

Inhibition of Morphine Withdrawal by the NMDA Receptor Antagonist MK-801 in Rat Is Age-Dependent

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ABSTRACT This study investigated the effects of the NMDA receptor antagonist MK-801 on the development of morphine dependence in 7-, 14-, and 21-day-old rat pups. For 6.5 days, starting at 1, 8, or 15 days of age, rats were pretreated with MK-801 (0.03 or 0.1 mg/kg, bid) or saline; 15 min later, morphine sulfate (10 mg/kg) or saline was injected to induce opiate dependence. On the afternoon of the seventh day, pups were injected with MK-801 (0.1 mg/kg) or saline and 15 min later with naltrexone (1 mg/kg) to precipitate withdrawal. Pups were then placed in a warm chamber with the litter and their behavior scan-sampled every 15 sec for a total of 15 min. MK-801 failed to inhibit morphine withdrawal in the 7-day-old rat, but did attenuate the development of morphine dependence in both the 14- and 21-day-old rats. These results suggest that the NMDA receptor is not functionally active in opiate withdrawal until around the second to third week of postnatal life in the rat and that there exists a transition period for the NMDA receptor to play a role in the development of opiate dependence and withdrawal. *Synapse* 40:282–293, 2001. © 2001 Wiley-Liss, Inc.

INTRODUCTION

Despite decades of research, the understanding of the mechanisms underlying opiate tolerance, physical dependence, and withdrawal is still very limited (Mayer and Mao, 1999; Trujillo, 1999) and multiple mechanisms may operate in these processes (Thorat et al., 1994). There is increasing evidence indicating that the noncompetitive NMDA receptor antagonist MK-801 inhibits the development of mu receptor-mediated opiate dependence in the adult rodent, and that the NMDA receptor and its second-messenger system play pivotal roles in these processes (Inturrisi, 1997; Mao, 1999; Trujillo, 1999; Trujillo and Akil, 1991b).

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 pregnancy or the pups are treated directly with opiates (Barr et al., 1998; Jones and Barr, 1995, 2000; Thornton and Smith, 1997; Thornton et al., 1997; Windh et al., 1995). 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;

Fitzgerald and Jennings, 1999; Marsh et al., 1997). 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 (Hori and Kanda, 1994; Kalb and Fox, 1997; Kalb et al., 1992). These include significant developmental alterations both in the density of the receptor (Morin et al., 1989; Represa et al., 1989; Tremblay et al., 1988), 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 EPSCs (Akaike and Rhee, 1997; Bardoni et al., 1998). Recently, due to advances in cloning technology and gene knockout technology, an explosion of studies have char-

Abbreviations: NMDA, N-methyl-D-aspartate; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate; mGluR, metabotropic glutamate receptor; NO, nitric oxide; NOS, nitric oxide synthase; ANOVA, analysis of variance; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[*c*]hepten-5,10-imine maleate, dizocilpine; EPSCs, excitatory postsynaptic currents.

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acterized the molecular structure of the NMDA receptor complex (Dingledine et al., 1999; Ozawa et al., 1998). The accumulating evidence indicates that the various subunits (NR1, NR2A-D, NR3A) composing the native NMDA receptor channel undergo dramatic differential changes during the first few weeks of postnatal life in the rat (Dingledine et al., 1999). It has been suggested that NMDA receptor is not functionally mature until the second postnatal week in the rat (Sircar, 2000). Thus, the pharmacological effects of NMDA antagonist in the infant may not necessarily be comparable to those in the adult. In fact, the acute administration of MK-801 is ineffective in suppressing the expression of withdrawal signs in the 4–7-day-old rat (Bell and Beglan, 1995; Zhu and Barr, 2000), which is in contrast to reports in the adult (Trujillo and Akil, 1991a).

The present study investigated the pharmacological effects of chronic coadministration of MK-801 with morphine on the acquisition of morphine dependence in the 7-, 14-, and 21-day-old preweanling rats. Our hypotheses were that: 1) MK-801 should be ineffective in attenuating the development of morphine dependence in newborn rat; 2) there exists a transition age for MK-801 to inhibit the establishment of morphine dependence; and 3) this transition age should temporally correlate with the maturational status of the native NMDA receptor. Since our previous study (Zhu and Barr, 2000) found that acutely administered MK-801 increased the occurrence of certain withdrawal behaviors (e.g., walking and wall climbing) in the 7-day-old rat, the present study also tested the effects of acutely administered MK-801 on withdrawal behaviors in 7-, 14-, and 21-day-old rats.

MATERIALS AND METHODS

Subjects

All animal procedures were in accordance with the *Principles of Laboratory Animal Care* (NIH, 1996) and approved by the Institutional Animal Care and Use Committee at Hunter College. The subjects were the offspring of Long-Evans hooded rats bred in our laboratory. Parent animals were housed in plastic tubs with wood chips in a colony room maintained at 22–24°C on a 12-h light/dark photocycle with lights on at 7 AM. The breeding colony existed in a separate room with minimal disturbances except for normal cleaning, feeding, and record keeping. Parent animals had Purina Lab Chow (Purina 5012) and water available ad libitum. Cages were checked twice daily, at approximately 10 AM and 6 PM. Pups found at either time were termed 0 days of age. After parturition, litters were culled to eight pups with an equal number of males and females. Pups were tattooed with India ink (Geller and Geller, 1966), which was

TABLE I. Behavioral definitions

Behavior	Definition
Burrow	Sliding the body under the shavings of the observation chamber
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
Together	Bodily contact with one or more littermates
Walking	Taking more than one step forward
Wall climbing	Placing at least two forepaws on the wall of the observation chamber

injected into one or two paws to label permanently individual pups in each litter.

Drug treatment and behavior observation

Morphine sulfate was obtained from the National Institute on Drug Abuse (Rockville, MD). All other chemicals were purchased from Tocris Cookson (Ballwin, MO).

From the first day on, when the rat pups were 1, 8, or 15 days old, all pups in a litter were removed from the dam, individually weighed, and injected with MK-801 (0.03 or 0.10 mg/kg) or saline (chronic pretreatment). Fifteen minutes after the pretreatment, morphine sulfate (10 mg/kg) or saline was injected (for details, refer to Table IV, "Chronic treatment" column). All drugs were delivered intraperitoneally. The treated pups were kept in an incubator maintained at approximately 33°C for about 30 min and then returned to the dam. The same procedure was conducted twice daily (10 AM and 6 PM; for the afternoon injection, body weight used was that of the morning) for 6.5 days. The last injection was in the morning of the seventh day. On 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. The pups were treated with MK-801 (0.10 mg/kg) or saline (acute pretreatment). After the acute pretreatment, the pups were then placed back into the observation chamber with the remainder of the litter (without the dam). Fifteen minutes later, naltrexone (1 mg/kg) was injected to precipitate withdrawal (for details, refer to Table IV, "Acute treatment" column). After the injection of naltrexone the behavior of the pup was identified every 15 sec and recorded on a checklist for 15 min (see Table I for definitions of behavior included in the checklist) by an observer who was blind to the treatment conditions. These behaviors have been shown to characterize the withdrawal repertoire of the infant rat (Jones and Barr, 1995). When the observation for a specific pup ended, the rectal temperature of the pup was measured by a thermometer probe (model: Type BT-1) connected to Physitemp digital thermometer (model: BAT-12; Physitemp Instruments, Clifton,

TABLE II. Baseline activities (mean \pm sem)

	7-day-old			14-day-old			21-day-old		
	Chronic MK-801 (0.03 mg/kg)	Chronic MK-801 (0.1 mg/kg)	MK-801 (0.1 mg/kg) + Acute MK-801	Chronic MK-801 (0.03 mg/kg)	Chronic MK-801 (0.1 mg/kg)	MK-801 (0.1 mg/kg) + Acute MK-801	Chronic MK-801 (0.03 mg/kg)	Chronic MK-801 (0.1 mg/kg)	MK-801 (0.1 mg/kg) + Acute MK-801
Burrow	N/A	N/A	N/A	.00 \pm .00	.00 \pm .00	.00 \pm .00	.00 \pm .00	.00 \pm .00	.00 \pm .00
Head moves	1.75 \pm .37	1.13 \pm .44	.63 \pm .26	.25 \pm .16	.00 \pm .00	.00 \pm .00	2.13 \pm .99	1.13 \pm .52	2.75 \pm 1.21
Moving paws	1.88 \pm .69	2.13 \pm .64	3.63 \pm 1.87	.25 \pm .16	.13 \pm .13	.00 \pm .00	N/A	N/A	N/A
Quiet	55.88 \pm 1.17	56.50 \pm .87	54.63 \pm 1.85	58.75 \pm .84	59.00 \pm .38	59.13 \pm .48	55.13 \pm 1.84	55.88 \pm 2.43	50.88 \pm 4.75
Rolling	.50 \pm .27	.00 \pm .00	.38 \pm .38	N/A	N/A	N/A	N/A	N/A	N/A
Together	.50 \pm .27	.50 \pm .27	.50 \pm .27	.00 \pm .00	.25 \pm .16	.13 \pm .13	.88 \pm .48	1.50 \pm .87	2.50 \pm 1.39
Walking	.00 \pm .00	.00 \pm .00	.00 \pm .00	.00 \pm .00	.00 \pm .00	.00 \pm .00	.38 \pm .26	.88 \pm .61	2.25 \pm 1.37
Wall climbing	.00 \pm .00	.00 \pm .00	.00 \pm .00	.13 \pm .13	.13 \pm .13	.25 \pm .25	.63 \pm .38	.13 \pm .13	.38 \pm .26

The data are from the MK-801-treated rats that did not receive chronic morphine treatment but received a single dose of naltrexone (1 mg/kg) challenge on the test day. Levels of behavior were low throughout and no significant differences were found among any treatment conditions. N/A: this behavior was not displayed.

NJ). The pup was anesthetized and placed back into the litter to keep the litter size unchanged. The next pup was tested until all treatment groups were completed. Animals were sacrificed with sodium pentobarbital at the conclusion of the experiments. Eight pups from each litter ($n = 8$ for each age) were used. The number of males and females in any treatment condition was balanced. The order of treatment conditions was assigned randomly within each experiment to minimize any potential order effect (Latin-square design).

Data analysis and statistics

Withdrawal behaviors

Separate statistical tests were conducted for the three age groups and for each of the withdrawal behaviors. Occurrences of each behavior were summed for the 15-min postnaltrexone observation period. All drugs were injected within a single litter and thus treatment effect was treated as a within-subjects variable. Data were first explored by orthogonal pair-wise comparisons to compare among the chronic morphine-treated group, the chronic MK-801 coadministration groups, and the chronic plus acute MK-801 coadministration groups. A one-way ANOVA was then conducted for the occurrence of the specific withdrawal behaviors. If significant results were detected, individual multiple comparisons were then conducted by Dunnett's two-tailed post-hoc test to compare each of the MK-801 pretreated groups that received chronic morphine treatment with the control group, which received chronic morphine treatment only. Since rats that did not receive chronic morphine treatment remained largely quiet and displayed only some baseline activities, to avoid confounding the statistical tests the data for these groups were analyzed separately from the data for those groups receiving chronic morphine.

Rectal temperature

For all three ages the rectal temperature scores of the rats that received MK-801 treatment but not morphine (a total of three groups, see Table IV for details)

were not significantly different from each other and they were combined as the control group. A one-way ANOVA was conducted for the five different combinations of chronic and acute coadministration of MK-801 with morphine and the control group. Fisher's PLSD were used for subsequent analysis. All values are expressed as mean \pm SEM.

Body weight

For all three ages the body weight scores of the rats that received MK-801 treatment but not morphine (a total of three groups, see Table IV for details) were combined as a control group. A separate one-way ANOVA was conducted for each day (a total of 7 days) for each of the three chronic treatment conditions. Fisher's PLSD were used. All values are expressed as mean \pm SEM.

RESULTS

Withdrawal behaviors

Chronic MK-801 but no morphine

Within each age cohort there were three groups that did not receive morphine treatment but received different MK-801 treatments. One group was treated with a chronic low dose of MK-801 (0.03 mg/kg); a second group received a chronic high dose of MK-801 (0.10 mg/kg); the third group was treated with a chronic high dose of MK-801 (0.10 mg/kg) plus an acute injection of MK-801 (0.10 mg/kg) 15 min before naltrexone challenge. For all ages, rats that received chronic MK-801 injections but were not treated with morphine remained largely quiet and displayed only some baseline activities (Table II). Within each age cohort, no statistically significant results were found among the three groups that did not receive any morphine treatment but did receive different MK-801 treatments. Thus, the occurrences for each of the behaviors for these three groups were averaged and are presented in Figures 1–3 as baselines (dashed lines horizontal to the abscissas).

Chronic MK-801 on the development of morphine dependence

Chronic coadministration of MK-801 had different pharmacological profiles on naltrexone-precipitated morphine withdrawal at the three ages tested. In the 7-day-old rat, compared with a control group that received chronic morphine treatment only, chronic coadministration of either dose of MK-801 with morphine failed to change significantly the frequency of most of the withdrawal behaviors (Fig. 1). These included head moves, moving paws, rolling, together, and wall climbing. On the other hand, both the low dose and the high dose of chronic coadministration of MK-801 with morphine significantly decreased quiet behavior, and the high dose of MK-801 significantly increased walking behavior. Therefore, as a whole MK-801 was ineffective in inhibiting the development of morphine dependence in the 7-day-old rat and exacerbated some of the withdrawal signs (e.g., increased walking and decreased quiet).

In the 14-day-old rat, chronic coadministration of the high dose of MK-801 (0.10 mg/kg) with morphine resulted in a decrease in head moves and moving paws and a significant increase in the frequency of quiet behavior (Fig. 2). No significant results were found for burrowing, together, walking, and wall climbing. The low dose of MK-801 (0.03 mg/kg) did not have a significant effect on any of the observed behaviors. Thus, overall, coadministration of MK-801 attenuated morphine withdrawal in the 14-day-old rat, but significant results were found for a limited number of withdrawal behaviors and for the high dose of MK-801 (0.10 mg/kg) only.

In the 21-day-old rat, both doses of MK-801 significantly decreased head moves and burrowing (Fig. 3). In addition, the high dose of MK-801 decreased walking behavior. Both doses of MK-801 also increased quiet. Occurrences of together and wall climbing behaviors were not significantly altered by either dose of MK-801. Thus, chronic coadministration of MK-801 suppressed most of the withdrawal signs, even at the low dose (0.03 mg/kg), although significant results were not reached for all withdrawal behaviors. Table III summarizes the effects of chronic coadministration of MK-801 with morphine compared to the chronic morphine-treated alone group on morphine withdrawal.

Acute MK-801 on the expression of morphine withdrawal

The effects of acute MK-801 prior to naltrexone challenge had different effects in different-aged animals. In the 7-day-old rat, acute MK-801 significantly increased rolling, walking, and wall climbing, as reported previously (Zhu and Barr, 2000). In the 14- and 21-day-old rat, compared with the chronic morphine-only control group, chronic MK-801 and morphine plus acute MK-

801 treatment resulted in less withdrawal in general. In the 14-day-old rat, although chronic coadministration of the low dose of MK-801 (0.03 mg/kg) failed to significantly increase quiet, the same treatment combined with an acute MK-801 (0.10 mg/kg) 15 min prior to naltrexone challenge did increase quiet significantly. In contrast, in the 21-day-old rat coadministration of the low dose of MK-801 (0.03 mg/kg) significantly increased quiet, yet the same treatment combined with an acute injection of MK-801 (0.10 mg/kg) decreased the occurrences of quiet and rendered the score not significantly different from the control group. The effects of chronic coadministration of two different doses of MK-801 with morphine and then one dose of acute MK-801 prior to naltrexone challenge on the various withdrawal behaviors for the 7-, 14-, and 21-day-old rat are shown in Figures 1, 2, and 3, respectively. In addition, the general patterns of chronic plus acute MK-801 on the development of morphine dependence compared with both morphine control group and the chronic MK-801 alone groups are summarized in Table III.

Rectal temperature

No significant differences in rectal temperature scores were found among any treatment conditions either in the 7-day-old, $F(5,58) = 2.33, P > 0.05$, or the 14-day-old rat, $F(5,58) = 1.99, P > 0.05$. In the 21-day-old rat, however, there were significant differences among the five groups that received different combinations of chronic and acute coadministration of MK-801 with morphine treatment and the groups that received MK-801 only, $F(5,58) = 5.66, P < 0.001$. Post-hoc tests revealed that although the rectal temperature scores of the five treatment groups that received both MK-801 and morphine treatment were not significantly different from each other, they were significantly lower than those of the control group, which did not receive morphine treatment (Table IV).

Body weight

Chronic morphine treatment inhibited the weight gain at all ages. Chronic coadministration of MK-801 did not ameliorate morphine's reduction of body weight gain. The effects of various treatments on body weight gain in the preweaning rat are depicted in Figure 4.

DISCUSSION

We previously reported that acutely administered MK-801 was ineffective in suppressing the expression of morphine withdrawal signs in the 7-day-old rat (Zhu and Barr, 2000). In the present study, we examined the pharmacological effects of MK-801 on the development of morphine dependence at different ages. Our results clearly show that coadministration of MK-801 did not inhibit the acquisition of morphine dependence in the

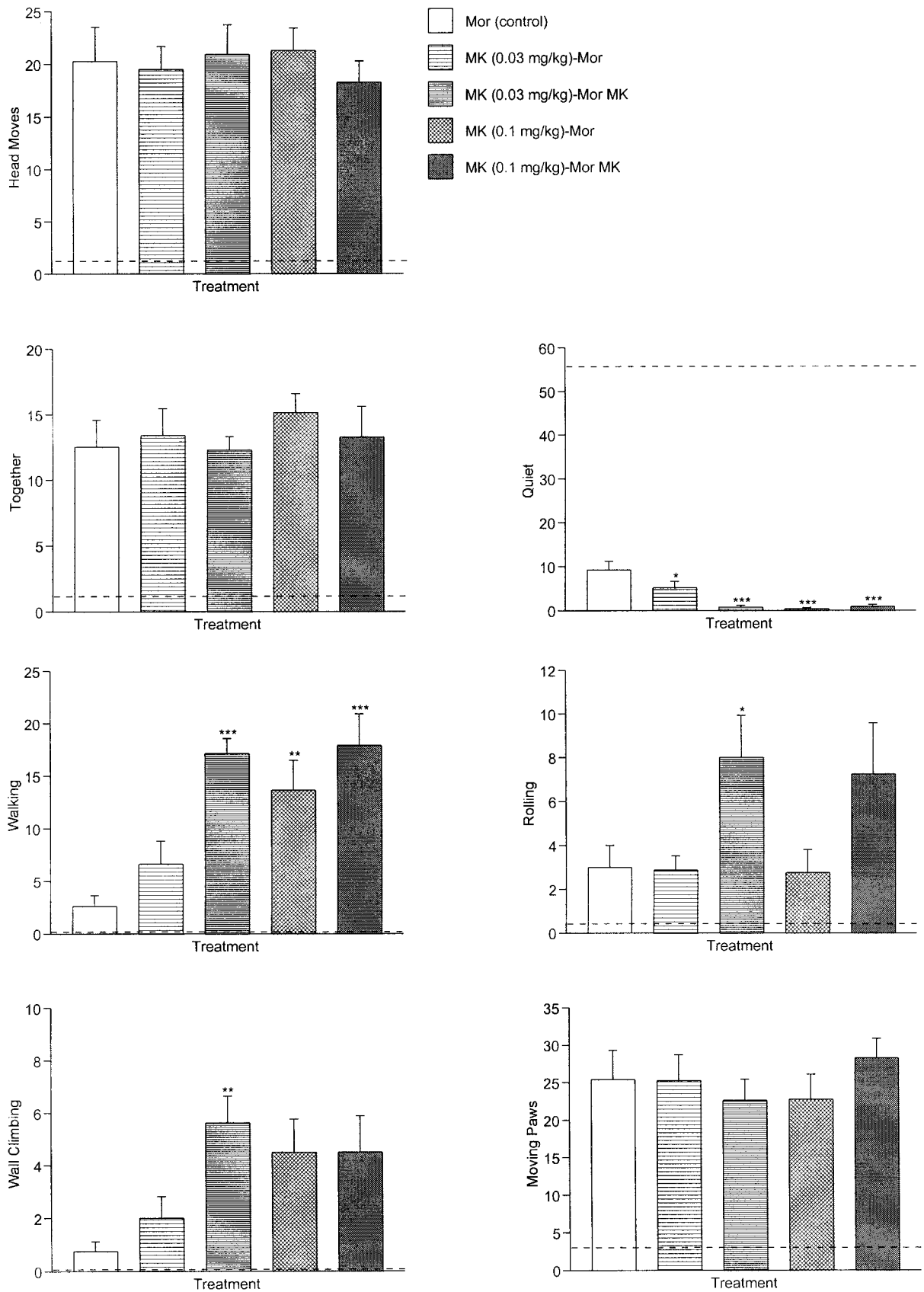


Fig. 1. The effects of chronic and acute MK-801 on naltrexone-precipitated morphine withdrawal in the 7-day-old rat. Ordinate: mean occurrences (mean \pm SEM) in 15 min of opiate withdrawal behaviors (definitions, see Table I). Abscissa: treatment conditions. All pups received chronic morphine treatment and the experimental groups also received chronic and/or acute MK-801 from postnatal days

1-7. Withdrawal was precipitated by naltrexone in all groups. Dashed line horizontal to the abscissa represents baseline activity of rats that did not receive morphine. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to control group that received chronic morphine treatment only.

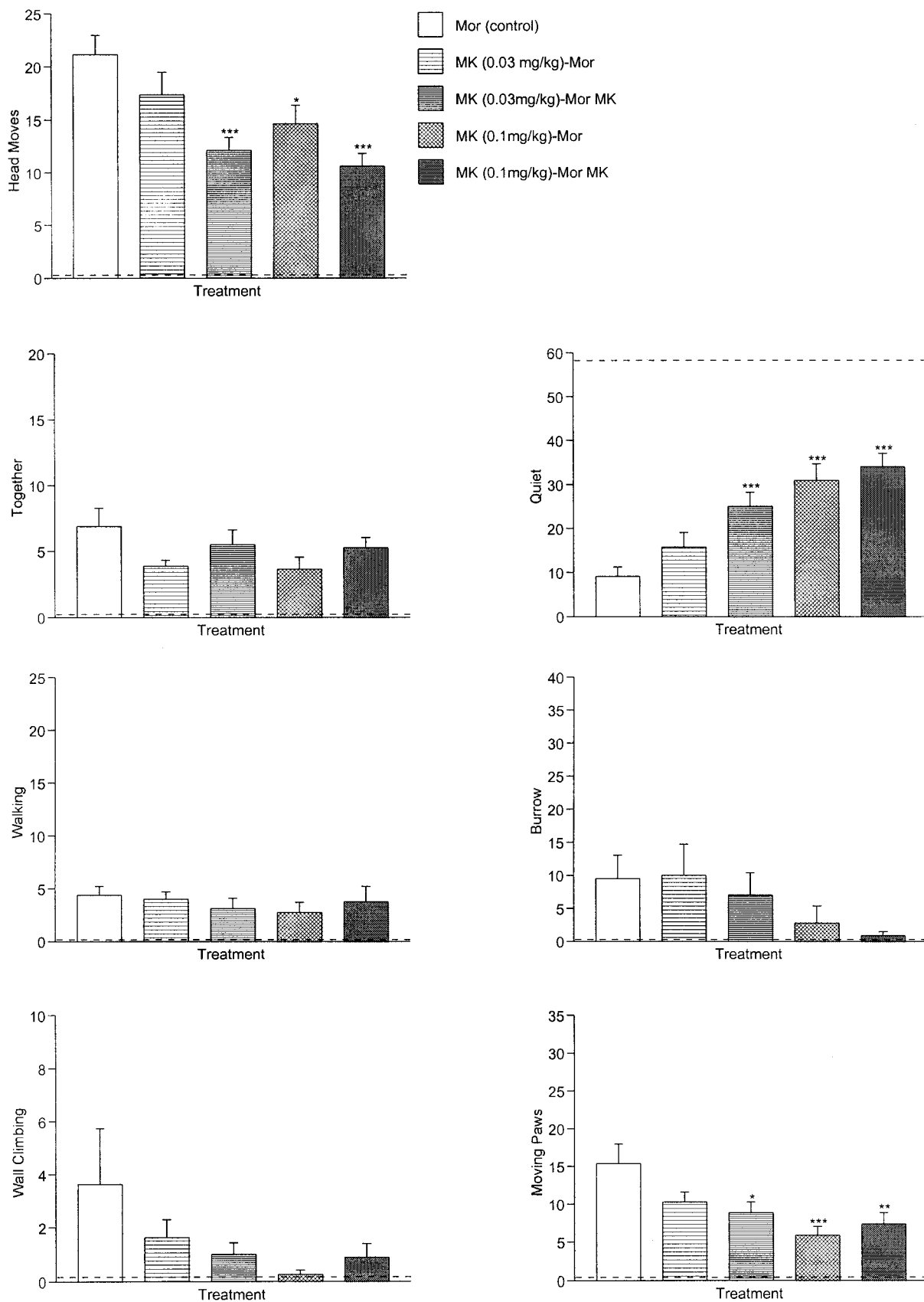


Fig. 2. The effects of chronic and acute MK-801 on naltrexone-precipitated morphine withdrawal in the 14-day-old rat. All pups received chronic morphine treatment and the experimental groups

also received chronic and/or acute MK-801 from postnatal days 8–14. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to control group that received chronic morphine treatment only. See Figure 1 for details.

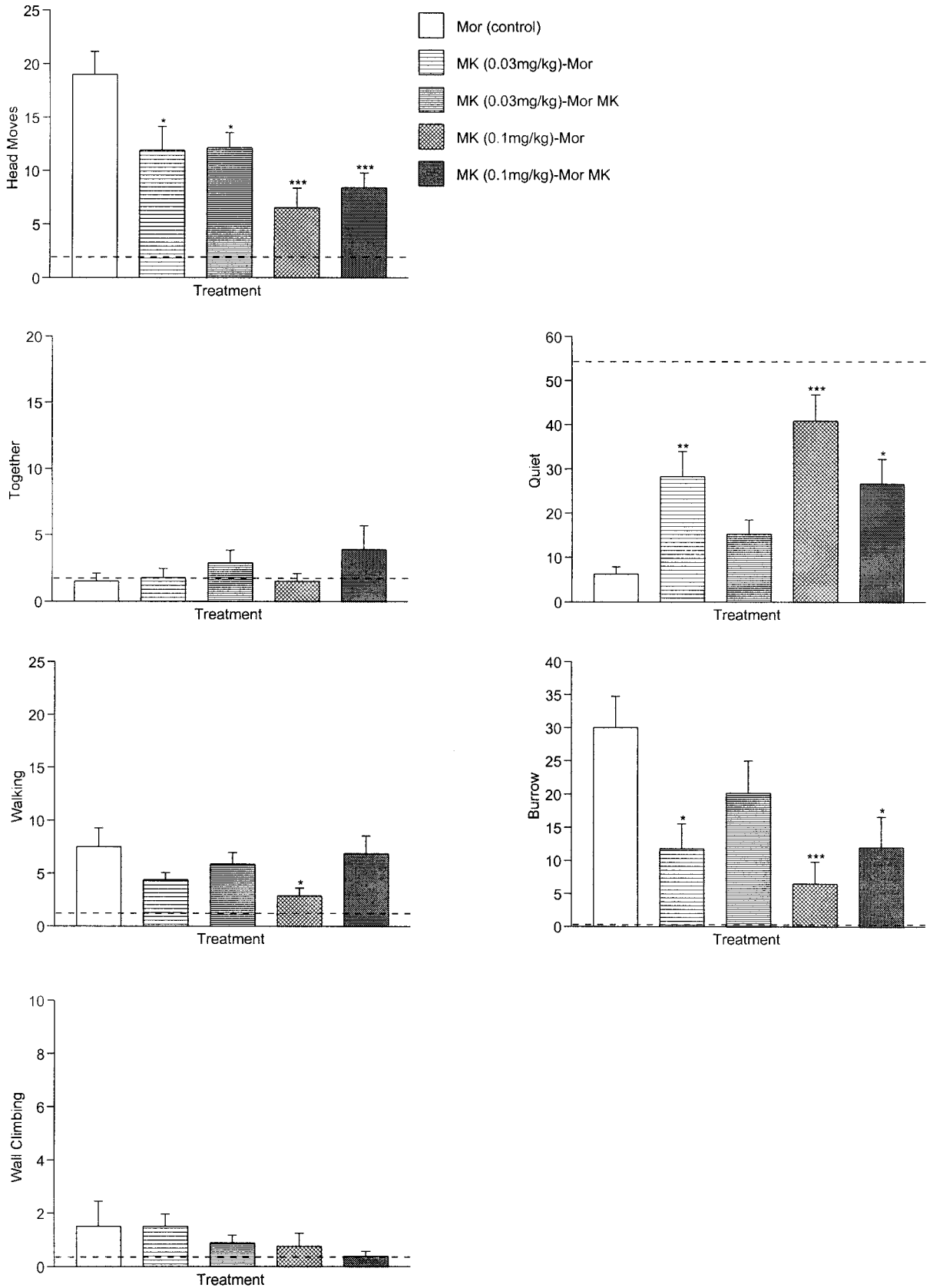


Fig. 3. The effects of chronic and acute MK-801 on naltrexone-precipitated morphine withdrawal in the 21-day-old rat. All pups received chronic morphine treatment and the experimental groups

also received chronic and/or acute MK-801 from postnatal days 15–21. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to control group that received chronic morphine treatment only. See Figure 1 for details.

TABLE III. Summary of the orthogonal pair-wise comparisons

	7-day-old			14-day-old			21-day-old		
	Chronic MK + Mor. vs. Chronic Mor.	Chronic MK + Mor. + acute MK vs. Chronic Mor.	Chronic MK + Mor. + acute MK vs. Chronic MK + Mor.	Chronic MK + Mor. vs. Chronic Mor.	Chronic MK + Mor. + acute MK vs. Chronic Mor.	Chronic MK + Mor. + acute MK vs. Chronic MK + Mor.	Chronic MK + Mor. vs. Chronic Mor.	Chronic MK + Mor. + acute MK vs. Chronic Mor.	Chronic MK + Mor. + acute MK vs. Chronic MK + Mor.
Burrow	N/A	N/A	N/A	↔	↔	↔	↓***	↓**	↔
Head moves	↔	↔	↔	↓*	↓***	↔	↓***	↓***	↔
Moving paws	↔	↔	↔	↓***	↓***	↔	N/A	N/A	N/A
Quiet	↓***	↓***	↓*	↓***	↓***	↑*	↑***	↑**	↓**
Rolling	↔	↑**	↑*	N/A	N/A	N/A	N/A	N/A	N/A
Together	↔	↔	↔	↓*	↔	↔	↔	↔	↔
Walking	↑**	↑***	↑***	↔	↔	↔	↓*	↔	↑*
Wall climbing	↑*	↑**	↔	↓*	↓*	↔	↔	↔	↔

Chronic MK + Mor. vs. Chronic Mor.: chronic coadministration of MK-801 with morphine vs. chronic morphine alone. Chronic MK + Mor. + acute MK vs. Chronic Mor.: chronic coadministration of MK-801 with morphine and acute MK-801 (0.10 mg/kg) before naltrexone challenge vs. chronic morphine alone. Chronic MK + Mor. + acute MK vs. Chronic MK + Mor.: vs. chronic coadministration of MK-801 with morphine and acute MK-801 (0.10 mg/kg) before naltrexone challenge; chronic coadministration of MK-801 with morphine.

N/A This behavior was not displayed.
 ↔ No significant differences found, $P > 0.05$.
 ↑* Significant increase, $P < 0.05$.
 ↑** Significant increase, $P < .01$.
 ↑*** Significant increase, $P < .001$.
 ↓* Significant decrease, $P < .05$.
 ↓** Significant decrease, $P < .01$.
 ↓*** Significant decrease, $P < .001$.

TABLE IV. Rectal temperature 15 min after naltrexone challenge (mean ± sem)

Grouping	Treatment conditions		Age of rat		
	Chronic treatment	Acute treatment	7-day-old	14-day-old	21-day-old
Morphine treated groups	Saline + morphine	Saline + naltrexone	36.40 ± .18	37.76 ± .15	37.56 ± .39***
	MK-801 (0.03 mg/kg) + morphine	Saline + naltrexone	36.41 ± .18	38.16 ± .11	37.89 ± .44**
	MK-801 (0.03 mg/kg) + morphine	MK-801 (0.10 mg/kg) + naltrexone	36.45 ± .26	38.01 ± .16	37.84 ± .25**
	MK-801 (0.10 mg/kg) + morphine	Saline + naltrexone	36.21 ± .19	37.88 ± .11	37.76 ± .40**
	MK-801 (0.10 mg/kg) + morphine	MK-801 (0.10 mg/kg) + naltrexone	36.31 ± .17	37.86 ± .15	37.98 ± .25**
Morphine untreated groups	MK-801 (0.03 mg/kg) + saline	Saline + naltrexone	36.76 ± .20	38.19 ± .26	38.53 ± .15
	MK-801 (0.10 mg/kg) + saline	Saline + naltrexone	36.79 ± .14	38.21 ± .22	39.15 ± .23
	MK-801 (0.10 mg/kg) + saline	MK-801 (0.10 mg/kg) + naltrexone	36.83 ± .15	38.41 ± .21	39.25 ± .17

Each cell entry represents the mean of eight rectal temperature scores. The scores of the three morphine untreated groups were not significantly different from each other and were combined as the control group.
 ** $P < 0.01$; *** $P < 0.001$.

7-day-old rat, but that MK-801 was effective in both the 14- and 21-day-old rats. We did not test older rats since the effectiveness of MK-801 in the adult is well documented. At the same time, as shown in the present study, the effects of chronic MK-801 in the 14- and 21-day-old rats were quite comparable, suggesting that the underlying mechanisms by which MK-801 reduces dependence are already stable at these ages. Our data thus suggest that there is a transition period for MK-801 in attenuating the development of morphine dependence in the rat and this period seems to be somewhere during the second postnatal week. Although age-related differences in pharmacokinetics and blood-brain barrier development may contribute to the differences in withdrawal behaviors seen for different ages, these factors are unlikely to contribute to the differences seen here (Thornton et al., 1998). Most importantly, the timing of the effectiveness of MK-801 in inhibiting the acquisition of morphine dependence coincides well with the maturation of the NMDA receptor

(Sircar, 2000). Therefore, it is possible that the different pharmacological properties of MK-801 in different-aged rats may reflect the different developmental stages of the NMDA receptor.

The NMDA receptor is composed of various subunits (NR1, NR2A-D, and NR3A) (Dingledine et al., 1999). All native NMDA receptors have at least one NR1 subunit but functional channels are formed only when NR1 is expressed together with one of the four NR2 subunits (Yamakura and Shimoji, 1999). The NR3A subunit may serve a regulatory function in NMDA receptors (Das et al., 1998). The various subunits of the NMDA receptor complex undergo dramatic differential maturation during the first few postnatal weeks (Guilarte and McGlothlan, 1998), resulting in considerably different functional states (Colwell et al., 1998). In the neonatal brain the predominant NMDA receptor subunits are the NR1, NR2B, and NR2D subunits (Goebel and Pooch, 1999). Expression of the NR1-NR2B subunit complex is high at birth, peaks at about day 14,

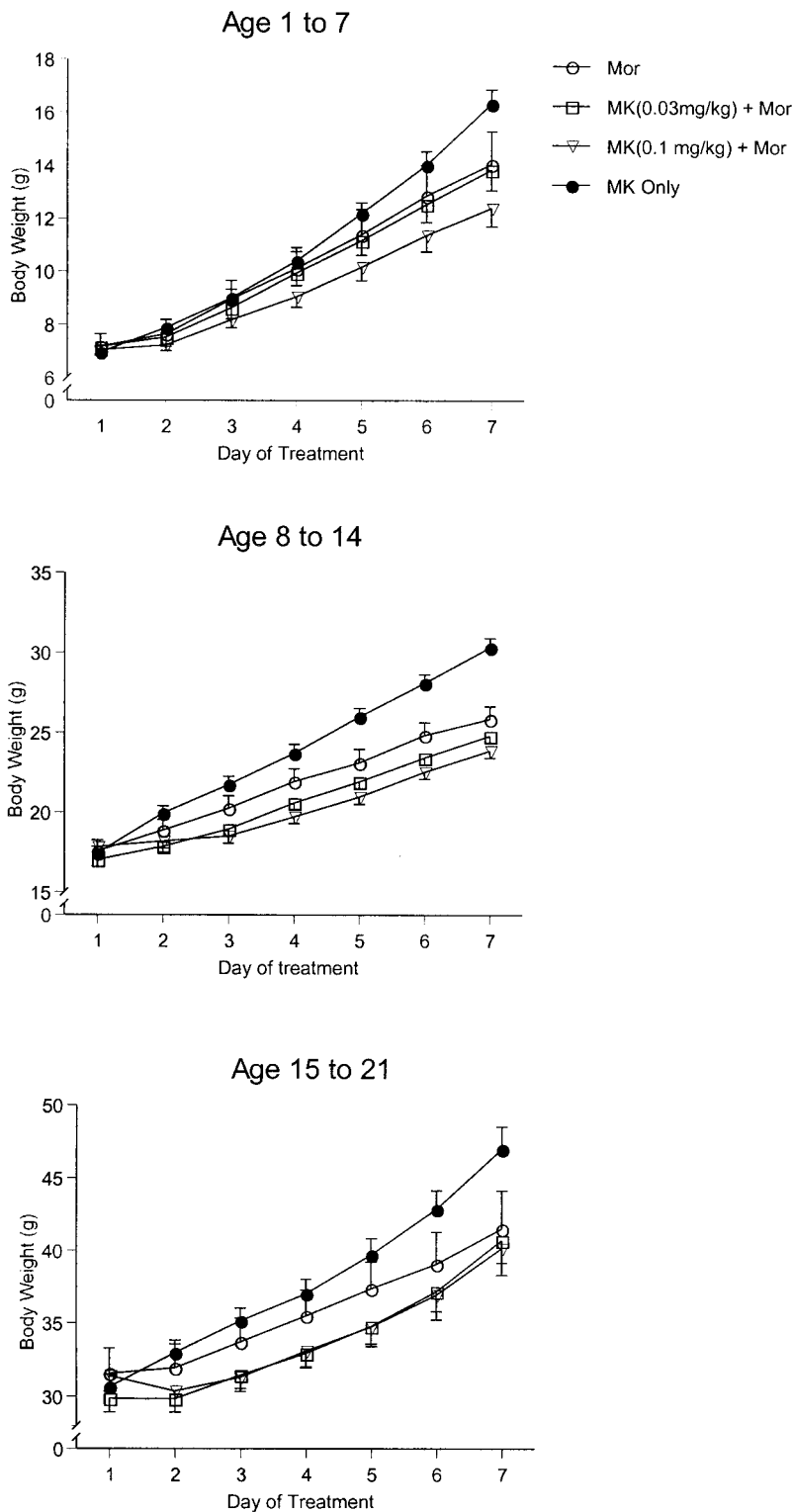


Fig. 4. The effects of chronic coadministration of MK-801 with morphine on body weight in the 7-, 14-, and 21-day-old rat. Ordinate: body weight (mean \pm SEM). Units: grams. Note ordinate scale changes for different aged rats. Abscissa: treatment time. Units: days.

and then declines after day 21. The NMDA receptor containing the NR2A subunit is almost nonexistent at birth, increases gradually with age, and reaches maximal level on day 21. Expression of the NR1 subunit

also increases with age and peaks on day 21 (Guilarte and McGlothlan, 1998; Sircar, 2000). It has recently been suggested that there are two types of NMDA receptors in the rat—an immature type and a mature

type—and that there exists a temporal switching of the NMDA receptor from the immature form to the adult mature form around the second postnatal week (Sircar, 2000). Thus, the pharmacological properties of the immature and mature NMDA receptor correlate well with the developmental profile of the NMDA receptor subunit configuration (Ozawa et al., 1998; Sircar, 2000). MK-801 was effective at 7 days of age—it augmented some withdrawal behaviors. This could be due to the blockade of the immature form of the NMDA receptor, or to nonspecific effects of MK-801 on other receptor types. It is important to note that as a noncompetitive NMDA receptor antagonist, MK-801 potently binds to the immature NMDA receptor (Sircar, 2000) and effectively blocks the ion-permeable channel (Dingledine et al., 1999; Ozawa et al., 1998; Yamakura and Shimoji, 1999). Thus, the ineffectiveness of MK-801 in inhibiting the development of morphine dependence in the 7-day-old rat is not a failure to block the immature channel, but rather is likely due to the lack of activity of that receptor in opiate withdrawal.

Compared with the clear transition of the pharmacological effects of MK-801 dependent on the age of the rat, the effects of acute MK-801 are more complicated. Like the effects of chronic MK-801, the effects of acute MK-801 on withdrawal are also age-dependent. For example, in the 7-day-old acute MK-801 decreased quiet behavior whereas it increased quiet in the 14-day-old. But surprisingly, acute MK-801 then decreased quiet behavior in the 21-day-old, suggesting that the acute effect of MK-801 on various morphine withdrawal in the preweaning rats is not a simple linear function of the developmental status of the NMDA receptor.

Our results do not support, at least in the neonatal rat, the widely cited Mao, Price, and Mayer model of opiate tolerance and dependence (Mao, 1999; Mao et al., 1995; Mayer and Mao, 1999). This model places the NMDA receptor at an indispensable position for the acquisition of opiate tolerance and dependence (Fundytus and Coderre, 1999; Trujillo, 1999). On the one hand, the role of the NMDA receptor in the establishment of opiate dependence in adult rodents has been firmly supported (Mao, 1999; Trujillo, 1999) and our data for the 14- and 21-day-old rats also support the role of the NMDA receptor in rats of these ages. But the model may need to be expanded to take into consideration the fact that the infant acquires and expresses withdrawal (Jones and Barr, 1995; Thornton et al., 1997; Windh et al., 1995) in the absence of functionally mature NMDA receptors.

One of the crucial assumptions in this model is that the activation of NMDA receptor results in Ca^{2+} influx into the cell and activates the second messenger, thus leading to long-lasting plasticity changes cascade (Mao, 1999; Trujillo, 1999). Yet the immature NMDA recep-

tor seems to have low Ca^{2+} permeability (Barrios and Liljequist, 1996; Didier et al., 1994; Frandsen et al., 1989; Frandsen and Schousboe, 1990; Goebel and Poosch, 1999; Resink et al., 1992). Thus, it seems that the immature NMDA receptor is not a vital element in the development of opiate dependence in rats of this age. We previously reported that, like in the adult, NOS inhibitors were very effective in depressing withdrawal signs in the 7-day-old rat (Zhu and Barr, 2000). Therefore, it seems that in the 7-day-old rat the establishment of opiate dependence relies on the same second-messenger system as in the adult. The difference between the infant and adult may lie somewhere more “upstream.” It is possible that other mechanisms may activate the Ca^{2+} -dependent second-messenger system such as NO production. If so, the essence of the Mao et al. model, which focuses on the role of the second-messenger system, including the influx of Ca^{2+} into the cell and the production of NO, may still hold in the infant. But in the infant there may exist some other mechanisms fulfilling the role of coupling to the activation of Ca^{2+} -dependent second-messenger system and at a later developmental time either confer this role to the NMDA receptor or play an adjunct role to the NMDA-mediated mechanism.

One of these mechanisms may be mediated by the metabotropic glutamate receptors (mGluRs), which are coupled to various second-messenger systems (Ozawa et al., 1998). Recently, it has been suggested that, in addition to NMDA receptors, mGluRs may be involved in the development and expression of opiate dependence in the adult rat and mouse (Fundytus and Coderre, 1997, 1999; Fundytus et al., 1997; Klodzinska et al., 1999; Vandergriff and Rasmussen, 1999). Therefore, it is possible that in the infant rat mGluRs play a more important role than the NMDA receptor and that these parallel mechanisms may coexist even in the adult, although NMDA receptor's importance increases after the early transition period.

The AMPA receptor may also activate the Ca^{2+} -dependent second-messenger systems in neural circuits involved in opiate dependence in the newborn rat. Depending on the region in the CNS, the immature AMPA receptor has high Ca^{2+} permeability (Ozawa et al., 1998) and activation of AMPA receptor produces a marked increase in cytoplasmic free Ca^{2+} (Ozawa et al., 1998). The role of Ca^{2+} entry through Ca^{2+} -permeable AMPA receptor has been hypothesized to activate downstream Ca^{2+} -dependent intracellular events (Jakowec et al., 1995a, 1995b; Ozawa et al., 1998), such as NO production (Kest et al., 1997), and there is both *in vitro* (Marin et al., 1993) and *in vivo* (Fedele and Raiteri, 1999) evidence the AMPA receptor is linked to the production of NO. Thus, it is possible that AMPA receptor is involved in the development of morphine dependence in the younger rat.

Lastly, care must be taken when applying these developmental changes in the receptor functional and molecular properties to any model on opiate tolerance, dependence, and withdrawal. The functional levels of expression of various subunits and their specific combinations vary markedly in different regions of CNS and the anatomical locus for opiate actions is multiple. Therefore, simple model systems to combine behavioral, electrophysiological, and molecular studies may be useful to elucidate the underlying mechanisms of opiate tolerance, dependence, and withdrawal.

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