl-2-aminoethanesulfonic acid, 10 mM EDTA, 0.3 M NaCl, 0.5% SDS and pH 7.4) that also contained 150,000 dpm of riboprobe. Samples were covered with two drops of mineral oil and hybridized at 75°C for 4 h. After hybridization, 300 μl solution containing a high salt buffer (0.3 M NaCl, 5 mM EDTA, and 10 mM Tris-HCl, pH 7.5), 40

**Table 1.** Behavioral Definitions

Behavior	Definition
Head Moves	Lateral and rotary motions of the head
Moving Paws	Continuous movement of the hindpaws without walking
Quiet	Sedated appearance with no movement
Rolling	Turning the body over at least one full rotation
Vocalizations	Emitting an audible sound
Walking	Taking more than one step forward
Wall Climbing	Placing at least two forepaws on the wall of the observation chamber

μg/ml RNase A (Worthington Biochemicals, Freehold, N.J.), and 2 μg/ml RNase T1 (Calbiochem, San Diego, CA) was added and samples were incubated at 30°C for 1 h to digest unhybridized probe. The ribonuclease reaction was terminated with 1 ml of 5% trichloroacetic acid (TCA) and 0.75% sodium pyrophosphate. One drop of 0.5% BSA was added to aid precipitation. This solution was mixed and the TCA precipitable dpms were collected onto glass microfiber filter paper (Reeves Angel 934AH, Brandel, Gaithersburg, MD) using a 24 place cell harvester (Brandel, Gaithersburg, MD). The filter was washed three times with 5% TCA, dried under an infra-red light and counted by liquid scintillation in 5 ml hydrofluor scintillation solution (National Diagnostics, Manville, NJ).

The standard calibration curve for *c-fos* mRNA is linear from 1.95 to 250 pg of the full length *c-fos* sense transcript (i.e., *c-fos* mRNA) with a correlation coefficient of 0.997. In ten consecutive experiments the interassay coefficient of variation averaged 7.4% and the intra-assay coefficient of variation average 3.8% for duplicate aliquots of 30 different extracts.

## Statistics

Withdrawal was analyzed by separate 1-way analysis of variance (ANOVA) for each withdrawal behavior followed by the Student-Newman-Keuls test for multiple comparison tests at the 0.05 level of significance.

All *c-fos* mRNA expression data points from the brain and spinal cord were analyzed with a 1-way ANOVA with subsequent group comparisons made using the Student-Newman-Keuls test at the 0.05 level of significance.

## RESULTS

### Withdrawal Behaviors

A single dose of morphine (10 mg/kg, s.c.) two hours later followed by a single injection of naltrexone (10 mg/kg, s.c.) induced a plethora of acute morphine withdrawal behaviors in the 7-day-old rat (see Figure 1). Compared with the saline control group, the morphine treated animals displayed significantly increased withdrawal behaviors and decreased quiet behavior. The increased withdrawal behaviors included head moves, moving paws, rolling, vocalizations, walking and wall climbing. The categories and intensity of these acute withdrawal behaviors are generally comparable to those seen in withdrawal after chronic morphine administration (Jones and Barr 1995).

The dose of LY235959 to cause a minimal motor function impairment in the 7-day-old rat we tested was 14 mg/kg as determined by the cumulative dosing experiment (see Table 2). Pre-treatment with the competitive NMDA receptor antagonist LY235959 at 10 mg/kg, a

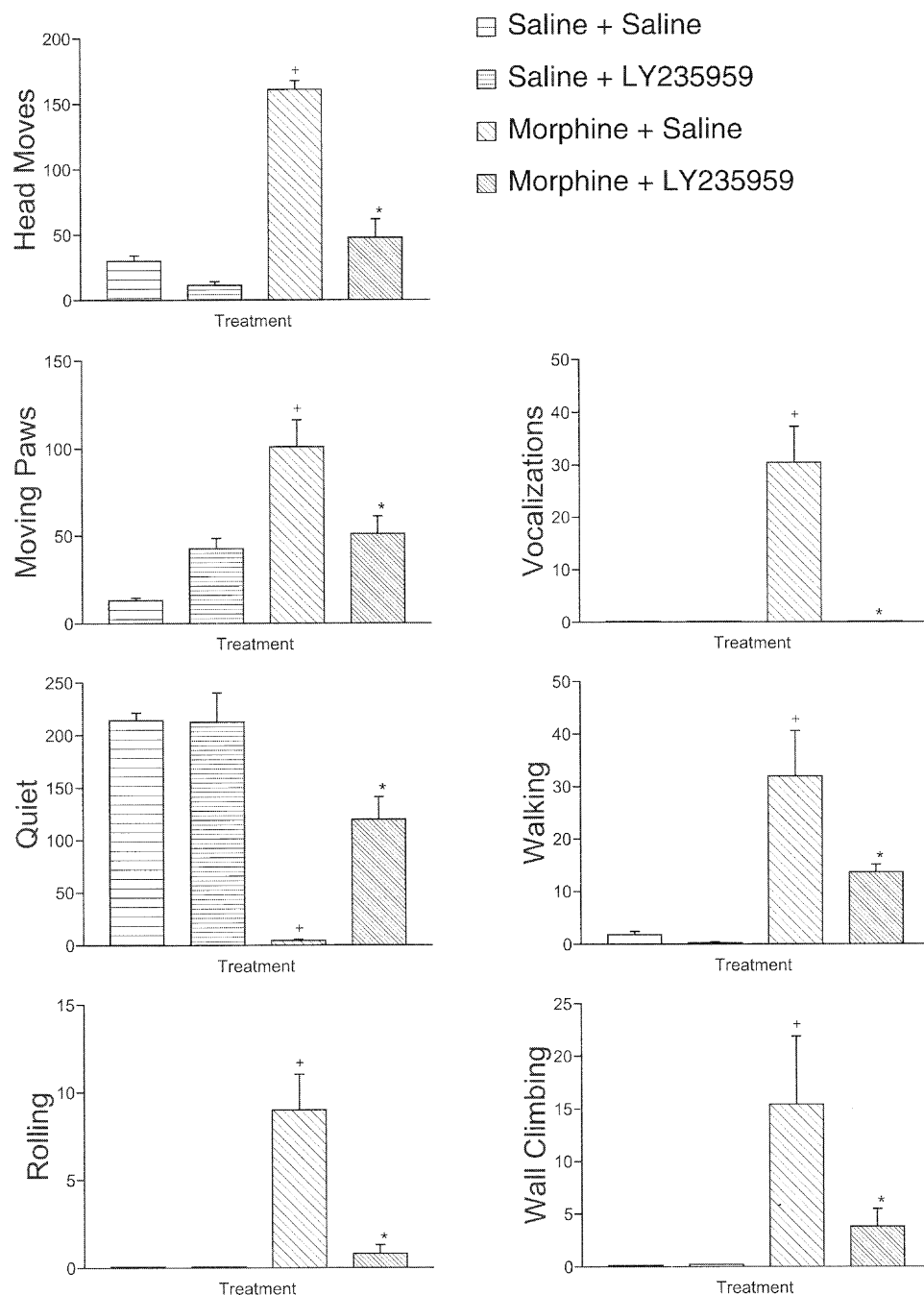
dose that did not cause any motor function impairment by itself (see Table 3), significantly reduced acute morphine withdrawal behaviors in the 7-day-old rat (see Figure 1). Compared with the control group that received morphine but no LY235959 prior to naltrexone challenge, all withdrawal behaviors in the LY235959 pre-treated pups were significantly attenuated and quiet behavior significantly increased. Withdrawal behaviors attenuated by pre-treatment of LY235959 included head moves, moving paws, rolling, vocalization, walking and wall climbing. In contrast, pre-treatment of 10 mg/kg LY235959 did not significantly change the occurrences of these behaviors in rats that did not receive morphine treatment, indicating that LY235959's effect in attenuating these behaviors is specific to acute morphine withdrawal rather than to any non-specific effect of this compound.

### Opiate Withdrawal Evoked Brain *c-fos* mRNA Expression

In the 7-day-old rat, brain *c-fos* mRNA expression was significantly elevated to approximately 300% of the control during acute morphine withdrawal. This acute morphine withdrawal-induced *c-fos* mRNA expression was significantly attenuated by the pre-treatment of LY235959 15 min prior to naltrexone injection (see Figure 2, upper panel). There are two important observations here: (1) This attenuation was specific to the acute morphine withdrawal syndrome, since LY235959 alone did not significantly alter the expression of *c-fos* mRNA in the brain (see Figure 2, upper panel). (2) Although pre-treatment of LY235959 significantly attenuated acute morphine withdrawal induced *c-fos* mRNA expression, *c-fos* mRNA expression did not return completely to baseline. Indeed, even though the dose of LY235959 (10 mg/kg) completely blocked NMDA induced *c-fos* mRNA expression in the brain (see Figure 2, lower panel), it attenuated the acute morphine withdrawal evoked increase of *c-fos* mRNA expression by less than 50% (see Figure 2, lower panel).

### Opiate Withdrawal Evoked Spinal Cord *c-fos* mRNA Expression

Similar to that in the brain, spinal cord *c-fos* mRNA expression was also significantly elevated during acute morphine withdrawal and this elevation was attenuated by the pre-treatment with LY235959 (see Figure 2, middle panel). The attenuation is specific to the acute morphine withdrawal syndrome, since LY235959 alone did not significantly alter the expression of *c-fos* mRNA in the spinal cord (see Figure 2, middle panel). Again, pre-treatment of LY235959 did not completely block (less than 50%) the elevation of *c-fos* mRNA expression evoked by acute morphine withdrawal (see Figure 2, middle panel).



**Figure 1.** The effect of pre-treatment with the competitive NMDA receptor antagonist LY235959 (10 mg/kg) on naltrexone-precipitated acute morphine withdrawal behaviors in the 7-day-old rat. Ordinate: Mean occurrences (Mean  $\pm$  one S.E.M.) in 60 min of opiate withdrawal behaviors (definition see Table 1). Abscissa: treatment conditions. Withdrawal was precipitated by naltrexone (10 mg/kg) in all groups. <sup>+</sup> $p < .05$  compared with the saline plus saline control group. <sup>\*</sup> $p < .05$  compared with the morphine plus saline group. For all tested behaviors, no significant results were found between the two groups that did not receive morphine injection.

Pre-treatment of 10 mg/kg LY235959 did not significantly alter the expression of *c-fos* mRNA in either the brain or the spinal cord in 7-day-old rats that did not receive morphine treatment, suggesting that LY235959's suppression of *c-fos* mRNA expression is restricted to acute morphine withdrawal.

## DISCUSSION

There are three major conclusions from the present study: (1) As manifested by both behavioral and mo-

lecular measures, acute morphine dependence can be induced by a single injection of morphine in the rat as young as 7 days of age. (2) Acute morphine withdrawal in the 7-day-old rat was attenuated by pre-treatment of a competitive NMDA receptor antagonist LY235959. (3) Although the dose of LY235959 was sufficient to have completely blocked NMDA receptor related neural transmission, its inhibition of acute morphine withdrawal was not complete. We explore the implications for each of these three observations.

**Table 2.** Behavioral Toxicity of LY235959 Using the Cumulative Dose Protocol. Pups Were Treated s.c. with LY2359595 and Tested for Motor Impairment Using the 30° Incline Test, Grasping and Righting Reflex (n = 15)

Cumulative Dose (mg/kg)	Timing (minutes after first injection)	Tests		
		30° Incline	Grasping	Righting
0	0	0	0	0
1.0	15	0	0	0
4.0	30	0	0	0
14.0	45	1	1	1
34.0	60	2	2	2
60.0	75	2	2	2

Note: Three tests were used to evaluate motor impairment produced by LY235959

1. The 30° incline test. Pups were placed facing downward on a screen positioned at 30° angle and allowed to position themselves upright.

2. Grasping. The forepaws of the pup were placed on a rod and tested for their ability to grasp.

3. Righting. Pups were placed on their back and observed for their ability to return to the upright position.

Pups were scored on a scale from 0 to 2:

0 = no motor impairment

1 = slight motor impairment

2 = severe motor impairment

The dose was increased every 15 minutes and each of the 15 pups showed the level of impairment indicated above.

### Acute Opiate Withdrawal Exists in Infant

Using the 7-day-old rat as a model, the present study has confirmed that opiate dependence can be observed after a single dose of morphine. At this age, 2 h after morphine injection, a myriad of withdrawal behaviors was precipitated by naltrexone challenge. In addition, *c-fos* mRNA expression levels were significantly increased in both the brain and the spinal cord. Thus, as in the adult rat, acute opiate withdrawal exists in the infant rat. Our results are consistent with the only other

available report on acute opiate withdrawal in the infant rat (Ceger and Kuhn 2000) and we extended the findings of that study by demonstrating that, in addition to withdrawal behaviors, molecular indices such as *c-fos* mRNA expressions are also increased.

### The NMDA Receptor Antagonist LY235959 Is Effective in Suppressing Acute Opiate Withdrawal

The existing literature on whether various NMDA receptor antagonists can inhibit the expression of opiate

**Table 3.** Behavioral Toxicity of 10 mg/kg LY235959 over 75-Min Period. Pups Were Treated s.c. with 10 mg/kg LY2359595 And Tested for Motor Impairment Using The 30° Incline Test, Grasping and Righting Reflex (n = 8)

Minutes after 10 mg/kg LY235959 injection	Rats showing each level of motor impairment								
	30° Incline Score			Grasping Score			Righting Score		
	0	1	2	0	1	2	0	1	2
0*	8	0	0	8	0	0	8	0	0
15	8	0	0	8	0	0	8	0	0
30	8	0	0	8	0	0	8	0	0
45	8	0	0	8	0	0	8	0	0
60	8	0	0	7	1	0	8	0	0
75	8	0	0	7	1	0	8	0	0

Note: Three tests were used to evaluate motor impairment produced by 10 mg/kg LY235959 (s.c.)

1. The 30° incline test. Pups were placed facing downward on a screen positioned at 30° angle and allowed to position themselves upright.

2. Grasping. The forepaws of the pup were placed on a rod and tested for their ability to grasp.

3. Righting. Pups were placed on their back and observed for their ability to return to the upright position.

Pups were scored on a scale from 0 to 2

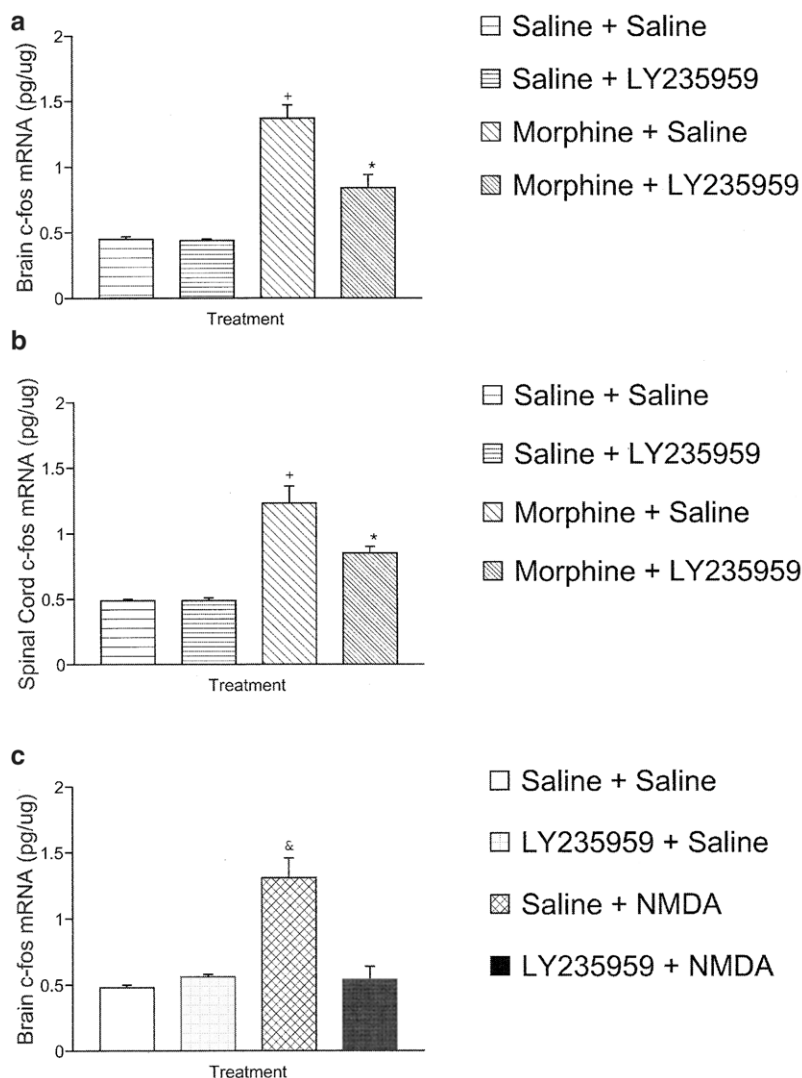
0 = no motor impairment

1 = slight motor impairment

2 = severe motor impairment

Each cell represents the number of rats (out of a total of 8 subjects) that earned the specific score. No significant results were found for the time effect.

\*:Immediately before LY235959 treatment.



**Figure 2.** Upper panel: The effect of pre-treatment with the competitive NMDA receptor antagonist LY235959 (10 mg/kg) on brain *c-fos* mRNA expression evoked by naltrexone-precipitated acute morphine withdrawal in the 7-day-old rat. Ordinate: Mean *c-fos* mRNA expression (pg/ $\mu$ g) in the brain (Mean  $\pm$  one S.E.M.). Abscissa: treatment conditions. Withdrawal was precipitated by naltrexone (10 mg/kg) in all groups. <sup>+</sup>  $p < .05$  compared with the saline plus saline control group. <sup>\*</sup>  $p < .05$  compared with the morphine plus saline group. No significant results were found between the two groups that did not receive morphine injection. Middle panel: The effect of pre-treatment with the competitive NMDA receptor antagonist LY235959 (10 mg/kg) on spinal cord *c-fos* mRNA expression evoked by naltrexone-precipitated acute morphine withdrawal in the 7-day-old rat. Ordinate: Mean *c-fos* mRNA expression (pg/ $\mu$ g) in the spinal cord (Mean  $\pm$  one S.E.M.). Abscissa: treatment conditions. Withdrawal was precipitated by naltrexone (10 mg/kg) in all groups. <sup>+</sup>  $p < .05$  compared with the saline plus saline control group. <sup>\*</sup>  $p < .05$  compared with the morphine plus saline group. No significant results were found between the two groups that did not receive morphine injection. Lower panel: The effect of LY235959 in inhibiting NMDA receptor responsible neural activity as assessed by NMDA-evoked *c-fos* expression in the 7-day-old rat's brain. Ordinate: Mean *c-fos* mRNA expression (pg/ $\mu$ g) in the brain (Mean  $\pm$  one S.E.M.). Abscissa: treatment conditions. <sup>&</sup>  $p < .05$  compared with the saline plus saline control group. No significant results were found among the two groups that did not receive morphine and the group that received LY235959 (10 mg/kg) 15 min prior to NMDA challenge.

withdrawal is not consistent (Herman et al. 1995; Trujillo and Akil 1995). Although most laboratories report that NMDA antagonists inhibit withdrawal symptoms (Tanganelli et al. 1991; Brent and Chahl 1993; Capendijk et al. 1993); others report that they do not

(Trujillo and Akil 1991a,b), and still others report mixed results (Rasmussen et al. 1991; Thorat et al. 1994). Two additional factors make comparison between our data with any available literature difficult. First, most of these studies were done in the adult rat or mouse and

there are significant developmental differences in the effectiveness of NMDA receptor blockers to attenuate withdrawal (Zhu and Barr 2000, 2001). Second, in all the studies investigating the pharmacology of various NMDA receptor antagonists on opiate withdrawal in infant animals, the chronic morphine administration paradigm was adopted (Bell and Beglan 1995; Zhu and Barr 2000, 2001). Thus, as far as we know, the present study represents the first report of the pharmacology of a NMDA receptor antagonist on acute opiate withdrawal in rat as young as 7 days old.

Earlier behavioral studies suggest that acute and chronic opiate withdrawal are quantitatively different. For example, the withdrawal syndrome resulting from chronic dependence tends to be more intense and of longer duration than that from acute dependence (Martin and Eades 1964). On the other hand, the observed signs and symptoms are qualitatively similar (Martin and Eades 1964). This qualitative similarity in withdrawal symptoms after acute and chronic opiate exposure suggests that the same mechanisms may underlie both processes (Heishman et al. 1989b). More recent molecular studies, however, suggest that the mechanisms underlying acute and chronic actions of opiates may be qualitatively different from, and in some cases opposing to, each other (Sharma et al. 1975; Nestler and Aghajanian 1997). Notably, acute morphine inhibits adenylate cyclase and results in a reduction of cAMP production in the cell whereas repeated treatment of morphine increases adenylate cyclase and cAMP-dependent protein kinase (Duman et al. 1988; Nestler and Tallman 1988; Nestler and Aghajanian 1997).

Interestingly, the acute morphine withdrawal behavioral repertoire of the 7-day-old rat characterized in the present study is qualitatively comparable of that documented in reports using the chronic morphine injection paradigm (Jones and Barr 1995; Windh et al. 1995; Thornton et al. 1997). Withdrawal behaviors representative of the acute morphine injection paradigm generally coincide with withdrawal behaviors typical of the chronic morphine administration model. For example, withdrawal behaviors such as head moves, moving paws, rolling, walking, wall climbing and vocalizations are typical of both withdrawal paradigms. However, our data do not support the hypothesis that the same mechanisms underlie both acute and chronic opiate dependence. Previously, our laboratory and others reported that neither competitive nor non-competitive NMDA receptor antagonists were effective in suppressing opiate withdrawal in the chronic morphine treatment paradigm in infant rats (Bell and Beglan 1995; Zhu and Barr 2000, 2001). Thus, it is possible that although behavioral indices of acute and chronic morphine withdrawal are similar, the underlying mechanisms differ. The differential short-term and long-term molecular changes (e.g. cAMP levels) resulting from

acute and chronic morphine treatment may account for the observed difference in the pharmacology of NMDA receptor antagonists in chronic and acute morphine withdrawal paradigms.

### Expression of Acute Opiate Withdrawal Involves Multiple Mechanisms

In both the brain and the spinal cord, LY235959 significantly but not completely blocked the *c-fos* mRNA expression evoked by acute morphine withdrawal. This finding is particularly interesting, since the dose of LY235959 that we used completely blocked the *c-fos* elevation induced by exogenously applied NMDA. Thus, our data suggest that mechanisms parallel to the NMDA receptor related mechanism may exist in the expression of acute morphine withdrawal. Specifically, in 7-day-old rats, the NMDA receptor plays an important, but not exclusive, role in the expression of acute morphine withdrawal. Other parallel mechanisms may co-exist with the NMDA receptor related mechanism. One such possibility is that the AMPA/kainate receptor could play an important role in the expression of acute opiate withdrawal. The AMPA/kainate receptor is colocalized with the NMDA receptor on many cell types (Gu et al. 1996). During ontogeny, AMPA/kainate receptors are known to mediate many cellular functions, such as long term potentiation (LTP) (Jia et al. 1996; Mahanty and Sah 1998), activation of the calmodulin-NOS cascade (Fedele and Raiteri 1999) and phosphorylation of cAMP response element-binding protein (Perkinton et al. 1999), that were thought to be the sole province of the NMDA receptor. Furthermore, several authors have reported that the AMPA/kainate receptor plays an very important, if not the most important, role in the expression of opiate withdrawal even in the adult (Rasmussen 1995; Rasmussen et al. 1996; Kest et al. 1997; McLemore et al. 1997). Thus, multiple and dynamic mechanisms may exist in mediating the acute opiate withdrawal. Further studies to elucidate the dynamic roles of various glutamate receptors in acute opiate withdrawal may prove to be beneficial.

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