

Research Report

**Inhibition of opiate tolerance by non-competitive *N*-methyl-D-aspartate receptor antagonists \*\***

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**Abstract**

Our laboratory and others have previously reported that the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, interferes with the development of tolerance to the analgesic effects of morphine. The present studies were performed in order to further characterize the role of NMDA receptors in opiate tolerance. The results demonstrate that opiate tolerance is inhibited rapidly, and at low doses, by four different non-competitive NMDA receptor antagonists (MK-801, ketamine, dextrorphan and phencyclidine), suggesting that this inhibition results from blockade of NMDA receptors rather than from the 'side-effect' of a particular drug. The NMDA antagonists were found to inhibit the development but not the expression of opiate tolerance; i.e. they were able to prevent but not reverse tolerance. Finally, the results suggest that NMDA receptor antagonists do not interfere with associative tolerance; instead it appears that these drugs may specifically inhibit non-associative tolerance. It thus appears that NMDA receptors may have a fundamental role in the development of opiate tolerance, and that non-competitive NMDA receptor antagonists may be effective adjuncts to opiates in the treatment of chronic pain.

*Key words:* Morphine; Analgesia; *N*-Methyl-D-aspartate receptor; Opiate tolerance; MK-801; Dizocilpine; Phencyclidine; Dextrorphan; Ketamine; Neuroplasticity

**1. Introduction**

Opiate tolerance and physical dependence are significant problems, both in the field of pain management and in relation to drug abuse. Tolerance is a decrease in the effect of a drug with repeated use. The clinical consequence of opiate tolerance is the need to increase the dose of the drug during chronic administration in order to maintain the desired effect. Physical dependence is a change in functioning following repeated use, whereby further drug administration is necessary to avoid physiological disturbance. The clinical consequence of physical dependence is that users will undergo an unpleasant withdrawal syndrome if opiate administration is rapidly terminated. Drug tolerance and dependence represent experience-dependent

changes in the brain and behavior, and are therefore very good examples of neural and behavioral plasticity.

Excitatory amino acid systems have been found to have an important role in neural and behavioral plasticity. In particular, *N*-methyl-D-aspartate (NMDA) receptors have been reported to be involved in neuronal development, kindling, long-term potentiation and learning [8,9,49]. Our laboratory [41,42,44] and others [4,28,29,39] have reported that the non-competitive NMDA receptor antagonist MK-801 (also known as dizocilpine) inhibits the development of tolerance to the analgesic effects of morphine without affecting pain responsiveness by itself, and without affecting the acute analgesic actions of morphine. MK-801 also interferes with the development of physical dependence on morphine [4,41,42,44]. These results suggest that, like other forms of neural and behavioral plasticity, opiate tolerance and dependence may require activation of NMDA receptors.

The purpose of the present studies was to further examine the potential role of NMDA receptors in

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\*\* Preliminary reports of this work have been presented [45–47].

opiate tolerance. The first aim of these experiments was to determine if the ability to inhibit opiate tolerance was a property specific to MK-801, or if other non-competitive NMDA receptor antagonists would produce similar effects. Although MK-801 is a potent and selective NMDA receptor antagonist, there are reports that this drug can produce effects on other systems [5,6]. It is thus possible that the ability of this drug to inhibit opiate tolerance and dependence results from actions other than NMDA receptor blockade. In order to definitively establish that NMDA receptors are involved in opiate tolerance and dependence, it is necessary to demonstrate that NMDA receptor antagonists other than MK-801 will inhibit these phenomena. In the present experiments we therefore examined the effects of four different non-competitive NMDA receptor antagonists, MK-801, phencyclidine, ketamine and dextrorphan, on the development of tolerance to the analgesic actions of morphine. Each of these drugs antagonize NMDA receptor-mediated actions by blocking the NMDA receptor ion channel [8,9,16,27,30,49].

The second aim of our studies was to examine whether non-competitive NMDA receptor antagonists inhibit the development or the expression of opiate tolerance. A specific role for NMDA receptors in the neural and behavioral plasticity resulting from chronic opiate exposure requires that NMDA antagonists inhibit the development, but not the expression of tolerance. In support of such a role, previous findings from our laboratory suggest that the non-competitive NMDA receptor antagonist MK-801 primarily affects the development of opiate tolerance [41,42,44,45]. In other words, MK-801 prevented the acquisition of tolerance, but did not reverse tolerance after it was established. Recent evidence from others, however, suggests that blockade of NMDA receptors may indeed 'reverse' tolerance under certain conditions [39]. We therefore felt it important to reexamine the effects of NMDA receptor antagonists on the development and the expression of opiate tolerance.

The final aim of the present studies was to determine if NMDA receptor antagonists inhibit opiate tolerance by interfering with associative processes or if these drugs have the ability to inhibit the more fundamental, non-associative mechanisms involved in tolerance. Two types of drug tolerance have been distinguished: associative (or behavioral) tolerance, in which the learning of associations between drug effects and environmental stimuli is key, appears to result from learning processes such as classical conditioning or habituation. Non-associative (or pharmacological) tolerance does not require that associations be made, but instead appears to involve more direct physiological changes in response to chronic pharmacological challenge [3,12,17,32,34,37]. Since NMDA receptor antago-

nists have been found to interfere with learning, it is possible that these drugs inhibit tolerance and dependence by affecting associative processes [42,44]. Alternatively, NMDA receptor antagonists may act more directly, by interfering with the primary adaptive changes that occur in response to the chronic drug treatment [42,44].

## 2. Materials and methods

### 2.1. General methods

Adult, male Sprague–Dawley rats (5–7 per group) were used in all experiments. Rats were group-housed in stainless steel cages, on a 12 h light/dark cycle. Food and water was available ad lib. Morphine sulfate (Mallinckrodt) was purchased from the University of Michigan Hospital Pharmacy, morphine and placebo pellets were obtained from NIDA, and (+)-MK-801 hydrogen maleate, ketamine hydrochloride, phencyclidine hydrochloride and dextrorphan D-tartrate were purchased from Research Biochemicals Inc. For repeated injection studies, drugs were dissolved in 0.9% saline and administered in a volume of 1 ml/kg. For continuous infusion studies, morphine or placebo was administered with pellets, and the NMDA antagonists were dissolved in 0.9% saline and administered with model 2001 or 2002 Alzet osmotic minipumps (Alza Co.).

Pain responsiveness was assessed by the tail-flick test [10], as previously described [42]. Briefly, a heat lamp was focused on the tail, between 2 and 8 cm from the tip, and the latency for the animal to remove its tail from the heat was assessed by a photocell-triggered timer. Two to three successive determinations were made for each rat, and the mean of these scores was used as the tail-flick latency for that animal. A 10-s cutoff was used to minimize tissue damage from the heat lamp.

### 2.2. Experiment 1: effect of phencyclidine on the development of morphine tolerance

This experiment was an initial attempt to determine whether an NMDA receptor antagonist other than MK-801 would affect the development of morphine tolerance. Methods were similar to those used previously [42]. Briefly, animals were taken to the experimental room each morning for 9 days, and after at least 15 min of habituation, were weighed. Animals then received an injection of saline, MK-801 (0.1 mg/kg i.p.) or phencyclidine (1.0 mg/kg i.p.), followed 30 min later by saline or morphine (10 mg/kg s.c.). Tail-flick latencies were determined 60 min following the second injection on odd-numbered days. Baseline tail-flick latencies were determined on day 1 prior to injections. On day 10 of the experiment, the first injection was eliminated; animals received morphine (10 mg/kg s.c.) alone, followed 60 min later by the tail-flick test. This strategy has been used previously to demonstrate that MK-801 affects the development, rather than the expression of opiate tolerance [41,42].

### 2.3. Experiment 2: effect of MK-801 on associative and non-associative morphine tolerance produced by repeated injections

In this experiment, drug injections were administered in an experimental room, in the presence of specific cues, to facilitate associations between the drug effects and environmental cues, or in the colony, in the absence of distinct cues, to minimize any such associations. In previous studies in which we examined the effect of MK-801 on morphine tolerance, a relatively high dose of morphine and a relatively short interdose interval was used (10 mg/kg, twice

daily) [41,42]. In the present study, in order to maximize the distinction between associative and non-associative tolerance [11,12,37,38], we lowered the dose to 3.0 mg/kg and increased the interdose interval to 24 h.

Each morning, one group of animals (the associative group) was taken to the experimental room in plastic cages, weighed, and injected with saline or MK-801 (0.1 mg/kg i.p.), followed 30 min later by morphine. This group remained in the experimental room for at least 90 min following the morphine injection. White noise masked outside sounds, and acted as an additional discriminative stimulus. Each morning the other group of animals (the non-associative group) was weighed in the colony, and injected with saline or MK-801, followed 30 min later by morphine. These animals were returned to their home cages immediately following injections. To promote explicit drug-environment pairing, each evening associative animals received administration of saline in the colony, while non-associative animals received saline in the experimental room. Testing was not performed until the final day of the experiment. On this day (test day), both groups were taken to the experimental room and assessed for baseline tail-flick latencies. Immediately following the test, animals were injected with morphine (3.0 mg/kg s.c.), and tail-flick latencies were assessed 60 min later (saline or MK-801 pretreatment was not administered on this day).

Since we had not previously utilized this injection protocol, a fifth group was used to assess the development of tolerance. This group (test group) was treated identically to the associative saline group, however, this group was tested for tail-flick latency each morning before the saline injection (baseline) and 60 min following the morphine injection. Testing was performed on the experimental groups one day following the demonstration of complete tolerance in the test group (i.e. when morphine responsiveness did not differ from baseline).

#### 2.4. Experiment 3: effect of MK-801 on non-associative morphine tolerance produced by chronic infusion

A second study was performed to examine whether MK-801 has the ability to inhibit non-associative tolerance. In this experiment, morphine was administered continuously with subcutaneous pellets, and MK-801 was administered continuously with osmotic pumps. Chronic administration of morphine with pellets prevents the establishment of associations of drug effects with environmental cues, and therefore produces a relatively pure non-associative tolerance [32].

Rats were implanted subcutaneously (s.c.), under light methoxyflurane anesthesia, with one 75 mg morphine pellet or placebo pellet, and one Model 2002 Alzet osmotic minipump containing either saline or 5  $\mu\text{g}/\mu\text{l}$  of MK-801 (this concentration of MK-801 was used to achieve an infusion dose of 0.1 mg/kg/day). Approximately 72 h after the first implant, animals were implanted with three additional morphine or placebo pellets. Tail-flick latencies were assessed in the animal colony 4 h after each of the pellet implants and once each day for 6 days.

#### 2.5. Experiment 4: effect of phencyclidine, dextrorphan and ketamine on non-associative morphine tolerance produced by chronic infusion

This experiment was performed to determine if non-competitive NMDA receptor antagonists other than MK-801 would inhibit non-associative morphine tolerance. Methods were similar to those in Expt. 3, except Model 2001 osmotic minipumps were used instead of Model 2002. In addition, pumps were 'primed' by incubating them in saline overnight at room temperature after loading. This was done to eliminate the 4 h delay typically required to achieve steady-state pumping rates (Alza Corporation, personal communication). Doses of the drugs were selected based on their relative ability to block NMDA receptors [30,36,40,51,52], using the 0.1 mg/kg/day dose of

MK-801 in Expt. 3 as a reference. Targeted doses were 1.0 mg/kg/day for phencyclidine, 5.0 mg/kg/day for dextrorphan and 10 mg/kg/day for ketamine. As such, pumps contained saline, 13.5  $\mu\text{g}/\mu\text{l}$  of phencyclidine, 67.7  $\mu\text{g}/\mu\text{l}$  of dextrorphan or 135  $\mu\text{g}/\mu\text{l}$  ketamine. Approximately 72 h after the first implant, animals were implanted with three additional morphine or placebo pellets. Tail-flick latencies were assessed once each day for 6 days after the initial implant.

In contrast to Expt. 3, tail-flick latencies were not determined 4 h after the implants; the first test occurred at 24 h. Advokat [1,2] has reported that animals that undergo tail-flick testing during the development of tolerance, even when analgesia is maximal (such as at the 4 h time-point in Expt. 3), show greater tolerance when later tested. We chose to eliminate the 4 h time-point to avoid any potential influence of this test on the initial determination of tolerance.

After the tail-flick test on the 6th day, pellets and pumps were removed under light methoxyflurane anesthesia. Tail-flick latencies were assessed each day for the next 4 days to examine withdrawal-induced changes in pain responsiveness. Animals remained unhandled for 6 additional days, whereupon they received a drug challenge: those that had previously received placebo pellets were challenged with saline, and those that had previously received morphine pellets were challenged with morphine (10 mg/kg s.c.). Tail flick latencies were determined 60 min later. A similar challenge with saline and 5 mg/kg morphine was performed one week later. These challenges were used to examine the persistence of the NMDA antagonist-mediated inhibition of tolerance.

#### 2.6. Experiment 5: effect of NMDA receptor antagonists on the expression of morphine tolerance

This experiment was designed to examine whether non-competitive NMDA receptor antagonists would 'reverse' tolerance after it had been established in the animals. Animals were implanted with one 75 mg morphine or placebo pellet under light methoxyflurane anesthesia. Tail-flick latencies were determined 24 and 48 h after the implant. Immediately after the 48 h test, animals received an injection of saline, MK-801 (0.1 mg/kg i.p.), phencyclidine (1.0 mg/kg i.p.), dextrorphan (5.0 mg/kg i.p.) or ketamine (10.0 mg/kg i.p.). Tail-flick latencies were again determined 60 min later.

### 3. Results

#### 3.1. Experiment 1: effect of phencyclidine on the development of morphine tolerance

Animals treated with phencyclidine (1.0 mg/kg i.p.) and saline each day showed no changes in tail-flick latency, when compared with baseline, demonstrating that this drug does not by itself influence pain responsiveness. Animals treated with morphine (10 mg/kg s.c.) and saline showed maximal analgesia on day 1, and decreasing levels of morphine analgesia on subsequent days, such that by day 9 tail-flick latencies had returned to baseline (Fig. 1a). In contrast, the phencyclidine/morphine group showed significant analgesia throughout treatment, and was significantly different from the saline/morphine group on days 7 and 9 (Fig. 1a). On day 10, when animals received morphine (10 mg/kg s.c.) alone, the phencyclidine/morphine group was significantly different from the saline/morphine group despite the omission of the phencyclidine injection.

tion (Fig. 1b). As previously observed [41,42,47], the MK-801/morphine group had very similar effects to the phencyclidine/morphine group; i.e. an inhibition of tolerance that extended to day 10, despite the omission of the antagonist on this day (data not shown).

### 3.2. Experiment 2: effect of MK-801 on associative and non-associative morphine tolerance produced by repeated injections

The test group showed rapid development of tolerance, such that by day 6 there was no difference between baseline and morphine tail-flick latencies (Fig. 2a). Testing in the experimental groups was therefore performed the following day. Two-factor analysis of variance for the experimental groups revealed a significant effect of treatment room ( $P < 0.0005$ ), a significant drug effect ( $P < 0.05$ ), and no interaction. Animals that received injections in the colony and testing in the experimental room (non-associative groups) showed greater morphine-induced analgesia (i.e. less morphine tolerance) than animals that received injections and testing in the experimental room (associative groups) (Fig. 2b). In addition, MK-801-treated animals showed greater analgesia (less tolerance) than saline-treated animals (Fig. 2b).

### 3.3. Experiment 3: effect of MK-801 on non-associative morphine tolerance produced by chronic infusion

Both the saline/morphine and the MK-801/morphine groups were completely analgesic at 4 h, and

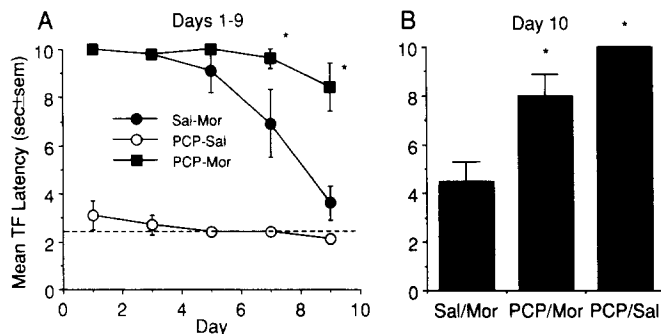


Fig. 1. Phencyclidine inhibits the development of morphine tolerance produced by repeated injections. A: on days 1–9, animals received daily injections of saline or phencyclidine (PCP; 1.0 mg/kg) followed 30 min later by saline or morphine (Mor; 10 mg/kg). Tail-flick (TF) latencies were determined 60 min following the second injection, on odd-numbered days. PCP, by itself, had no effects on TF latencies, however, this drug inhibited the development of Mor tolerance. B: on day 10, animals received Mor (10.0 mg/kg) alone, without the pre-injection. Animals that received PCP and Mor on days 1–9 showed inhibition of tolerance on day 10 despite the absence of the PCP injection on this day. These results demonstrate that PCP need not be present during testing to observe the inhibition of tolerance, and suggest that PCP inhibits the development, rather than the expression of morphine tolerance. \* = the PCP-Mor group was significantly different ( $P < 0.05$ ) from the Sal-Mor group on days 7, 9 and 10.

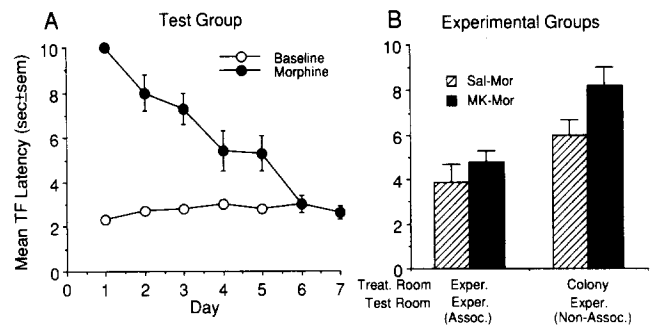


Fig. 2. MK-801 inhibits non-associative morphine tolerance produced by repeated injections. A: the test group was used to follow the time-course of tolerance, in order to determine the optimal day for assessing tolerance in the experimental groups. Animals received daily injections of saline, followed 30 min later by morphine. Tail-flick (TF) latencies were determined immediately prior to saline injections (Baseline), and 60 min following morphine injections (Morphine). Since complete tolerance in this group (defined by morphine TF latency = baseline TF latency) occurred on day 6, the experimental Groups were compared on day 7. B: experimental groups received injections of saline (Sal) or MK-801 (MK; 0.1 mg/kg), followed 30 min later by morphine (Mor; 3.0 mg/kg), each morning for 6 days in either the experimental room (Exper.) or the animal colony (Colony). In order to promote explicit pairing of environmental cues and drug effects, each evening animals received an injection of saline paired with the opposite environment. TF latencies were not determined in these groups until day 7. On day 7, animals were taken to the experimental room, were administered Mor (3.0 mg/kg), and TF latencies were determined 60 min later. Two-way ANOVA revealed a significant effect of treatment room ( $P < 0.0005$ ), a significant drug effect ( $P < 0.05$ ), and no interaction. The results indicate that MK-801 inhibited morphine tolerance similarly in both the associative (Assoc.) and the non-associative (Non-Assoc.) groups. See text for further discussion.

showed evidence of tolerance by 24 h. The saline/morphine group was significantly different from the saline/placebo control group only at the 4 h, 24 h and 76 h time-points. The MK-801/morphine group was significantly different from the saline/placebo control group at all time points examined. In addition, the MK-801/morphine group showed greater analgesia (less tolerance) than the saline/morphine group by the 24 h time-point, and this effect was evident throughout the week of treatment (Fig. 3). Placebo-pelleted animals treated with MK-801 showed no effect on tail-flick latency when compared to the saline/placebo group (Fig. 3). MK-801, therefore, inhibited non-associative morphine tolerance produced by chronic treatment, without affecting pain responsiveness on its own.

### 3.4. Experiment 4: effect of phencyclidine, dextrorphan and ketamine on non-associative morphine tolerance produced by chronic infusion

Similar to Expt. 3, the saline/morphine group in the present experiment showed significant analgesia relative to the saline/placebo group only at the 24 h

time-point (the first time-point examined in this experiment). Although mean tail-flick latency increased slightly for this group on the day following the second pellet implant, this increase was not significant (Fig. 4). The NMDA receptor antagonists produced no significant effects on the tail-flick test in placebo-pelleted animals, relative to the saline/placebo control group (Fig. 4a,b,c). Animals that received the NMDA receptor antagonists together with morphine showed significant analgesia relative to their respective placebo controls. In addition, each of these groups was significantly different from the saline/morphine group at several time points during treatment (Fig. 4a,b,c).

As observed by others in previous studies with morphine pellets [54], there was no significant withdrawal hyperalgesia on the days following removal of the pellets. There was, however, significant weight loss in morphine-pelleted animals on the 4 days following removal of pellets and pumps. This weight loss was observed both in saline-treated animals and in those that had received the NMDA receptor antagonists (data not shown).

Ten days following pellet removal, there was no difference between the groups on baseline tail-flick response. There was also no significant effect of saline injection in animals that had previously been treated with placebo pellets. Morphine administration (10 mg/kg s.c.) produced potent analgesia in animals that had been treated with morphine pellets 10 days earlier. Tail-flick latencies were near maximal for all four groups, and there was no significant difference between groups (data not shown). Because the high levels of morphine analgesia produced a ceiling effect, which

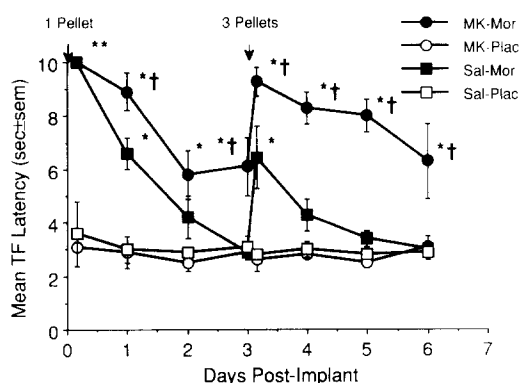


Fig. 3. MK-801 inhibits non-associative morphine tolerance produced by chronic infusions. At the beginning of the experiment, animals were implanted with one 75 mg morphine (Mor) or placebo (Plac) pellet, and one osmotic minipump delivering either saline (Sal) or MK-801 (MK; 0.1 mg/kg/day). Animals received three additional pellets of the same type on day 3. Tail-flick (TF) latencies were determined 4 h after each pellet implant, and daily thereafter. MK had no effect on TF latencies in Plac-pelleted animals, however, this drug significantly inhibited the development of tolerance to Mor. \* = significantly ( $P < 0.05$ ) different from respective Plac group. † = significantly ( $P < 0.05$ ) different from Sal-Mor group.

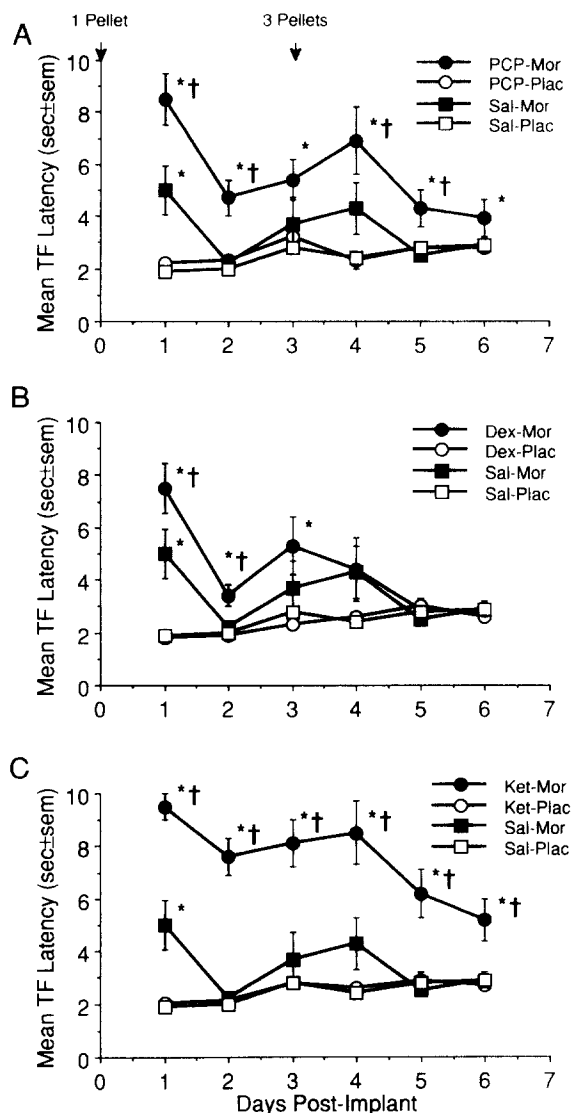


Fig. 4. Phencyclidine, dextrorphan and ketamine inhibit non-associative morphine tolerance produced by chronic infusions. Methods were similar to those in Fig. 3, however animals were not tested for TF latency 4 h after the pellet implants; the first TF test was 24 h following implant. Animals were implanted with Mor or Plac pellets and an osmotic pump infusing saline (Sal), phencyclidine (PCP; 1.0 mg/kg/day), dextrorphan (Dex; 5.0 mg/kg/day), or ketamine (Ket; 10.0 mg/kg/day). PCP, Dex nor Ket had any effect on TF latencies in Plac-pelleted animals, however, each of these drugs significantly inhibited the development of tolerance to Mor. \* = significantly ( $P < 0.05$ ) different from respective Plac group. † = significantly ( $P < 0.05$ ) different from Sal-Mor group.

may have masked differences between the groups, a second challenge was performed 1 week later using a lower dose of morphine. On this day (17 days after removal of pumps and pellets) animals that had previously been implanted with placebo pellets received a saline injection, while those that had previously been implanted with morphine pellets received 5.0 mg/kg of morphine. This dose of morphine uniformly produces maximal analgesia in the tail-flick test in opiate-

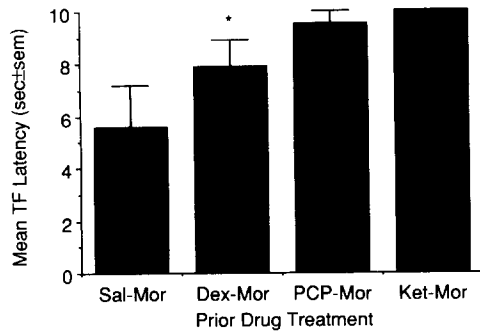


Fig. 5. The inhibition of tolerance by NMDA receptor antagonists persists in the absence of drug administration. The data represent Mor-induced analgesia 17 days after the removal of pumps and pellets in the animals shown in Fig. 4. Pumps and pellets were removed after the TF test on day 6. Seventeen days later, animals that had previously had Mor pellets received an injection of Mor (5.0 mg/kg), in the absence of the NMDA receptor antagonists (the designations under each bar represents the drugs that the animals had received 17 days earlier). Animals that had earlier received dextrorphan (Dex-Mor), phencyclidine (PCP-Mor), or ketamine (Ket-Mor) during Mor administration showed significantly greater Mor analgesia than those that had received saline (Sal-Mor), suggesting that the inhibition of tolerance by these drugs persisted for at least 17 days after drug administration was terminated. \* = significantly different from Sal-Mor group.

naive animals (Trujillo and Akil, unpublished results). There was no significant effect of saline in animals that had been treated with placebo pellets 17 days earlier (data not shown). Morphine administration produced a moderate analgesic response in animals that had previously received saline and morphine (Fig. 5). Groups

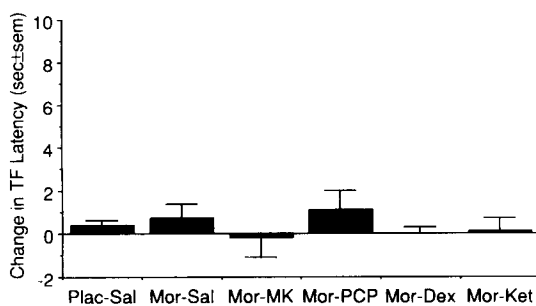


Fig. 6. Non-competitive NMDA receptor antagonists do not reverse morphine tolerance. Animals were implanted with one 75 mg morphine (Mor) or placebo (Plac) pellet, and tail-flick (TF) latencies were determined 24 and 48 h later. Immediately after the 48 h TF test, animals received an injection of saline (Sal), MK-801 (MK; 0.1 mg/kg), phencyclidine (PCP; 1.0 mg/kg), dextrorphan (Dex; 5.0 mg/kg) or ketamine (Ket; 10.0 mg/kg), and TF latencies were assessed 60 min later. The results are shown as the antagonist-induced change in TF latency (post-injection TF minus pre-injection TF). No change in TF latency was produced by any antagonist in animals in which tolerance had already developed, demonstrating that these drugs do not affect the expression of tolerance, and do not reverse tolerance.

that had received PCP, dextrorphan or ketamine together with morphine 17 days earlier showed greater analgesia than the saline/morphine group (Fig. 5). Thus, morphine tolerance was evident in the saline/morphine group 17 days after administration of the drugs was terminated, as was inhibition of tolerance by the three NMDA receptor antagonists.

### 3.5. Experiment 5: effect of NMDA receptor antagonists on the expression of morphine tolerance

As observed in Expt. 4, animals implanted with morphine pellets showed tolerance developing across the 24 and 48 h time-points (data not shown). By 48 h, tail-flick latencies in morphine-pelleted animals were not significantly different from those in placebo-pelleted animals (data not shown). Administration of the NMDA receptor antagonists at the 48 h time-point produced no significant effect on tail-flick latencies in animals that were tolerant to morphine (Fig. 6).

## 4. Discussion

The present results demonstrate that NMDA receptor blockade with non-competitive antagonists inhibits the development of morphine tolerance. Inhibition of tolerance was found with four different antagonists, MK-801, ketamine, dextrorphan and phencyclidine. The NMDA receptor antagonists were found to inhibit the development and not the expression of opiate tolerance; i.e. they were able to prevent but not reverse tolerance. Importantly, these drugs were found by two different methods to block non-associative or pharmacological tolerance.

### 4.1. NMDA receptor antagonists inhibit morphine tolerance

Our laboratory [41,42,45,47] and others [4,28,29,39] have previously reported that the non-competitive NMDA receptor antagonist MK-801 interferes with opiate tolerance. However, although MK-801 is a potent and selective NMDA receptor antagonist, no drug is absolutely specific for a particular receptor. Moreover, there have been reports that MK-801 may produce actions unrelated to NMDA receptor blockade [5,6]. Thus, one aim of the presents experiments was to examine whether other non-competitive NMDA receptor antagonists would produce similar effects.

In Expt. 1, the non-competitive NMDA receptor antagonist phencyclidine was observed to inhibit morphine tolerance in a manner similar to MK-801. The dose of phencyclidine (1.0 mg/kg i.p.) found to be effective is quite significant. We have previously established that 0.1 mg/kg of MK-801 is the most effective

dose for inhibiting the development of morphine tolerance without producing untoward side-effects [42]. Both behavioral experiments [40] and *in vitro* studies [51] have shown that MK-801 is 10-fold more potent than phencyclidine at the NMDA receptor. This difference directly corresponds to the 10-fold higher dose of phencyclidine that inhibited morphine tolerance in the present experiment. Although dose–response studies with phencyclidine are necessary to determine the most effective dose of this drug, the present results suggest that the inhibition of morphine tolerance by phencyclidine and MK-801 corresponds to their relative affinities at the NMDA receptor.

It might be argued that the effect of phencyclidine on morphine tolerance resulted from accumulation of the drug over the repeated injections, leading to higher doses, and causing impairment of the animal. The apparent inhibition of tolerance in such a situation would result from a non-specific impairment-induced increase in tail-flick latency. However, evidence suggests that this is highly unlikely. First, the tail-flick response is very resistant to non-specific impairment. In our laboratory, animals completely immobilized by flaccid paralysis with 3.0 mg/kg of MK-801 showed no changes in tail-flick latency [41,42], demonstrating that this response is highly resistant to disruption by NMDA receptor antagonists. Additionally, studies on the disposition of phencyclidine in rats have demonstrated that this drug is 99% cleared from the brain by 24 h following injection of a moderate dose [31]. A similar rapid clearance of MK-801 has been observed, with 90% of the drug being cleared from the brain by 6 h after injection [48]. Since injections in the present experiment occurred at 24 h intervals, it is unlikely that significant accumulations occurred. More importantly, no changes in pain responsiveness were observed in the phencyclidine/saline group over the nine days of treatment. If the increased analgesia seen in the phencyclidine/morphine group was the result of impairment produced by accumulated phencyclidine, then the phencyclidine/saline group should also have been affected.

In Expts. 3 and 4, MK-801, phencyclidine, dextrorphan, and ketamine were each found to inhibit opiate tolerance when the drugs were administered by chronic infusion. Although there was some variability in the effectiveness of the non-competitive antagonists, the fact that each drug inhibited tolerance at a dose selected for NMDA receptor antagonism indicates that it is the ability of these drugs to block NMDA receptors, and not a ‘side-effect’ of a particular drug, that is responsible for the effect. It is important to note that the doses required to inhibit the development of tolerance in these experiments are quite low relative to the doses of these drugs that produce other effects. At the doses examined, these drugs had no effect on pain

responsiveness in the presence of placebo pellets. The doses were also below those that produce phencyclidine-like behavioral effects, such as head weaving and ataxia [36,40]. The use of chronic infusion in these experiments, in particular, emphasizes that very low doses are required to inhibit the development of tolerance. By spreading the dose over 24 h, this method allowed for much lower concentrations of the drug to be present at any particular time-point than when bolus injections were used. For example, when repeated injections were used to study the development of tolerance, the 0.1 mg/kg dose of MK-801 was found to be most effective for inhibiting morphine tolerance without producing untoward effects [41,42]. In the present studies, this same dose, spread over a 24 h infusion was also effective. It thus appears that chronic infusion may be an excellent method for inhibiting tolerance, while minimizing untoward side-effects.

In addition to minimizing the dose necessary to inhibit tolerance, chronic infusion also decreased the time necessary to observe the inhibition of tolerance. In previous studies in which repeated injections were used, because of the slower development of tolerance, the effects of MK-801 were not evident until several days following the onset of drug administration [28,29,41,42]. In contrast, the inhibition of tolerance in Expts. 3 and 4 was manifest as little as 24 h following the first pellet implant. Ben-Eliyahu and coworkers [4] recently reported that the effects of MK-801 on morphine tolerance may be seen as little as 6 h following coadministration of the drugs in a sustained-release preparation. Thus, the effects of NMDA receptor antagonists on opiate tolerance are quite rapid in onset, and occur following exposure to very low cumulative doses of the drugs.

The ability of non-competitive NMDA receptor antagonists to inhibit opiate tolerance rapidly, and at very low doses, supports and strengthens previous suggestions that NMDA receptors may have an important role in opiate tolerance and dependence [4,28,29,41,42,44,47]. These suggestions are further strengthened by the recent report by Tiseo and Inturrisi [39] that the competitive NMDA receptor antagonist LY274614 also inhibits the development of morphine tolerance. The involvement of NMDA receptors in opiate tolerance and dependence suggests that these phenomena are very similar to other forms of neural and behavioral plasticity, such as learning, long-term potentiation, kindling and development [8,9,49].

#### *4.2. NMDA receptor antagonists inhibit the development, but not the expression of morphine tolerance*

Three of the present experiments were designed to determine whether NMDA receptor antagonists inhibit

the development or the expression of opiate tolerance. In Expts. 1 and 4, we examined whether the inhibition of tolerance by these antagonists would persist beyond administration of the drugs. If NMDA antagonists simply inhibited the expression of opiate tolerance then the inhibition of tolerance should not be evident in their absence. On the other hand, if these drugs inhibited the development (or acquisition) of tolerance, then the inhibition of tolerance should persist beyond NMDA antagonist administration. In both experiments, inhibition of tolerance by the antagonists was observed to persist beyond administration of the drugs; in the case of Expt. 4 for up to 17 days. Since it is highly unlikely that any of the NMDA antagonists remained in the body following 17 days without treatment, the results strongly demonstrate that the drugs need not be present at the time of testing to observe the inhibition of tolerance. These results support previous findings on the persistence of the inhibition of opiate tolerance in the absence of continued NMDA antagonist administration [4,39,41,42,47].

In Expt. 5 an alternative procedure was used to determine whether the non-competitive NMDA receptor antagonists inhibit the development or the expression of opiate tolerance. In this experiment, the antagonists were administered to animals in which tolerance had already been acquired. If these antagonists inhibited the expression of tolerance, then it would be expected that these drugs would increase opiate analgesia in tolerant animals. However, if these drugs specifically inhibited the development of tolerance, then they should not affect analgesia in animals in which tolerance was already established. The results, in which no effects of the NMDA receptor antagonists were observed in tolerant animals, demonstrate that these drugs do not affect the expression of opiate tolerance. Moreover, in contrast to the recent conclusions of Tiseo and Inturrisi [39], the results suggest that NMDA receptor antagonists do not have the ability to 'reverse' tolerance. There are some notable differences between our experiments and those of Tiseo and Inturrisi that may be responsible for the different conclusions. First, while we found no reversal of tolerance with non-competitive NMDA receptor antagonists, they found reversal of tolerance with the competitive antagonist LY274614. Second, we used the tail-flick test for assessing analgesia, while they used the hot-plate test. Finally, we used a bolus injection of the NMDA receptor antagonists to test for reversal, whereas they used chronic infusion. Thus, although there is some disagreement over whether NMDA receptor antagonists have the ability to reverse tolerance, the evidence available thus far strongly supports our suggestion that NMDA receptor antagonists inhibit the development, and not the expression of opiate tolerance [41,42,44,45,47].

#### *4.3. NMDA receptor antagonists inhibit non-associative, but not associative morphine tolerance*

We previously suggested two alternatives for the inhibition of opiate tolerance by NMDA receptor antagonists [43,44]. Since these drugs have been found to interfere with learning, we suggested that they might inhibit tolerance by interfering with the associations between drug effects and environmental cues sometimes involved in tolerance (associative tolerance). Alternatively, these drugs might act more directly, by interfering with the primary adaptive changes that occur in response to the chronic drug treatment (non-associative tolerance). Evidence from three of the present experiments (Expts. 2, 3 and 4) suggests that the latter may be the case.

In Expt. 2, in which the effects of morphine were explicitly paired or unpaired with specific environmental cues, there was a significant effect of treatment room (demonstrating a difference between the associative and non-associative conditions), a significant effect of drug treatment (demonstrating that MK-801 was indeed effective in inhibiting tolerance), but no interaction (demonstrating similar effects of the drug in both treatment rooms). Whereas the unpaired group represents a relatively pure non-associative condition, the paired group has the combined influences of the non-associative (or pharmacological) effects of morphine and the associative cues. Since MK-801 was effective under both conditions, the results suggest that this drug has the ability to inhibit non-associative morphine tolerance. In fact, closer examination of the pattern of results suggests that MK-801 may not affect associative tolerance. Since the difference between the paired and unpaired groups represents the influence of associative cues, if MK-801 specifically affected associative tolerance, then the MK-801-treated animals in the paired condition should have shown the same level of analgesia as the MK-801-treated animals in the unpaired condition. The fact that this difference was not abolished by MK-801 suggests that the drug does not influence associative morphine tolerance.

In Expts. 3 and 4, the effects of NMDA receptor antagonists were examined on morphine tolerance produced by pellet implantation. Chronic administration of morphine with pellets, by continuously exposing the animals to the drug, prevents the establishment of associations between drug effects and environmental cues, and therefore produces a relatively pure non-associative tolerance [32]. Each of the NMDA receptor antagonists examined had the ability to inhibit tolerance produced by morphine pellets, further demonstrating that these drugs have the ability to inhibit non-associative tolerance. Taken together with previous findings [4,18,19,44,46,47], the present results suggest that NMDA receptor antagonists may directly



interfere with the adaptive neural changes that occur during chronic opiate administration, rather than by 'simply' interfering the learning of drug-environment associations.

#### 4.4. Concluding comments

For many years researchers have searched for the perfect opiate analgesic: one that produces potent pain relief, but that does not produce tolerance and dependence. The present results, taken together with those of previous studies, suggests that an alternative strategy is to coadminister an adjunct that inhibits the development of tolerance and dependence, but which allows the opiate to retain its full analgesic potency. Non-competitive NMDA receptor antagonists appear to have this ability. Importantly, the present studies demonstrate that these antagonists inhibit the development of opiate tolerance at doses well-below those that produce untoward side-effects. In addition to the ability to inhibit opiate tolerance and dependence, NMDA receptor antagonists have the ability to inhibit 'wind-up', a process that leads to pathological hyperalgesia [7,13,15,26,53]. These combined abilities suggest that NMDA receptor antagonists may be quite promising in the field of pain management.

The specific role of NMDA receptors in opiate tolerance is presently unclear. For example, since studies published thus far have focused on tolerance to opiate analgesia, it is unknown whether NMDA receptor antagonists will inhibit tolerance to other opiate effects. Observations of animals in the present studies, as well as in previous experiments [41,42,45–47], however, indicate that NMDA receptor antagonists may inhibit tolerance to the locomotor-depressant effects of morphine (Trujillo, unpublished observations). Therefore, NMDA receptors may be involved in tolerance to opiate actions other than analgesia, although this remains to be more systematically studied. Since previous experiments have utilized behavioral measures, it is presently unknown whether the interaction between NMDA receptor antagonists and opiates occurs at the level of individual cells, or whether it is a function of interacting cells or circuits. Because of the well-described role of calcium ions in NMDA receptor-mediated neural and behavioral plasticity [8,9,49], we have previously suggested that NMDA receptor-activated, calcium-dependent processes may be involved in the development of opiate tolerance [44]. The recent finding that a nitric oxide synthase inhibitor will inhibit opiate tolerance suggests that, like other NMDA receptor-mediated processes, this phenomenon may involve the diffusible messenger nitric oxide [25]. Although it is tempting to speculate about potential cellular mechanisms, further studies are required to deter-

mine the specific role of NMDA receptors in opiate tolerance and dependence.

It is important to note that opiate tolerance and dependence are not the only drug-induced adaptive responses that are affected by NMDA receptor antagonists. Non-competitive NMDA antagonists have been found to interfere with tolerance to ethanol [22–24] and cocaine [14], and with sensitization (or reverse tolerance) to amphetamine [20,21,35,50], cocaine [14,20] and nicotine [33]. NMDA receptors therefore appear have an important role in the neural and behavioral changes produced by chronic administration of a variety of drugs that are used in the clinic and on the streets. Further research will help elucidate the role of NMDA receptors in the adaptive responses to psychoactive drugs, as well as the potential clinical utility of NMDA receptor antagonists in the treatment of substance abuse and chronic pain.

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