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Long-Term Morphine Delivery via Slow Release Morphine Pellets or Osmotic Pumps: Plasma Concentration, Analgesia, and Naloxone-Precipitated Withdrawal

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Abstract

Aims—Slow-release morphine sulfate pellets and osmotic pumps are common routes of chronic morphine delivery in mouse models, but direct comparisons of these drug delivery systems are lacking. In this study, we assessed the efficacy of slow-release pellets versus osmotic pumps in delivering morphine to adult mice.

Main methods—Male C57BL/6NCr mice (8 weeks old) were implanted subcutaneously with slow-release pellets (25 mg morphine sulfate) or osmotic pumps (64 mg/mL, 1.0 μ L/hour). Plasma morphine concentrations were quantified via LC-MS/MS, analgesic efficacy was determined by tail flick assay, and dependence was assessed with naloxone-precipitated withdrawal behaviors (jumping) and physiological effects (excretion, weight loss).

Key findings—Morphine pellets delivered significantly higher plasma drug concentrations compared to osmotic pumps, which were limited by the solubility of the morphine sulfate and pump volume/flow rate. Within 96 hours post-implantation, plasma morphine concentrations were indistinguishable in pellet vs. pump-treated samples. While osmotic pump did not have an antinociceptive effect in the tail flick assay, pumps and pellets induced comparable dependence symptoms (naloxone-precipitated jumping behavior) from 24–72 hours post-implantation.

Significance—In this study, we compared slow-release morphine pellets to osmotic minipumps for morphine delivery in mice. We found that osmotic pumps and subcutaneous morphine sulfate pellets yielded significantly different pharmacokinetics over a 7-day period, and as a result significantly different antinociceptive efficacy. Nonetheless, both delivery methods induced dependence as measured by naloxone-precipitated withdrawal.

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Introduction

Opioids remain one of the most effective classes of analgesics available for treatment of acute and chronic pain conditions, as well as one of the most abused prescription drugs [1,2]. Since 2000, fatal opiate overdoses have increased by an estimated 200% in the United States. Patients who experience a non-fatal overdose are twice as likely to receive a new prescription for opioids, and four times as likely to overdose again [3]. Opioids induce a range of negative side-effects including respiratory depression [4] and constipation [5]. Prolonged opioid use/misuse can worsen clinical outcomes in other diseases including human immunodeficiency virus (HIV) infection [6] and neuropathic pain [7,8]. Indeed, there is growing concern over the long-term use of opioids, particularly in chronic non-cancer pain patients, where prolonged opioid use shows limited analgesic efficacy; instead, opioid therapy in these patients leads to increased risk of overdose, depression, and opioid-induced hyperalgesia [9,10]. Thus, we must continue to improve our understanding of the negative effects of prolonged opioid exposure in both the periphery and the central nervous system.

Morphine, a prototype of the opioid class, remains in clinical use today. Morphine is also the primary active metabolite of heroin, which began as a therapeutic drug in the 1800s and is now solely a drug of abuse in the United States [11]. Morphine acts primarily as a μ -opioid receptor agonist with lesser affinity for the δ -opioid and κ -opioid receptors [12]. These G-protein coupled receptors are expressed in multiple regions, including the central nervous system [13,14], the gastrointestinal tract [15], and immune system [16], and can induce effects ranging from analgesia and addiction to peripheral immunosuppression [17–20].

Various rodent models have been developed to mimic human opioid use/misuse and its consequences (e.g., tolerance and physical dependence). Subcutaneous injections provide convenient, reasonable approximation to typical opioid self-administration in humans: morphine dosages can be escalated over time to accommodate for tolerance, and the time between treatments can be varied to mimic dosage-withdrawal cycles [21]. Self-administration paradigms have also been developed for opioids such as oxycodone [22]. To assess chronic opioid exposure – comparable to patients treated with long-acting opioids or extended-release prescriptions – rodent models regularly employ slow-release morphine pellets provided by the National Institute on Drug Abuse (NIDA) drug supply program. In mice, the most common dosages are 25 mg or 75 mg [23–25]. These pellets are implanted subcutaneously and have been reported to provide a steady dose of morphine for up to one week [26]. Another approach is the use of osmotic pumps that are designed to release a set volume of preloaded drug at the microliter level per hour, providing a finer control of drug delivery than slow-release pellets [27–29]. However, compound solubility, pump volume, and the slow flow-rate necessary to achieve 3–7 days of drug delivery all limit the amount of drug an osmotic pump can deliver [30].

Despite the frequent use of morphine pellets and osmotic minipumps in preclinical models of opioid use/misuse, direct comparisons of these delivery routes in mice are lacking. To assess these treatment paradigms, we compared the pharmacokinetics, analgesia, and dependence associated with morphine pellets and osmotic pumps over 7 days. We report that subcutaneous pellets delivered a maximum concentration of plasma morphine at 24 hours

post-implantation, and declined rapidly. Osmotic pumps did not supply enough morphine to induce analgesia, but both pump- and pellet-implanted mice exhibited naloxone-precipitated withdrawal behaviors for up to one week post-implantation.

Materials and Methods

Animals and Treatment

Seven-week-old male C57BL/6NCr mice (National Cancer Institute, Frederick, MD, USA) were provided food and water *ad libitum* and kept on a 12-hour light/dark cycle. After a week of habituation, mice were implanted with either morphine sulfate (25 mg) pellets (NIDA, Bethesda, MD, USA) or Alzet pumps loaded with morphine sulfate (64 mg/mL) suspended in saline (#2001, 1.0 μ L/hour, Durect Corporation, Cupertino, CA, USA). Pellets and pumps were implanted s.c. in mice anesthetized by isoflurane. For the tail-flick assay, mice were injected subcutaneously (s.c.) with 20 mg/kg morphine sulfate twice daily at 12-hour intervals. At the conclusion of the experiment, animals were euthanized by CO₂ inhalation. All procedures were in compliance with the Animal Welfare Act and NIH Guide for the Care and Use of Laboratory Animals and approved by the University of New England Institutional Animal Care and Use Committee.

Plasma Drug Exposure

To evaluate the pharmacokinetic profile of morphine delivery, mice were administered morphine pellets or pumps as described above. Blood was collected into EDTA-treated tubes at multiple time points (0.25, 0.5, 1, 2, 3, 4, 6, 24, 36, 48, 60, 72, 96, 120, 144, 168 hours post-implantation) by retro-orbital bleed or by terminal cardiac puncture. Plasma was stored at -80°C until bioanalysis. Samples were analyzed at the UNE School of Pharmacology or by Illinois Institute of Technology Research Institute (IITRI, Chicago, IL, USA) under the conditions described below.

Concentrations of morphine in plasma (total drug concentrations) were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Waters Corporation, 2008). In brief, a 20 μ L aliquot of each plasma sample was mixed with 40 μ L blank mouse plasma, 30 μ L 0.2% methanol-water containing 60 ng internal standard (ISTD; morphine-*d*3, Sigma-Aldrich, St. Louis, MO) and 30 μ L water. After conditioning with 0.5 mL methanol and 0.5 mL water, a 100 μ L aliquot was transferred into an Oasis® HLB Extraction Plate (Waters Corporation, Milford, MA, USA). Wells were washed with 0.4 mL of 5% methanol in water, dried under vacuum (5 min) and then eluted with 200 μ L acetonitrile (ACN):isopropyl alcohol(IPA):formic acid(FA) (40/60/0.2, v/v/v). Extracts were collected in a clean 96-well plate, dried under nitrogen flow at RT and reconstituted in 100 μ L 70% ACN in water.

Freshly prepared morphine (Sigma-Aldrich, St. Louis, MO, USA) standard calibration samples were analyzed coincident with study sample analysis. Calibration samples and quality control (QC) samples were prepared as 60 μ L of blank mouse plasma with 30 μ L stock morphine in 0.5% methanol and water. Calibrations were prepared at: 0.5, 1, 5, 25, 100, 250, 500, 1000 and 2500 ng/mL. QC samples were prepared at: 2.5, 1000, and 2500

ng/mL. Calibration and QC samples were processed by the same method as study samples (described above).

Samples were analyzed on an API 3000 LC-MS-MS (Applied Biosystems/MDS Sciex, Foster City, CA, USA) equipped with an 1100 HPLC (Agilent Technologies, Wilmington, DE, USA). For HPLC, column temperature was maintained at 25°C. Samples were run at an isocratic flow rate of 300 µL/min. Phase A was 5 mM ammonium formate buffer and 0.2% formic acid in water. For MS/MS, ion spray voltage was maintained at 4500 volts with ion source temperature of 400°C.

Samples were analyzed using a standard curve with two replicates at each QC level. Calibrators and quality control samples fell within an acceptable range, at 95.7–101% accuracy. Analytes were not detected above the LLOQ (0.5 ng/mL) in blank samples.

Tail-Flick Assay

Morphine-induced antinociception was assessed using the warm-water tail-flick assay in morphine-treated mice from 15 minutes to 168 hours post-treatment, mirroring the timepoints taken in the plasma drug exposure study described above. A baseline measure was taken immediately prior to morphine treatment. Mice (n=10) received morphine via s.c. injection (20 mg/kg, twice daily, with an interval of 12 hours between each injection), osmotic pump (64 mg/ml, 1.0 µL/hour), or slow release pellet (25 mg). Control mice (n=5 per control group) received a placebo pellet or a placebo (saline-filled) pump. At each timepoint, tail flick behavior was assessed by submerging the tail in a 50°C water bath and measuring the time until the mouse withdrew its tail. A 10 second cutoff was used to avoid tail damage.

Naloxone Precipitated Withdrawal Behavioral Assay

To assess morphine dependence, mice (n=5) received an intraperitoneal injection of naloxone (10 mg/kg) at 1, 3, and 7 days post-pellet or pump implantation. Morphine-treated mice (n=5) received morphine via s.c. injection (20 mg/kg, twice daily, with an interval of 12 hours between each injection), osmotic pump (64 mg/ml, 1.0 µL/hour), or slow release pellet (25 mg). Control mice received: (a) naloxone alone or (b) neither naloxone nor morphine.

At the time of the test, mice were weighed and injected i.p. with 10 mg/kg naloxone. Mice were then observed for the jumping withdrawal behaviors described by Way et al. 1969 [32]. Mice were placed on filter paper within a clear Plexiglas cylinder and observed for jumping behavior for 20 minutes. Following completion of the jump test, the filter paper was weighed to quantify feces and urine excretion. Mice were weighed to compare to pre-test weight and then euthanized via CO₂.

Statistical Analysis

Statistical analysis was performed in SigmaStat 11.0 (Systat Software, Inc., San Jose, CA, USA). Factors 'Time' and 'Delivery Method' were compared via two-way ANOVA

followed by a Student-Newman-Kewls post-hoc test. Significance was determined as $p < 0.05$.

Results

Pharmacokinetics of morphine delivery in slow release pellets vs. osmotic pumps

Fig. 1 shows the plasma concentration of morphine, as measured by LC-MS/MS. Male C57BL/6NCr mice ($n=3$) were implanted s.c. with a morphine sulfate pellet (25 mg) or an osmotic pump (64 mg/mL, 1.0 μ L/hour). To compare with a 1-week slow release morphine pellet, we utilized an Alzet #2001 osmotic pump, which can sustain a flow rate of 1.0 μ L/hour for one week. Loaded with morphine at its maximum solubility of 64 mg/mL, these pumps were expected to release a total of 10.8 mg morphine. These 7-day pumps, which are the largest that can be implanted in adult C57BL/6 mice, provide the closest side-by-side comparison with implantation of a single 25 mg morphine sulfate pellet. Blood was drawn from 15 minutes to 168 hours (7 days) post-implantation. As expected - due to the limitations of morphine sulfate's solubility and the osmotic pump's size and capacity - pumps delivered significantly less morphine over the 7-day period than 25 mg pellets (time $\times$ delivery: $F_{15,58}=7.202$, $p<0.001$). However, the pharmacokinetics of these two delivery methods over the week of observation was surprising: while morphine was detectable within 15 minutes of implantation in all mice (pellet= 382.0 ± 91.0 ng/mL, pump= 850.0 ± 106.9 ng/mL), plasma drug concentrations in pellet-treated mice peaked at 24 hours post-implantation (2695.3 ± 785.1 ng/mL), while plasma concentrations peaked at 30 minutes post-implantation in pump-treated animals (1115.3 ± 133.8 ng/mL). By 2 hours post-implantation, pellets delivered significantly more morphine than pumps ($p<0.001$). Pellets continued to deliver significantly higher levels of morphine until 96 hours (4 days) post-implantation. By day 4, plasma concentrations in pellet- and pump-implanted mice had fallen to 392.0 ± 84.5 ng/mL and 80.7 ± 48.8 ng/mL, respectively. At day 5 post-implantation, plasma levels of morphine were similar in pellet-implanted (280.7 ± 126.4 ng/mL) and pump-implanted mice (237.0 ± 29.5 ng/mL). Using area under the plasma concentration curve (AUC) analysis (not shown), we found that morphine sulfate pellets delivered, on average, 1.52×10^5 ng-hr/mL, while pumps delivered 2.71×10^4 ng-hr/mL. A previous study found that 25 mg pellets released on average 15.4 mg over 7 days, as measured by removing pellets and assessing the amount of remaining morphine by spectrophotometry (Dighe 2009). At 64 mg/mL and 1.0 μ L/hour, the highest potential morphine amount delivered by osmotic pumps would be 10.8 mg over the course of the week, at a rate of 64 μ g per hour. The solubility limitations of morphine (64 mg/mL in saline) limited the amount of drug we could deliver by osmotic pump, but in both cases the majority of morphine is released within the first 48 hours, rather than the slow release over 5–7 days previously shown [33].

Effects of delivery method on analgesia in the tail flick assay

To assess antinociception, the distal 1/3rd of each mouse's tail was immersed in a 50°C water bath and observed for the tail flick (withdrawal) response (figure 2). Tails were removed from the bath after 10 seconds to prevent burns. Male C57BL/6NCr mice ($n=10$) received pumps (64 mg/mL, 1.0 μ L/hour) or pellets (25 mg). Controls received subcutaneous morphine injection (20 mg/kg, twice daily, $n=10$), placebo pellets ($n=5$), or placebo (saline)

pumps (n=5). Pumps, pellets and subcutaneously-injected morphine led to significantly different antinociceptive responses over time (time x delivery, $F_{64,561}=15.200$, $p<0.001$).

For subcutaneous injection, mice received their first morphine dosage at time 0, concurrently with pump and pellet implantation; injections were repeated twice daily at 12-hour intervals throughout the week. These injections maintained antinociception in mice for the observation period ($p<0.001$ morphine pumps, $p<0.001$ morphine pellets, 7 days), but a tolerance-induced decline in latency was detectable within 24 hours ($p<0.001$, 15 minutes vs. 24 hours). Within 15 minutes of implantation, pellet-implanted mice exhibited antinociception, with a tail-flick latency of 7.2 ± 0.5 seconds compared to 4.2 ± 0.2 seconds at baseline ($p<0.001$). At 48 hours post-implantation, tail-flick latency in mice with morphine pellets (4.3 ± 0.5 sec) did not differ significantly from placebo pellet controls (3.4 ± 0.2 sec, $p=0.363$). Pump-implanted mice did not show a significant change in latency, with an average latency of 4.7 ± 0.4 sec at baseline, 4.8 ± 0.5 sec at 15 minutes, and 4.3 ± 0.4 sec at 1 hour post-implantation. Morphine pump-implanted mice never showed a significant increase in latency to withdrawal compared to saline pump-implanted controls, suggesting the dose delivered was either not sufficient to induce antinociception or the limits of solubility of the concentrated morphine solution caused issues with the pump delivery.

Effects of delivery method on naloxone-precipitated withdrawal

To assess morphine dependence, mice received subcutaneous injection, morphine pellets or morphine pumps (n=5) as described above. Withdrawal was precipitated with a 10 mg/kg injection of naloxone at 24 hours (1 day), 72 hours (3 days), and 168 hours (7 days) after initiation of morphine treatment. Control mice received no treatment ("no naloxone nor morphine") or naloxone alone ("naloxone"). After naloxone injection, mice were placed in a Plexiglas cylinder and the number of vertical jumps quantified over 20 minutes post-injection (Fig. 3A). Afterwards, excretion (feces and urine) were quantified by weighing filter paper at the bottom of the cylinder (Fig. 3B). Mice were weighed directly before and after the jumping test to examine weight loss (Fig. 3C).

Despite the lack of antinociceptive effects seen in the tail flick assay, both pellet- and pump-implanted mice exhibited significantly more naloxone-precipitated jumping behavior compared to non-morphine-treated controls at 7 days post-implantation. Delivery methods differed significantly in their effects over time on jumping behavior ($F_{8,60}=2.73$, $p=0.012$, Fig. 3A) and weight loss ($F_{8,60}=7.524$, $p<0.001$ Fig. 3C); simple main effects for timepoint ($F_{2,60}=31.548$, $p<0.001$) and delivery method ($F_{2,60}=5.100$, $p=0.009$) were observed in excretion (Fig. 3B). At 168 hours (7 days) post-implantation, pellet-implanted mice jumped an average of 154.2 ± 21.1 jumps compared to 84.6 ± 12.6 in pump-implanted mice ($p=0.004$). Weight loss was likewise significantly higher in pellet-implanted mice compared to pump-implanted mice at 168 hours post-implantation ($p<0.001$), while more excretion was observed in pump-implanted mice at 24 hours ($p=0.022$) and 72 hours ($p=0.039$) post-implantation compared to pellet-implanted mice. Interestingly, pellets and pumps induced significantly more jumping behavior than s.c.-injected mice at 72 hours, although the s.c. injection group and pump-implanted mice were no longer significantly different by 7 days. Overall, pellet-implanted mice exhibited more jumping behavior and weight loss at 168

hours post-implantation, indicating the 25 mg pellet induces longer-lasting dependence than the osmotic pump. Both the pellet and pump were sufficient to produce naloxone-precipitated withdrawal symptoms comparable to, if not greater than, that produced by 20 mg/kg morphine injection at 7 days post-implantation.

Discussion

In this study, we compared two subcutaneous morphine delivery systems in mice: slow-release morphine sulfate pellets and osmotic pumps. Despite their common use, there are no studies directly comparing these two routes of morphine delivery. Here, we show that while osmotic pumps did deliver detectable plasma concentrations of morphine – enough to elicit naloxone-precipitated withdrawal responses – they delivered significantly less morphine than 25 mg pellets, and the concentrations were not sufficient to induce antinociception in the tail flick test. In contrast, pellets delivered substantially more morphine, but showed a sharp peak in plasma concentration at 24 hours, followed by a gradual decline over the 7 days observed. The effects of these delivery systems on pharmacokinetics, anti-nociception, and physical dependence are summarized in Table 1.

Mouse and human plasma pharmacokinetics correlate well for multiple opioids [34]. In the clinical setting, morphine and other opiates are generally available as acute injections, oral and buccal tablets [35,36], and intrathecal and intracerebrovascular pumps [37,38]. After a 10 mg/kg intravenous dosage of morphine, humans had an average plasma AUC_{0–12} of 91.9±6.3 ng·hr/mL [36]. Pharmacokinetics vary in morphine slow-release formulations; AUC_{0–12} in humans ranged from 71.9 µg·hr/L to 167.4 µg·hr/L over the first 12 hours post-administration [35].

In mice, both 25 mg [25,39,40] and 75 mg pellets [23,24,41] have been used in strains ranging from C57BL/6 to ICR, generally 20 g or greater in weight. As measured by radioimmunoassay, 75 mg pellets provided a steady plasma concentration of morphine for 24 hours post-implantation, reaching maximum concentration 4 hours post-implantation at ~1650 ng/mL [42].

Osmotic pumps are limited by their volume, the solubility of the compound, and their size, which can be prohibitive to implantation in adult mice. Investigators have worked around these issues by implanting multiple pumps [43]; using pumps that must be replaced after 3 days, but deliver a higher volume per hour rate [44]; or by exceeding the 64 mg/mL solubility limits of morphine [28]. We selected the 7-day pump to more closely approximate the 25 mg pellet, as it – like the pellet – is implanted once over the 7-day period. While pumps with larger delivery rates per-hour are available, the physical size of larger pumps limits which models can be implanted in adult mice. Using a higher flow-rate pump with the same volume as our 7-day pump would require repeated surgeries, limiting a direct comparison between the single pellet and the pump. Exceeding morphine's solubility could lead to precipitation within the pump, cutting off flow altogether. Intracerebroventricular infusion is one alternative for delivering adequate amounts of morphine for studies of analgesia, addiction, and dependence, as these pumps allow direct delivery of an effective dose of morphine directly to the brain [45]. However, the more local intracerebroventricular

infusion approach has limitations, as opioid receptors in the spinal cord and peripheral nerves have been shown to play a role in tolerance, dependence and withdrawal [46–48]. Due to these solubility and volume limitations, the osmotic pump is better suited for the study of more potent opioid analgesics, such as oxycodone or fentanyl [44].

Brain concentrations of 100 ng morphine per g of brain tissue were sufficient to induce analgesic effects in Swiss-Webster mice implanted with 75 mg pellets, which produced antinociception within 20 minutes of implantation, although the increasing ED₅₀ values after 24 hours indicate the development of tolerance as discussed below [41]. In the present study, pellets induced antinociceptive effects within 30 minutes post-implantation, while pumps never delivered sufficient morphine to induce antinociception. By 48 hours post-implantation, pellet-implanted mice no longer showed antinociception, as determined by tail flick. Mice injected twice daily with 20 mg/kg morphine continued to exhibit analgesia for the full week, although tolerance began to develop within 24 hours as indicated by declining latency.

Withdrawal behaviors as defined by Way et al. include stereotyped jumping, defecation/urination, increased motor activity, and paw tremors/body shakes. These symptoms are shorter in duration but greater in intensity when precipitated by naloxone [49]. In their 1975 study, Patrick and Dewey observed tolerance, as measured by tail-flick latency, in mice implanted with 75 mg pellets within 24 hours post-implantation; they observed dependence, as indicated by naloxone-precipitated withdrawal, within 6 hours of implantation [41]. Despite the non-detectable analgesic effect of the dosage of morphine delivered by osmotic pump, we observed significant withdrawal behaviors in both pump- and pellet-implanted mice at 24, 72, and 168 hours post-implantation. 25 mg morphine pellets induced a slow rise in morphine ED₅₀ that plateaued 3 days post-implantation [40]. Way et al. [21] observed a 70-fold drop in the ED₅₀ for naloxone between 3 hours and 72 hours post-implantation. In comparing these results to other methods of delivery (acute injection, daily injection), Dighe et al. [15] suggested that the steady infusion of morphine provided by the subcutaneous pellet induced a greater magnitude of tolerance than injection delivery. Delivery-dependent differences in tolerance may have contributed to the decline in analgesic efficacy in pellet-implanted mice, as well as the development of dependence in pump-implanted mice (Figure 2). Dependence in the absence of any detectable antinociceptive effects in osmotic pump-implanted mice is intriguing. Within chronic pain populations, opioids often fail to provide effective analgesia from the underlying pain condition while continuing to contribute towards tolerance, dependence, and ultimately addiction [9,10,50]. Further studies to assess levels of morphine provided by these delivery methods in critical brain regions – e.g., periaqueductal gray, nucleus accumbens – and how these measures correlate to antinociception, tolerance and dependence would be beneficial to our understanding of the effects of chronic morphine treatment in the central nervous system and periphery.

Conclusion

Subcutaneous slow-release pellets and osmotic pumps differed significantly in plasma drug concentrations delivered, analgesic efficacy, and dependence measures of morphine treatment in mice. Osmotic pumps were limited by their volume and the solubility

limitations of morphine, and did not provide an antinociceptive dosage. Pellets delivered the bulk of their dosage within 24 hours post-implantation, but maintained antinociception for up to 36 hours post-implantation. Overall, both methods induced physical dependence as measured by naloxone-precipitated withdrawal behaviors for up to one week post-implantation. These factors should be considered when chronic morphine experiments are designed, and these data highlight the importance of monitoring plasma morphine concentrations throughout chronic morphine treatment paradigms. Alternatively, more potent clinically relevant opioid analgesics could be formulated as pellets and/or used in osmotic minipumps to facilitate future studies on the chronic use of opioids under a variety of conditions.

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Figure 1. Pharmacokinetics of slow-release pellet vs. osmotic pump delivery of morphine
Male C57BL/6NCR mice (n=3) were implanted with a morphine sulfate pellet (25 mg) or an osmotic pump (64 mg/mL, 1.0 μ L/hour). Blood was drawn at multiple timepoints over 7 days, either retro-orbitally or through a terminal cardiac puncture. Morphine plasma levels were measured by LC-MS/MS. Significance is shown as * $p < 0.05$ vs. pump, as determined by a two-way ANOVA with a Student-Newman-Keuls post hoc test.

Figure 2. Osmotic pumps provide a non-analgesic dosage of morphine

Male C57BL/6Ncr mice (n=10) were treated with three distinct paradigms: (i) acute injection (20 mg/kg, s.c., twice daily); (ii) osmotic pump (64 mg/mL, 1.0 μ L/hour); and (iii) slow-release pellet (25 mg). Control mice (n=5) received placebo pumps or pellets. Immediately prior to morphine treatment, animals' baseline response was measured ('baseline'). Following morphine implantation/injection, mice were tested for 7 days with tail flick test at identical timepoints to those taken for the pharmacokinetics study (figure 1). For each test, animals' tails were submerged in 50°C water and observed for tail withdrawal. Tails were removed after 10 sec to prevent damage. Significance is shown as * $p < 0.05$ compared to control (placebo pellet or pump), # $p < 0.05$ compared to morphine S.C. injection, \$ $p < 0.05$ compared to osmotic pump, as determined by a two-way ANOVA with a Student-Newman-Keuls post hoc test.

Figure 3. Morphine delivery systems induce different withdrawal behaviors

Male C57BL/6NCR mice (n=5) were implanted with a morphine sulfate pellet (25 mg), an osmotic pump (64 mg/mL, 1.0 μ L/hour), or injected subcutaneously twice daily (20 mg/kg). Control mice received no treatment (“no naloxone”) or a naloxone injection without prior morphine treatment (“naloxone”). Withdrawal was induced at 1, 3, or 7 days post-implantation with an i.p. injection of 10 mg/kg naloxone. (A) After naloxone injection, mice were placed within a Plexiglas column and video-recorded for 20 minutes. Vertical jumps were assessed over the 20-minute period. (B) After completion of the jumping test, filter paper at the bottom of the column was weighed to measure feces and urine excreted. (C) Animal weight was compared before and after naloxone injection. Significance is shown as * $p < 0.05$ compared to non-morphine controls, # $p < 0.05$ compared to morphine S.C. injection, \$ $p < 0.05$ compared to osmotic pump within each timepoint, as determined by a two-way ANOVA with a Student-Newman-Keuls post hoc test. Bracketed groups indicate a significant difference between timepoints within the delivery method.

Table 1

Summary of results.

Time	Morphine Sulfate Pellet (25 mg, s.c.)	Osmotic Pump (64 mg/mL, 1.0 μ L/hour)	Subcutaneous injection (20 mg/kg, twice daily)
<i>Plasma Concentration (ng/mL)</i>			
24 hours	2695.3 $\pm$ 785.1 ^{\$}	209.7 $\pm$ 50.0	N/A
72 hours	1039.5 $\pm$ 73.5 ^{\$}	57.3 $\pm$ 39.1	N/A
168 hours	229.5 $\pm$ 92.5 ^{\$}	45.8 $\pm$ 25.4	N/A
<i>Antinociception (tail-flick latency, sec)</i>			
24 hours	6.4 $\pm$ 0.6 ^{,\$,#,*}	2.5 $\pm$ 0.2 [#]	7.9 $\pm$ 0.8 [*]
72 hours	4.2 $\pm$ 0.5 [#]	3.2 $\pm$ 0.2 [#]	7.1 $\pm$ 0.4 [*]
168 hours	3.2 $\pm$ 0.3 [#]	3.5 $\pm$ 0.3 [#]	5.5 $\pm$ 0.2 [*]
<i>Naloxone-precipitated withdrawal (number of jumps)</i>			
24 hours	61.4 $\pm$ 11.7 [*]	72.0 $\pm$ 16.6 [*]	35.4 $\pm$ 5.1
72 hours	130.6 $\pm$ 21.8 ^{,\$,*}	107.8 $\pm$ 32.2 [*]	51.8 $\pm$ 12.2 [*]
168 hours	154.2 $\pm$ 21.1 ^{,\$,#,*}	84.6 $\pm$ 12.6 [*]	89.6 $\pm$ 12.2 [*]

Significance is shown as

*
p < 0.05 compared to non-morphine-treated controls,#
p < 0.05 compared to morphine S.C. injection,\$
p < 0.05 compared to osmotic pump as determined by a two-way ANOVA with a Student-Newman-Keuls post hoc test.