

Short communication

## The clinically available NMDA receptor antagonist dextromethorphan attenuates acute morphine withdrawal in the neonatal rat

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### Abstract

We investigated the ability of dextromethorphan, a clinically available NMDA receptor antagonist, to attenuate the behaviors and the expression of *c-fos* mRNA associated with acute morphine withdrawal in the 7-day-old rat. The intensity of the acute morphine withdrawal behaviors and the elevation in *c-fos* mRNA expression in the brain induced by acute morphine withdrawal were reduced by dextromethorphan. Thus, dextromethorphan can attenuate acute morphine withdrawal in the developing organism.

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*Theme:* Neural basis of behavior

*Topic:* Drugs of abuse: opioids and others

*Keywords:* *c-fos* mRNA; Morphine withdrawal; Dextromethorphan; NMDA receptor antagonist

Improvements in the short- and long-term clinical outcomes of critically ill neonates have led to the widespread use of opioids for analgesia and sedation [28]. Consequently, large numbers of neonates are now receiving progressively increasing amounts of opioids [24,28–30]. These clinical practices have led to an increasing incidence of opioid dependence during therapy, with consequent increases in the number of patients showing the clinical signs of acute opioid withdrawal when therapy is discontinued [28].

The acute effects of opioid withdrawal can adversely affect the infants' alertness, visual responsiveness, and sleep regulation [8]. In addition, because neonatal withdrawal occurs during the period when the maternal–infant interaction patterns are first established, withdrawal can induce far-reaching dysfunctional changes that can affect not only attachment but also cognitive development [8]. Since physicians are now more likely to use opioids to manage pain in neonates and infants than in the past [28],

the need is much greater for a clearer understanding of the mechanisms of acute opioid withdrawal in the developing organism.

NMDA receptor antagonists inhibit opioid withdrawal syndrome in the mature organism [9,16,18,31]. Indeed, clinically available NMDA receptor antagonists such as memantine and dextromethorphan have been shown in animal models [15,17,19,22,23,21] and clinical trials [5,4,12,13,26] to attenuate opioid withdrawal. However, little is known about NMDA receptor antagonists' effect on acute morphine withdrawal in the developing organism.

We previously reported that a competitive NMDA receptor antagonist LY235959 can attenuate acute morphine withdrawal in the infant rat [11]. The present study further investigates the ability of dextromethorphan, a clinically available NMDA receptor antagonist, to attenuate the behavioral and *c-fos* mRNA expression associated with acute morphine withdrawal. The *c-fos* gene is expressed in the central nervous system in response to neuronal stimuli and its elevation has been demonstrated to be a reliable indication of morphine withdrawal [11,25].

The subjects were the offspring of Sprague–Dawley rats. Pregnant dams were purchased on gestational day 19

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or 20 and 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. After parturition, litters were culled to six male pups. Pups were tattooed with India ink [7], which was injected into one or two paws to label permanently individual pups in each litter.

In the afternoon of the 7th postnatal day, pups were weighed and assigned to one of four treatment groups ( $n=15$  for each group). Two groups received morphine (10 mg/kg, s.c.) and two groups received saline injection. Then 2 h later pups were injected with either dextromethorphan (50 mg/kg, s.c.) or saline. This dose of dextromethorphan is typical in animal models of morphine withdrawal [17]. This dosage is also comparable to the doses used in human neonates clinically [1,3,14]. Then 15 min later, naltrexone (10.0 mg/kg, s.c.) was injected to precipitate withdrawal. The behavior of the pups was then scan-sampled for 60 min after the injection of naltrexone. Every 15 s, the withdrawal behaviors appropriate to rats of this age [10,11] were identified and recorded on a checklist in real time by an observer who was blind to treatment (see Table 1 for definitions of behavior included in the checklist).

Immediately after withdrawal behavior testing, animals were sacrificed by decapitation and brains (minus cerebellum) removed. Each tissue was immediately homogenized and the RNA recovered using the Trizol reagent (Life Sciences, Gaithersburg, MD) and *c-fos* mRNA levels were determined in tissue extracts of total cellular RNA using a previously described solution hybridization assay for *c-fos* mRNA [36]. A  $^{32}\text{P}$ -labeled antisense riboprobe was prepared by in vitro transcription. The plasmid for the rat *c-fos* riboprobe contained a 970-bp BglIII-ScaI fragment [36] obtained from a full-length cDNA [6]. *c-fos* Riboprobe transcripts (specific activity= $6.5 \times 10^8$  dpm/ $\mu\text{g}$ ) were applied to a CF11 column [36,37], 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 [37]. Non-radiolabeled mRNA 'sense' standards were prepared by in vitro transcription [37] using the full-length cDNA described above [6]. 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  $\mu\text{g}$  of hybridization buffer (10 mM *N*-tris[hydroxymethyl]methyl-2-amino-ethanesulfonic acid, 10 mM EDTA, 0.3 M NaCl, 0.5% SDS, 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  $\mu\text{l}$  solution containing a high salt buffer (0.3 M NaCl, 5 mM EDTA, and 10 mM Tris-HCl, pH 7.5), 40  $\mu\text{g}/\text{ml}$  RNase A, and 2  $\mu\text{g}/\text{ml}$  RNase T1 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 using a 24-place cell harvester. 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. 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 intraassay coefficient of variation average 3.8% for duplicate aliquots of 30 different extracts.

All data were analyzed by one-way analysis of variances (ANOVA) followed by the Student–Newman–Keul's test for multiple comparison tests at the 0.05 level of significance.

A single dose of morphine (10 mg/kg, s.c.) 2 h 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 (Fig. 1). Compared with the saline control group, the morphine treated animals displayed significantly increased withdrawal behaviors, including head moves, moving paws, rolling, vocalizations, walking and wall climbing. Thus, overall, pups experiencing acute morphine withdrawal remained less quiet than the controls.

Pre-treatment with 50 mg/kg dextromethorphan significantly reduced acute morphine withdrawal behaviors in the 7-day-old rat (Fig. 1). Compared with the control

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

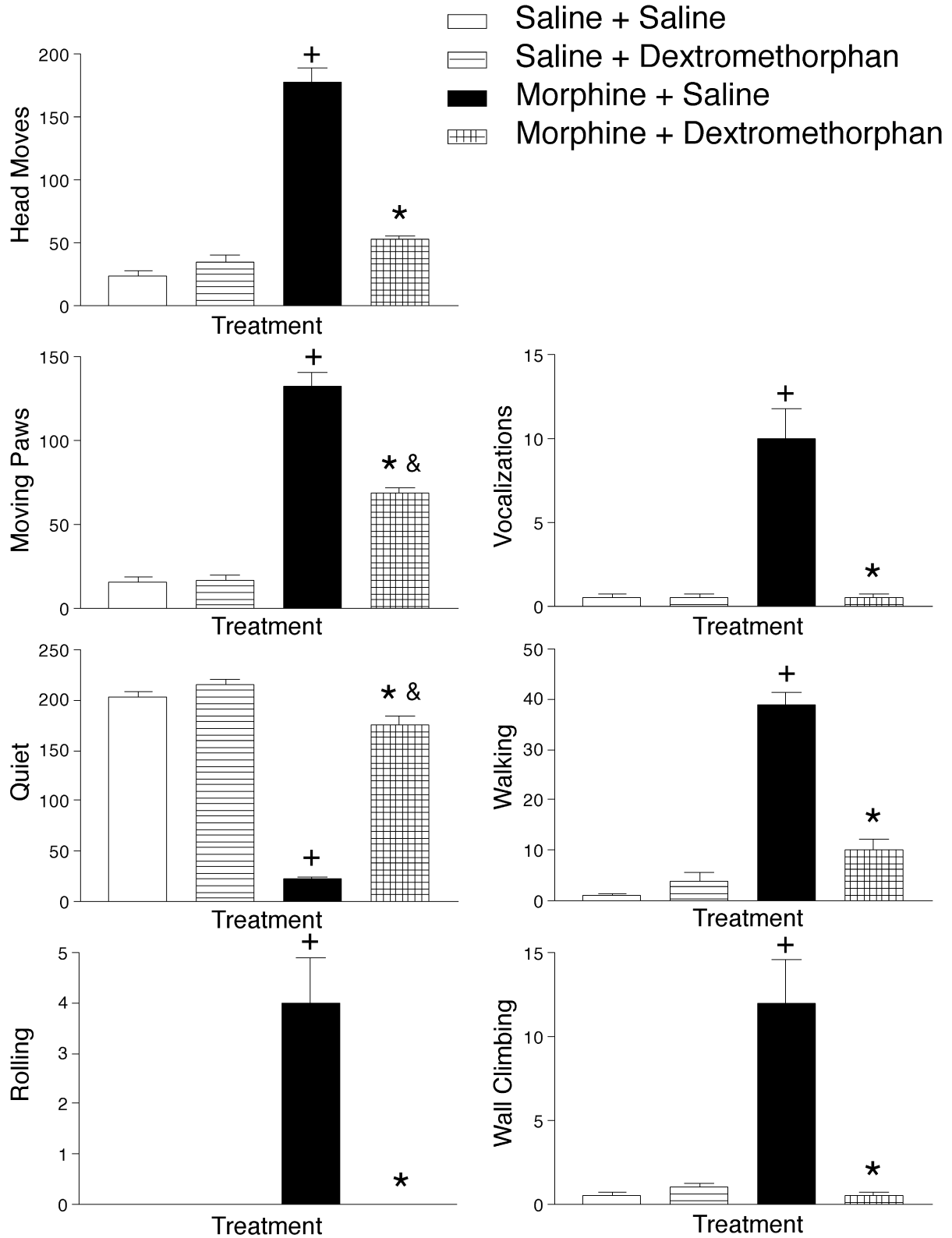


Fig. 1. The effect of pre-treatment with the NMDA receptor antagonist dextromethorphan (50 mg/kg, s.c.) on naltrexone precipitated acute morphine withdrawal behaviors in the 7-day-old rat. Ordinate: mean occurrences (mean±1 S.E.M.) in 60 min of opioid withdrawal behaviors (for definitions see Table 1). Abscissa: treatment conditions. Acute morphine withdrawal was precipitated by naltrexone (10 mg/kg, s.c.) in all groups. <sup>+</sup>*P*<0.05 compared to the saline plus saline control group; <sup>\*</sup>*P*<0.05 compared to the morphine plus saline group; <sup>&</sup>*P*<0.05 compared to the saline plus dextromethorphan group. For all tested behaviors, no significant differences were found between the two groups that did not receive morphine.

group that received morphine but no dextromethorphan prior to naltrexone challenge, all withdrawal behaviors in the dextromethorphan pre-treated pups were significantly attenuated and quiet behavior significantly increased. In contrast, pre-treatment with dextromethorphan did not cause any motor function impairment by itself, since pre-treatment of dextromethorphan did not significantly change the occurrences of behaviors in rats that did not receive morphine treatment (Fig. 1). Therefore, dextromethorphan's effect in attenuating these behaviors is specific to acute morphine withdrawal rather than any non-specific motor effect of this compound.

In the 7-day-old rat, brain *c-fos* mRNA expression was significantly elevated during acute morphine withdrawal (Fig. 2). This acute morphine withdrawal induced *c-fos* mRNA expression was significantly attenuated by the pre-treatment of dextromethorphan (Fig. 2). Importantly, this attenuation was specific to the acute morphine withdrawal syndrome, since dextromethorphan alone did not signifi-

cantly alter the expression of *c-fos* mRNA in the brain in pups that did not receive morphine treatment (Fig. 2). However, although dextromethorphan significantly attenuated acute morphine withdrawal induced *c-fos* mRNA expression, *c-fos* mRNA expression did not return completely to baseline (Fig. 2).

Since physicians are now more likely to use opioids to manage pain in neonates and infants than in the past [28], there are increasing concerns about the adverse effects of acute opioid withdrawal in this population [28,29]. Due to the complexity of the human setting, animal models are necessary to better study the mechanism and potential treatment of opioid withdrawal in human infant [2,8,35].

The present study has further confirmed our previous finding that acute morphine withdrawal can be observed after a single dose of morphine then followed by naltrexone challenge [11]. We conclude that the neonatal rat can be used as a reliable model system of acute opioid withdrawal. The establishment of this model system provides a stepping-stone for further study of the mechanism and potential treatment of acute opioid withdrawal in human infants.

Using this model system, we examined the clinically available NMDA receptor antagonist dextromethorphan on acute morphine withdrawal in the developing organism. Our data clearly demonstrated that dextromethorphan was effective in suppressing behaviors associated with acute morphine withdrawal in the neonatal rat. Indeed, all withdrawal behaviors returned to baseline partially (moving paws and quiet) or completely (head moves, rolling, vocalization, walking, and wall climbing) by dextromethorphan pre-treatment. Importantly, dextromethorphan's effect on acute morphine withdrawal is selective since in no case did this dose of dextromethorphan alter the normal motor behavior in the 7-day-old rat. Similarly, dextromethorphan selectively attenuated the elevation of brain *c-fos* mRNA expression associated with acute morphine withdrawal in the 7-day-old rat. Thus, the results of the present study extended the available literature on dextromethorphan's ability in inhibiting acute morphine withdrawal to include the developing organism.

We previously reported that NMDA receptor antagonists were not effective in suppressing morphine withdrawal resulted from chronic postnatal morphine treatment [33,34], although dextromethorphan was reported to attenuate certain signs of chronic morphine withdrawal if morphine was delivered prenatally [32]. The reason why NMDA receptor antagonists attenuate acute but not chronic morphine withdrawal [33,34] is unknown. It could be that the mechanisms underlying acute and chronic actions of morphine are qualitatively different from, and in some cases opposing to, each other [11,20,27]. Further studies are needed to identify how acute and chronic morphine interact with the NMDA receptor, particularly the immature NMDA receptor in the developing organism [35], leading to the different pharmacological effects of NMDA

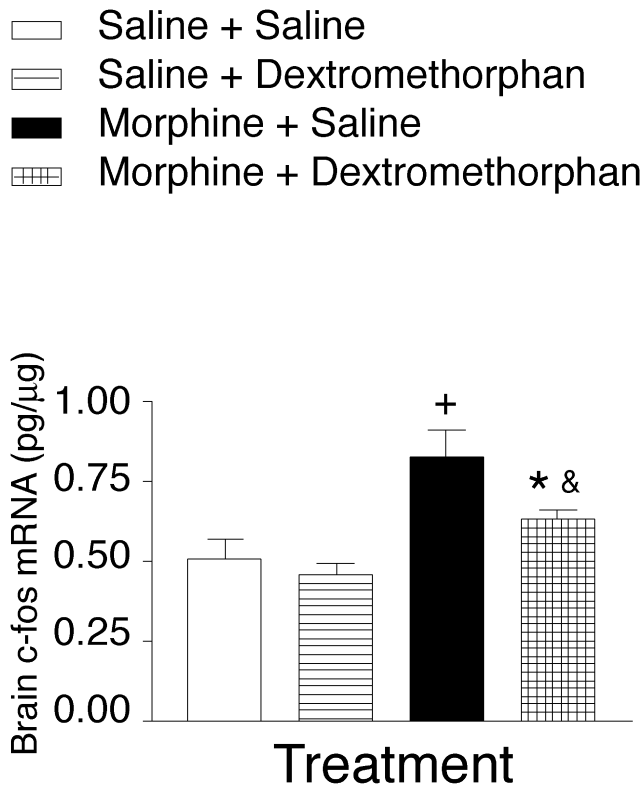


Fig. 2. The effect of pre-treatment with the NMDA receptor antagonist dextromethorphan (50 mg/kg, s.c.) on brain *c-fos* mRNA expression evoked by naltrexone precipitated acute morphine withdrawal in the 7-day-old rat. Ordinate: mean *c-fos* mRNA expression (pg/μg) in the brain (mean ± 1 S.E.M.). Abscissa: treatment conditions. Acute morphine withdrawal was precipitated by naltrexone (10 mg/kg, s.c.) in all groups. <sup>+</sup> $P < 0.05$  compared to the saline plus saline control group; <sup>\*</sup> $P < 0.05$  compared to the morphine plus saline group; <sup>&</sup> $P < 0.05$  compared to the saline plus dextromethorphan group. For *c-fos* mRNA levels, no significant difference was found between the two groups that did not receive morphine.

receptor antagonists on acute and chronic morphine withdrawal.

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