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Pharmacology, Biochemistry and Behavior 84 (2006) 487–496
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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# Effects of morphine on circadian rhythms of motor activity and body temperature in pig-tailed macaques

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Received 17 October 2005; received in revised form 1 June 2006; accepted 15 June 2006
Available online 21 July 2006

**Abstract**

Previous studies of the effects of opiates on motor activity and body temperature in nonhuman primates have been limited in scope and typically only conducted with restrained animals. The present study used radio-telemetry devices to continuously measure activity and temperature in unrestrained pig-tailed macaques for 24 h following morphine administration. Two dose–response functions (0.56 to 5.6 mg/kg, i.m.) were determined, one with morphine administered at 9 a.m. and one with morphine administrated at 3 p.m. Under both the 9 a.m. or 3 p.m. administration schedules, body temperature and activity were increased acutely. Activity was also reduced the following morning after morphine administered at either time. In other regards, morphine's effects on both temperature and activity differed between 9 a.m. and 3 p.m. injection, including periods of decreased activity immediately after the acute increases after 9 a.m. but not 3 p.m. administration. Surprisingly, motor activity also increased 9–12 h post-injection following morphine administered at 9 a.m., but not at 3 p.m. These results clearly show an interaction between timing of morphine administration and effects on temperature and activity. These results also underscore the fact that single injections of drugs may have multiple and delayed effects on circadian rhythms in macaques.


*Keywords:* Morphine; Pig-tailed macaque; Motor activity; Body temperature; Radio-telemetry; Circadian rhythm

## 1. Introduction

Morphine administration affects locomotion in several species and generally has biphasic effects on locomotor activity (Swerdlow et al., 1986; Uhl et al., 2002). Low and moderate doses of morphine stimulate locomotor activity while higher doses of morphine produce sedation (Swerdlow et al., 1986; Uhl et al., 2002). The vast majority of data on locomotor activity come from rodents where simple and inexpensive techniques for locomotor measurement are common, and where some species differences are known to exist. In rats, for instance, higher doses of morphine produce acute decreases in activity followed by delayed periods of hyperactivity 3–5 h after injection (Browne and Segal, 1980; Dafters and Taggart, 1992). In mice, however, morphine's effects may be strain-dependent with some strains demonstrating dose-related increases in motor activity (e.g. C57BL/6J) (Browne and Segal, 1980), and other strains demonstrating decreased activity following morphine administration (e.g. DBA/2J) (Frischknecht et al., 1988). Biphasic responses, with decreases followed by increases, are also common in mice (Belknap et al., 1998; Patti et al., 2005). In contrast to rodents, few studies have investigated the effects of morphine on locomotor activity in nonhuman primates. Morphine has been shown to increase activity in an observational study of freely moving marmosets (Guard et al., 2002). Additionally, the long-acting opiate acetylmethadol (LAAM) given every other day, has been shown to increase motor activity on drug days and decrease activity on non-drug days in pig-tailed macaques (Crowley et al., 1985).

Morphine and other opiates also affect thermoregulation, and these effects are distinct from the effects of increased activity on body temperature (Adler et al., 1988; Dafters and Taggart, 1992; Baker and Meert, 2002). In rodents, morphine can produce hypothermia or hyperthermia depending upon dose, species, methodologies and ambient temperature (Clark, 1979; Adler et al., 1988; Gonzalez, 1993). Despite the variation in the literature, most often, hyperthermia occurs at low to moderate doses and

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doi:10.1016/j.pbb.2006.06.012

hypothermia at higher doses (Clark, 1979; Adler et al., 1988; Dafters and Taggart, 1992). Hyperthermic and hypothermic actions of low and high doses of morphine and other opioids in rats are likely due to predominately mu-opioid receptor influences at lower doses with higher doses having kappa-opioid, hypothermic, effects (Geller et al., 1983; Clark and Lipton, 1985; Adler et al., 1988; Spencer et al., 1988).

One possible reason for the varied reports in the effects of opiate on thermoregulation is that the measurement of body temperature is typically invasive and stressful to the animal, causing heart rate and body temperature changes (Chen and Herbert, 1995). Procedures used to measure temperature range from mildly stressful restraint and infrared measurement of ear canal temperatures to highly stressful restraint and rectal temperature measurement (Clark, 1979; Adler et al., 1988). Restraint equipment may also vary from study to study, and the type of equipment may interact with the effects of drugs such as morphine to significantly alter its pharmacologic effects (McDougal et al., 1983). Reports vary as to whether restraint alters the effects of drugs on temperature due to the inducement of stress, or to procedural factors such as insufficient heat dissipation (McDougal et al., 1983; Wright and Katovich, 1996); duration of restraint is typically short, however, and therefore measurement of body temperature over long periods of time is less common than acute measurements.

Studies of opiate effects on body temperature have been more prolific with rodents than with nonhuman primates. The published nonhuman primate studies have the potential confounds of chair restraint and rectal temperature measurements and the stress associated with these procedures (Holtzman and Villarreal, 1969). The development of radio telemetry procedures has allowed for the determination of both locomotor activity and body temperature in freely moving animals (Essler and Folk, 1961; Winget and Fryer, 1966). In addition to the removal of confounds such as restraint stress, telemetric measurement allows for convenient measurement over longer periods of time. For animals living in cages equipped with telemetry receivers, activity and temperature can be measured continuously for many months, enabling time course studies of longer-term changes in circadian rhythms. Given the frequency of species differences reported previously among rodents, and the dearth of data from primates, the present studies were designed to investigate the effects of morphine on activity and temperature over a full 24-h circadian period.

## 2. Methods

Four male pig-tailed macaques (3–5 kg) were subjects in this study. The monkeys were implanted with radio telemetry transmitters (TA-D70, Data Sciences International, St. Paul, MN) that transmitted body temperature and locomotor activity. Using aseptic surgical techniques in anesthetized animals, the transmitters were placed in the inter-peritoneal (i.p.) cavity, secured to the right front abdominal wall in 3 monkeys. Due to surgical complications following initial i.p. placement, a transmitter was placed subcutaneously (s.c.) in 1 monkey. For the s.c. placement, the

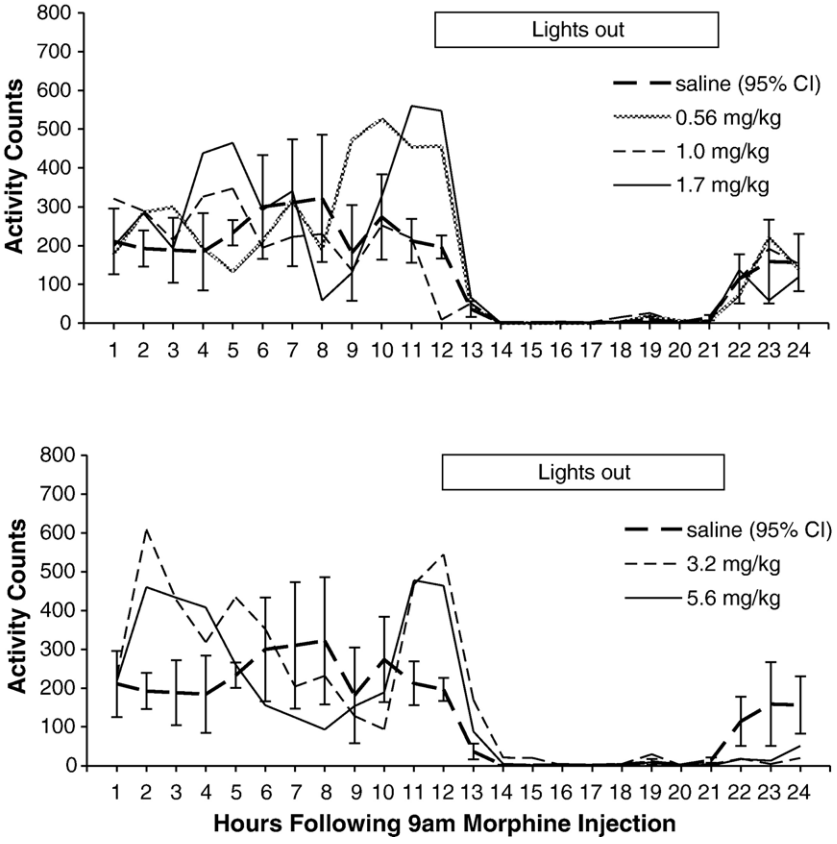


Fig. 1. Circadian patterns of locomotor activity following 9 a.m. injections of morphine. Group means ($N$=4) are represented on the $Y$-axis and hours following injection are represented on the $X$-axis. Error bars around saline means are 95% confidence intervals.

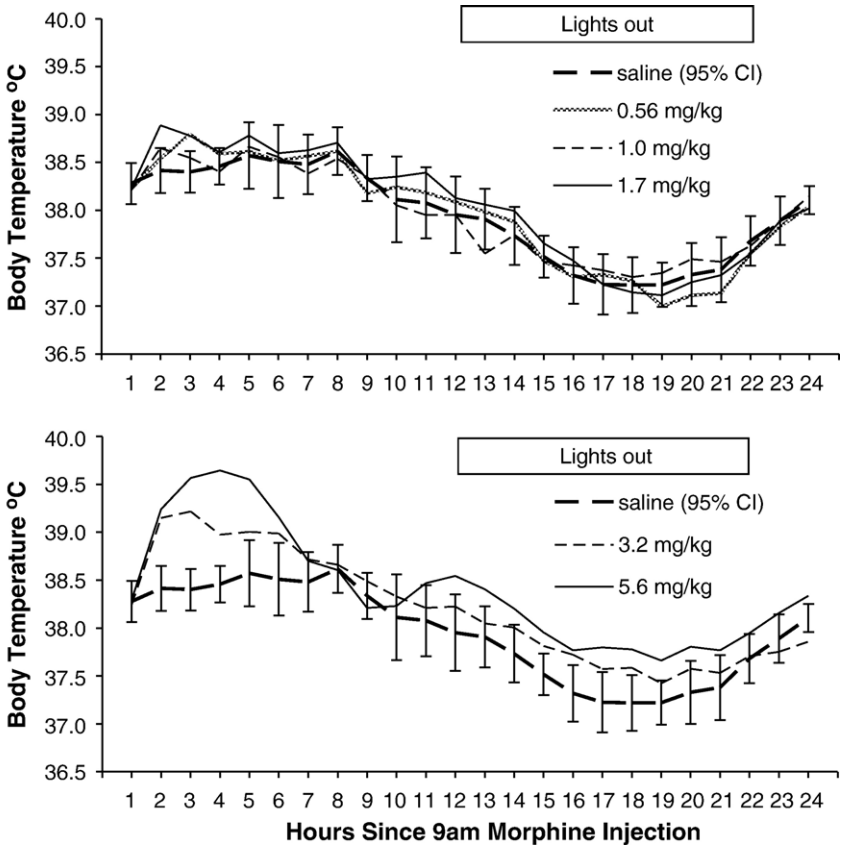


Fig. 2. Circadian patterns of body temperature following 9 a.m. injections of morphine. Group means ($N=4$) are represented on the *Y*-axis and hours following injection are represented on the *X*-axis. Error bars around saline means are 95% confidence intervals.

transmitter was placed on the right side of the monkey's back 5 cm above the pelvic bones (Horn et al., 1998). No significant differences in activity and temperature data were observed between this subject and the other 3 subjects (data not shown).

Each monkey's home cage was equipped with antenna plates (Receiver plate RLA 2000) and connected to the hardware and software of the Data Sciences International data acquisition system. Data collection was computer controlled and occurred every 10 min. Body temperature was read at 200 Hz for a 10-s interval and the average temperature during this interval was recorded every 10 min. An activity count corresponded to a crossing of the midline of the cage. Activity counts were summed over the same 10 min intervals. Room temperatures were recorded on the same sampling schedule and averaged 27 °C.

Monkeys were provided with water *ad lib.* and housed in rooms illuminated from 7 a.m. to 9 p.m. The monkeys were fed an excess of food at approximately 4 p.m. M–F. The monkeys were housed individually with visual contact with each other. Principles of laboratory animal care (Guide for the Care and use of Laboratory Animals, National Academy Press, 1996) were followed, and all protocols were approved by the Institutional Animal Care and Use Committee of The Johns Hopkins University School of Medicine.

### 2.1. Drug administration

Morphine sulfate was obtained from the National Institute on Drug Abuse. Doses were determined from the salt. Morphine was administered intramuscularly with at least 2 days separating drug determinations. Morphine doses were administered in the following order: 3.2, saline, 1.7, saline, saline, 5.6, saline, 1.0, 0.56 mg/kg for morphine administered at 9 a.m. When the 9 a.m. dose–response function was finished another dose–response function was generated in the same fashion with administrations at 3 p.m.

### 2.2. Data analysis

Telemetry data were organized using custom software in MATLAB (The Mathworks, Inc, Natick, MA) that aligned the telemetry data for the time of each day's injection (i.e. 9 a.m. or 3 p.m. injections). Confidence intervals (95%) around the means of data following the four saline administrations were calculated for each of the twenty-four 1-h bins. With large datasets such as these, the use of confidence intervals alone can introduce type I errors based simply on the number of comparisons. In addition, an important assumption of ANOVAs is the homogeneity of variances between cells. Circadian data typically involves period where values are low and periods where values are high. Variances during the low and high periods are often systematically different, as with the activity data from this study. Therefore, the dataset was reduced into 4-h time bins and analyzed using separate repeated measures ANOVAs for each time bin. The use of separate one-way ANOVAs for each time bin met the requirements for homogeneity of variances between cells.

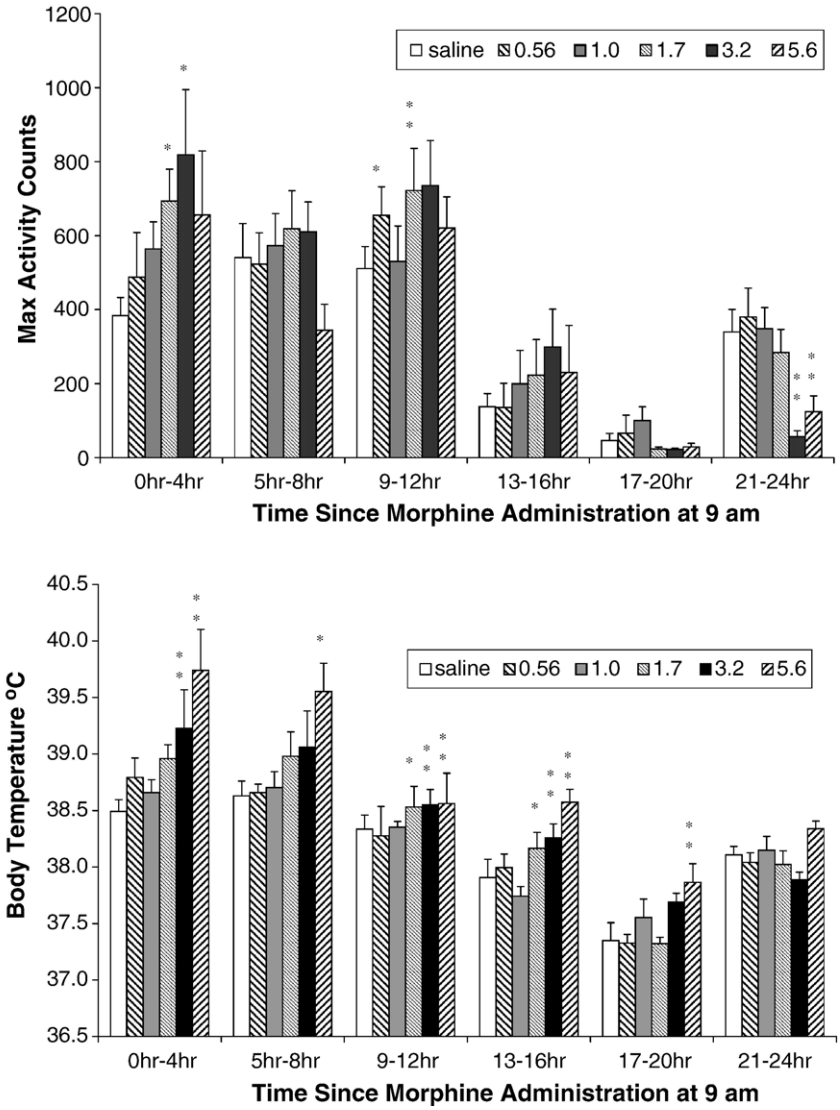


Fig. 3. Locomotor activity (top panel) and body temperature (bottom panels) over selected time bins following 9 a.m. injections of morphine. *X*-axes represent time from morphine administration at 9 a.m. *Y*-axis represents group mean of individual animals' maximum activity count within each time bin. Activity following saline and 5 doses of morphine, mg/kg i.m. are presented for each time bin. Bottom panel, *Y*-axis represents body temperature. Body temperature following saline and 5 doses of morphine, mg/kg i.m. are presented for each time bin. Error bars represent standard error of the mean. *Group mean following that dose differed from saline at the $p<0.05$ level. **Dose differed from saline at the $p<0.01$ significance level.

For the activity data, peak effect analyses were performed by choosing the highest hourly values for individual monkeys in 4-h time bins: (i.e. 0–4 h following injection, 5–8 h, 9–12 h, 13–16 h, 17–20 h, and 21–24 h following injection). The peak effect analysis is useful for data that vary within a time bin. For data such as the activity data, there were often rises followed by falls within a time bin that led to an unchanged bin average. Using peak analysis instead of averages thus provides a more accurate estimate of increases (or decreases by analyzing nadirs) than does averaging for biphasic data. Area under the curve (AUC) analyses were also performed and produced similar results (data not shown); however, as with averaging, AUC analyses are insensitive to biphasic changes within a given time bin; therefore, AUC analyses are not reported. Temperature data did not display the dramatic within-bin changes seen with the activity data and therefore analyses of bin mean temperature and bin peak temperature produced identical results (data not shown). Mean temperatures in 4-h time bins are presented for simplicity.

Peak effect data were analyzed statistically using repeated measures (RM) ANOVAs (GB-Stat software; Dynamic Microsystems, Inc., Silver Spring, MD). Activity data were subject to a logarithmic transform prior to ANOVA to increase homogeneity of variance between cells. Between cell variance for temperature data was homogeneous and therefore temperature data were not transformed. Planned, *a priori*, post-hoc comparisons were performed to compare morphine doses to saline using Dunnet's procedure. Statistically significant post-hoc results are reported when a given dose differed from saline.

Formal analysis of data was limited to 24 h post-injection for logistical reasons (i.e. there were veterinary or husbandry procedures carried out the 2nd day after data collection that influenced activity or temperature data).

Mean saline data from all four saline administrations (4-h bins) were analyzed with RM ANOVAs [3,20] (within factor of time) to ensure that there were no residual changes in residual activity or body temperature patterns following the repeated morphine administrations. These analyses included data from both 9 a.m. and 3 p.m. time points for all four saline administrations.

## 3. Results

### 3.1. Morphine administered at 9 a.m.

Fig. 1 indicates that mean locomotor activity was significantly increased following morphine administration at 9 a.m., based on the group mean temperature exceeding the 95% confidence intervals of the mean following saline. Fig. 1, lower panel, indicates that the two highest doses, 3.2 mg/kg and 5.6 mg/kg significantly increased activity for 5–6 h. The initial increase was followed by a return to baseline activity levels after all but the 5.6 mg/kg dosage where activity was decreased in hours 6–8. Surprisingly, there was an additional increase in activity prior to the dark period around 10–12 h after morphine administration. The morning after morphine administration at 9 a.m., activity was decreased following the 3.2 and 5.6 mg/kg doses. Fig. 2 indicates that body temperature was significantly increased following morphine administration. Body temperatures remained elevated for approximately 7 h following either 5.6 or 3.2 mg/kg morphine.

Fig. 3 illustrates the peak or maximum activity (upper panel) and mean temperature (lower panel) in 4-h bins following saline and morphine administration. Repeated measures ANOVA confirmed the significant increases in activity levels and body temperature relative to saline administration apparent in Fig. 1. There was a significant main effect on maximum activity during the 0–4 h bin following morphine administration at 9 a.m., $F(3,20)=3.13$, $p<0.05$. Post-hoc tests revealed that 1.7, and 3.2 mg/kg morphine differed from saline ($p<0.05$ each). The lack of significant differences in peak activity between morphine and saline in the 5–8 h bin suggests a return to baseline levels of activity; however, there was a significant main effect of the repeated measures ANOVA on the activity nadir during the 5–8 h bin, $F(3,20)=4.75$, $p<0.05$ (data not shown). Post-hoc tests confirmed that the activity nadir of the 5–8 h bin was decreased following 1.0, 1.7 and 5.6 mg/kg morphine, relative to saline ($p<0.05$). These results also highlight the usefulness of peak or nadir analyses in the analyses of biphasic data, as peak activity was unchanged but the nadir decreased in the 5–8 h bin.

The delayed peak in activity 9–12 h following morphine administration evident in Fig. 1 was confirmed by a significant main effect, $F(3,20)=6.17$, $p<0.01$ of morphine on activity in this bin. Post-hoc tests confirmed increases in activity in the 9–12 h bin for 0.56 ($p<0.05$) and 1.7 ($p<0.01$) mg/kg morphine. Morphine administration at 9 a.m. reduced peak activity in the 21–24 h bin, main effect $F(3,20)=10.61$, $p<0.01$. The 3.2 and 5.6 mg/kg doses of morphine given at 9 a.m. significantly reduced activity 20–24 h following administration ($p<0.01$, each).

Morphine produced significant main effects on mean temperature in each time bin during the 24 h recordings: main effects in

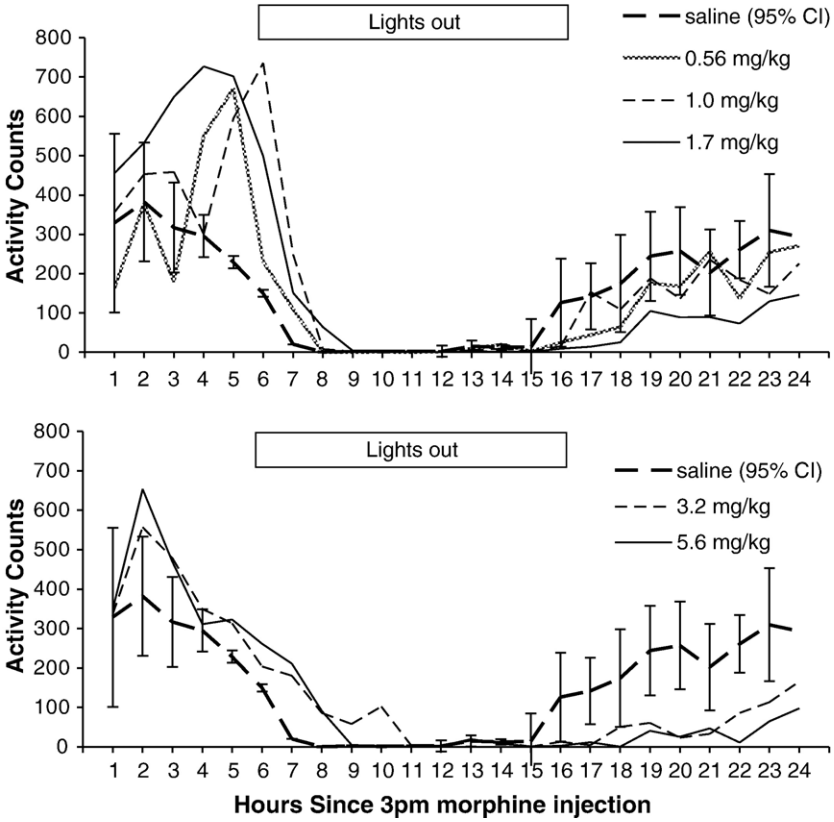


Fig. 4. Circadian patterns of locomotor activity following 3 p.m. injections of morphine. Graph details are the same as Fig. 1.

0–4 h bin $F(3,20)=9.89$, $p<0.01$; in 5–8 h bin $F(3,20)=3.4$, $p<0.05$; in 9–12 h bin $F(3,20)=6.87$, $p<0.01$; in 13–16 h bin $F(3,20)=8.82$, $p<0.01$; in 17–20 h bin $F(3,20)=3.67$, $p<0.05$; and in 21–24 h bin $F(3,20)=5.62$, $p<0.01$. Post-hoc tests confirmed that temperature following 3.2 and 5.6 mg/kg morphine was significantly above saline in the 1–4 h bin ($p<0.01$ for both). In the 5–8 h bin, 5.6 mg/kg morphine significantly increased temperatures ($p<0.05$). In the 9–12 h bin 1.7, 3.2 and 5.6 mg/kg morphine all significantly increased body temperature ($p<0.05$ for both 1.7 and 3.2 and $p<0.01$ for 5.6 mg/kg). Similarly, in the 13–16 h bin 1.7, 3.2 and 5.6 mg/kg morphine all significantly increased body temperature ($p<0.05$ for 1.7 and $p<0.01$ for both 3.2 and 5.6 mg/kg). Body temperature was increased by 5.6 mg/kg morphine in the 17–20 h bin, and there was a significant main effect of temperature without significant drug vs. saline post-hoc comparisons in the 21–24 h bin.

### 3.2. Morphine administered at 3 p.m.

Locomotor activity data presented in Fig. 4 indicate that activity also increased following morphine administration at 3 p.m. Following an acute increase in activity there was increased activity in the dark phase and decreased activity the following morning (e.g. activity following 3.2 mg/kg was increased above saline levels in hours 7–10 and activity following 5.6 mg/kg morphine was above saline levels in hours 7 and 8). Fig. 5 presents temperature data indicating an acute increase in temperature that remained elevated for 10–12 h with the higher doses of 3.2 and 5.6 mg/kg.

Repeated measures ANOVA of data in 4 h bins confirmed these interpretations (Fig. 6). Although the acute increase in activity did not reach statistical significance in the 0–4 h time bin $F(3,20)=1.78$, $p>0.05$, activity was significantly increased in the 5–8 h bin $F(3,20)=9.16$, $p<0.01$. Post hoc tests confirmed significant increases following 0.56, 1.0 and 1.7 mg/kg morphine ($p<0.05$, $p<0.01$, and $p<0.05$, respectively). Peak activity was later significantly reduced in the 13–16 h and 17–20 h time bins ($F(3,20)=4.25$, $p<0.05$; $F(3,20)=11.99$, $p<0.01$ for the main effects, respectively). Peak activity was used to analyze the reductions in these bins because the activity nadirs were zero, introducing floor effects. In the 13–16 h time bin peak activity was decreased following doses of 1.0 mg/kg ($p<0.05$), 1.7 mg/kg ($p<0.01$), 3.2 mg/kg ($p<0.05$), and 5.6 mg/kg doses ($p<0.05$). In the 17–20 h time bins peak activity was decreased for 1.7 mg/kg, 3.2 mg/kg and 5.6 mg/kg doses ($p<0.01$ for each). Peak activity in the 21–24 h bin was not significantly reduced relative to saline.

Fig. 6 also confirms that body temperatures was elevated through the 0–4 and 5–8 h time bins ($F(3,20)=4.82$, $p<0.01$; $F(3,20)=4.24$, $p<0.05$ for the main effects, respectively). Post

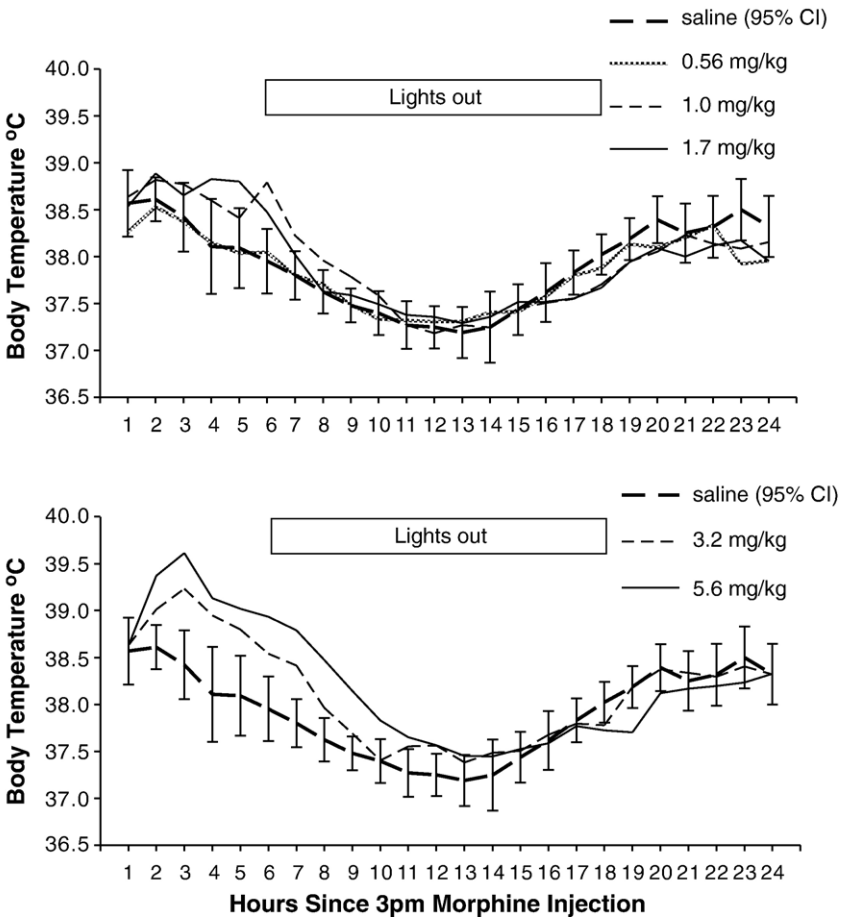


Fig. 5. Circadian patterns of body temperature following 3 p.m. injections of morphine. Graph details are the same as Fig. 2.

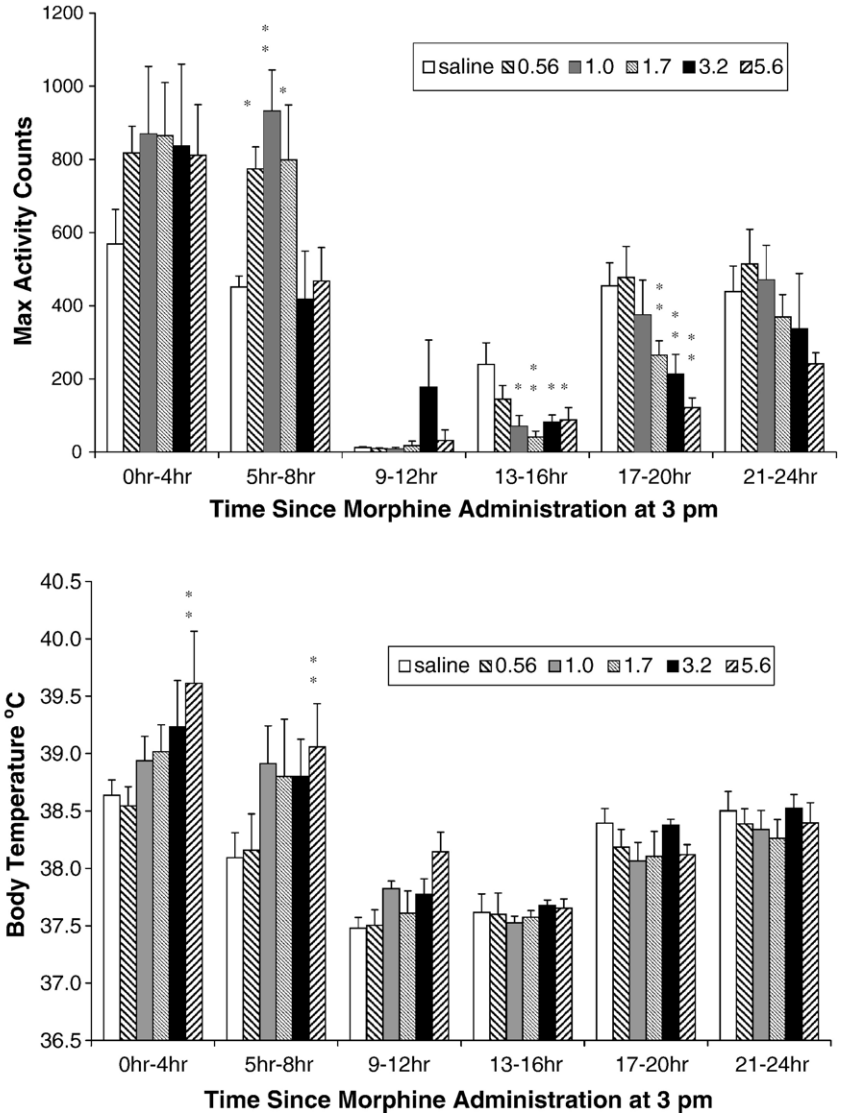


Fig. 6. Locomotor activity (top panel) and body temperature (bottom panels) over selected time bins following 3 p.m. injections of morphine. *X*-axes represent time from morphine administration at 3 p.m. *Y*-axis represents group mean of individual animals' maximum activity count within each time bin. Activity following saline and 5 doses of morphine, mg/kg i.m. are presented for each time bin. Bottom panel, *Y*-axis represents body temperature. Body temperature following saline and 5 doses of morphine, mg/kg i.m. are presented for each time bin. Error bars represent standard error of the mean. *Group mean following that dose differed from saline at the $p<0.05$ level. **Dose differed from saline at the $p<0.01$ significance level.

hoc tests confirmed that 5.6 mg/kg morphine increased body temperature relative to saline in both the 0–4 h and 5–8 h bins ($p<0.01$ for each).

Morphine administered at 3 p.m. did not change peak locomotor activity or body temperature in the 9–12 h bin ($F(3,20)=1.57$, $p>0.05$; $F(3,20)=2.88$, $p>0.05$, respectively), in contrast to morphine's effects 9–12 h after administration at 9 a.m. These results suggest that morphine's effects on locomotor activity and body temperature 9–12 h after injection differ depending upon whether the drug is administered at 9 a.m. or 3 p.m.

### 3.3. Saline administrations

One-way RM ANOVAs with the factors of saline administration (4 administrations) for each 4-h time bin indicated that there were no systematic changes in the circadian patterns of either activity or temperature between the saline administrations at both the 9 a.m. and 3 p.m. time points (data not shown). The lack of systematic changes across the saline administrations indicates that there were no residual effects of the drug-dosing schedule.

## 4. Discussion

These results clearly show that morphine has complex and long-lasting effects on circadian rhythms of spontaneous locomotor activity and core body temperature in pig-tailed macaques. As expected, there was an acute increase in both activity and temperature after morphine administration. These acute increases were followed by periods of reduced activity – the 'acute rebound' periods – reminiscent of psychomotor stimulant 'crashes' (Gauvin et al., 1994, 1997), a phenomenon

not previously reported in primates. The finding of a second period of increased locomotor activity was unexpected; this is likely because the methodology previously used to study locomotor activity and/or body temperature did not lend itself to prolonged data acquisition.

Rebound crashes have been previously reported in rodents and sometimes linked to rebound anhedonia (Gauvin et al., 1994, 1997). One suggested mechanism is simply fatigue related to diminished available neurotransmitters. Another suggestion is a homeostatic mechanism that produces effects counter to that of the drug (Solomon and Corbit, 1974; Koob and Le Moal, 2001). When the drug clears the system the homeostatic mechanism would remain in place and produce an overshoot in the opposite direction. Delayed effects of drugs are not uncommon, even at times when pharmacokinetically one would expect the drugs to have been long eliminated. While not well characterized, delayed effects are acknowledged widely enough to influence common behavioral pharmacology methodologies that employ 'wash-out' days between drug injections (e.g. cognitive effects of drugs, drug-discrimination, etc.). The nature of the mechanisms behind delayed increases in locomotor activity reported here remain unclear.

Studies of circadian rhythms have previously shown a number of drugs to have effects lasting longer than the direct effects of the drugs, including ethanol's effects on temperature in humans the night following administration (Eastman et al., 1994; Wasielewski and Holloway, 2001) and common 'hangover' effects the day after alcohol consumption. Morphine administration can induce a phase shift on circadian rhythms of locomotor activity in mice (Marchant and Mistlberger, 1995), and the authors concluded that the effect was due to homeostatic mechanisms responding to the disruptions in circadian rhythms. Delayed effects of ethanol including rebound hyperactivity and hyperthermia 30–40 h following drug administration have been attributed to homeostatic responses following acute hypoactivity and hypothermia (Holloway et al., 1993). Such homeostatic mechanisms would be consistent with the results of the current study in that disruptions in circadian patterns were seen following acute changes in activity produced by morphine.

The finding of hyperthermia following the higher doses of morphine was somewhat unexpected as hypothermia has been reported following high doses of morphine in rodents (Baker and Meert, 2002). Baker and Meert (2002) reported 2–3 degree drops in temperature in mice following 10 or 40 mg/kg morphine (Baker and Meert, 2002). Estimates of interspecies scaling would place these mouse doses equivalent to 2–8 mg/kg morphine in the macaque (Mordenti and Chappell, 1989); therefore it would not be unreasonable to have expected hypothermia. However, the present study did not include high enough doses to fully address this issue.

The unexpected results of this study highlight the advantages of noninvasive measurement procedures over long periods of time. Once a transmitter is implanted, data acquisition becomes noninvasive and does not restrict movement or stress the animal. When measured in an animal's home cage, this technology allows for extended monitoring of activity and temperature, as reported in this study. Previous studies using telemetric techniques in rodents have used test chambers equipped with receivers (as opposed to home cages), limiting the length of data collection to 4–8 h recordings (Dafters and Taggart, 1992; Ansah et al., 1996).

Pig-tailed macaques administered cocaine daily at 9 a.m. showed a pattern of acute and delayed increases in activity similar to those found in the current study (Weed, unpublished observations). In addition, previously published research in this laboratory on cocaine's locomotor effects in baboons showed some similarities to the present study (Hienz et al., 1992). Baboons with collar-mounted activity monitors were administered cocaine i.m. at 1 p.m. For one baboon, 0.32 mg/kg cocaine increased activity acutely for approximately 1 h (during the operant training session) then again 4 h post-injection (after the baboon was returned to its home cage). Activity returned to baseline for hours 4–8 and was increased in hours 8–10 around lights-out and into the dark phase. In another baboon, a dose of 1.8 mg/kg cocaine produced acute *decreases* in activity for 2–3 h followed by an increase in activity relative to saline injection (4–8 h). Although, procedural differences make formal comparisons between these studies problematic (e.g., differing drug administration times, behavioral testing after drug administration) they do indicate that similar delayed increases in activity have been reported previously.

Morphine has been posited to increase locomotor activity via dopaminergic mechanisms (Swerdlow et al., 1986). Together, the observations of delayed activity increases after morphine and cocaine administration are consistent with involvement of a dopaminergic mechanism; however, this assertion is preliminary, and there is admittedly no obvious pharmacologic mechanism for the delayed locomotor and thermoregulatory effects observed for morphine. There are active metabolites of morphine that have locomotor activating effects (Handal et al., 2002); however, there is no pharmacokinetic reason to expect a surge of metabolites needed to cause activity increases in the 10–13 h bin. One possibility for the delayed increases is that the animals are having trouble going to sleep. This hypothesis is unlikely because morphine administered at 9 a.m. did not appear to retard the onset of sleep (or near-zero activity) in the dark phase, as the first hour with near-zero activity was hour 14 for saline and morphine alike (Fig. 1). Activity in hour 14 was unchanged despite the activity increase in hours 10–13 in the light phase. Additionally, morphine administered at 3 p.m. did appear to retard onset of sleep, but it did not produce increases in activity when the 10–13 h bin occurred during the dark phase. Therefore, it is difficult to reconcile the delayed increases in activity with changes in sleep patterns.

However, the reductions in activity the following morning after morphine may well result from changes in sleep patterns. This is most apparent following 3 p.m. administration of the higher doses (3.2 and 5.6 mg/kg) where sleep onset is delayed, but morphine at 9 a.m. also reduced activity the following morning. In addition to delaying onset, sleep quality may have been affected. Morphine can affect sleep architecture, reducing rapid-eye-movement sleep in humans (Shaw et al., 2005) and in rats (Robert et al., 1999), and while the present study did not address sleep architecture, reduction of sleep quality may have negatively influenced activity the next morning.

The study of how the time of day affects a given drug's pharmacology has given rise to a new field of study, namely chronopharmacology. Morphine's pharmacology differs depending upon when in the light/dark cycle morphine is administered. For instance, the magnitude of morphine's analgesic effect in mice is larger in the dark phase than in the light phase for a given dose (Bornschein et al., 1977; Yoshida et al., 2003) (although there may be strain differences in the timing of the diurnal changes in morphine analgesia (Cui et al., 2005)). Photoperiod can also affect other pharmacological effects of morphine: both acute and chronic alterations in the light/dark schedule can attenuate morphine's ability to engender conditioned place preference in mice (Tahsili-Fahadan et al., 2005). The mechanisms behind these chronopharmacologic effects have yet to be elucidated, but they may involve interactions with hormones that show diurnal variation (e.g. melatonin).

In summary, morphine produced acute increases in gross motor activity and body temperature in pig-tailed macaques. A second bout of increased activity approximately 9–12 h after injection was seen with morphine given at 9 a.m. The delayed increase in activity depended upon when in the circadian rhythm the drug was administered, in that morphine given at 3 p.m. did not produce delayed increases in activity. Thus the delayed increase in activity was seen when some motor activity was present, (approximately 7–9 p.m. at the end of the light phase) but not during periods of minimal activity (2–3 a.m.) in the dark phase. Morphine produced significant 'hangover' effects evidenced by decreased activity the following morning post injection whether administered at 9 a.m. or 3 p.m. That the 'hangover' effects of morphine were more pronounced following 3 p.m. administration is also consistent with circadian influences on drug effects.

## Acknowledgements

This paper was supported by NIH grants DA05831 (MRW), and DA13343 (MRW and RDH), as well as a gift from the Susan R. Scherer Educational Foundation.

## References

Adler MW, Geller EB, Rosow CE, Cochin J. The opioid system and temperature regulation. Annu Rev Pharmacol Toxicol 1988;28:429–49.

Ansah TA, Wade LH, Shockley DC. Changes in locomotor activity, core temperature, and heart rate in response to repeated cocaine administration. Physiol Behav 1996;60:1261–7.

Baker AK, Meert TF. Functional effects of systemically administered agonists and antagonists of mu, delta, and kappa opioid receptor subtypes on body temperature in mice. J Pharmacol Exp Ther 2002;302:1253–64.

Belknap JK, Riggan J, Cross S, Young ER, Gallaher EJ, Crabbe JC. Genetic determinants of morphine activity and thermal responses in 15 inbred mouse strains. Pharmacol Biochem Behav 1998;59:353–60.

Bornschein RL, Crockett RS, Smith RP. Diurnal variations in the analgesic effectiveness of morphine in mice. Pharmacol Biochem Behav 1977;6:621–6.

Browne RG, Segal DS. Behavioral activating effects of opiates and opioid peptides. Biol Psychiatry 1980;15:77–86.

Chen X, Herbert J. Regional changes in c-*fos* expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothermic and endocrine responses. Neuroscience 1995;64:675–85.

Clark WG. Influence of opioids on central thermoregulatory mechanisms. Pharmacol Biochem Behav 1979;10:609–13.

Clark WG, Lipton JM. Changes in body temperature after administration of acetylcholine, histamine, morphine, prostaglandins and related agents: II. Neurosci Biobehav Rev 1985;9:479–552.

Crowley TJ, Macdonald MJ, Zerbe G. Variability in simian motor and social behavior with alternating-day acetylmethadol. Psychopharmacology (Berl) 1985;85:353–60.

Cui Y, Sugimoto K, Araki N, Sudoh T, Fujimura A. Chronopharmacology of morphine in mice. Chronobiol Int 2005;22:515–22.

Dafters R, Taggart P. Biotelemetric investigation of morphine's thermic and kinetic effects in rats. Psychopharmacology 1992;106:195–201.

Eastman CI, Stewart KT, Weed MR. Evening alcohol consumption alters the circadian rhythm of body temperature. Chronobiol Int 1994;11:141–2.

Essler WO, Folk Jr GE. Determination of physiological rhythms of unrestrained animals by radio telemetry. Nature 1961;190:90–1.

Frischknecht HR, Siegfried B, Waser PG. Opioids and behavior: genetic aspects. Experientia 1988;44:473–81.

Gauvin DV, Goulden KL, Holloway FA. A three-choice haloperidol–saline–cocaine drug discrimination task in rats. Pharmacol Biochem Behav 1994;49:223–7.

Gauvin DV, Briscoe RJ, Baird TJ, Vallett M, Carl KL, Holloway FA. Physiological and subjective effects of acute cocaine withdrawal (crash) in rats. Pharmacol Biochem Behav 1997;57:923–34.

Geller EB, Hawk C, Keinath SH, Tallarida RJ, Adler MW. Subclasses of opioids based on body temperature change in rats: acute subcutaneous administration. J Pharmacol Exp Ther 1983;225:391–8.

Gonzalez LP. Cocaine alters body temperature and behavioral thermoregulatory responses. NeuroReport 1993;4:106–8.

Guard HJ, Newman JD, Roberts RL. Morphine administration selectively facilitates social play in common marmosets. Dev Psychobiol 2002;41:37–49.

Handal M, Grung M, Skurtveit S, Ripel A, Morland J. Pharmacokinetic differences of morphine and morphine–glucuronides are reflected in locomotor activity. Pharmacol Biochem Behav 2002;73:883–92.

Hienz RD, Turkkan JS, Spear DJ, Sannerud CA, Kaminski BJ, Allen RP. General activity in baboons measured with a computerized, lightweight piezoelectric motion sensor: effects of drugs. Pharmacol Biochem Behav 1992;42:497–507.

Holloway FA, Miller JM, King DA, Bedingfield JB. Delayed ethanol effects on physiological and behavioral indices in the rat. Alcohol 1993;10:511–9.

Holtzman SG, Villarreal JE. Morphine dependence and body temperature in rhesus monkeys. J Pharmacol Exp Ther 1969;166:125–33.

Horn TF, Huitron-Resendiz S, Weed MR, Henriksen SJ, Fox HS. Early physiological abnormalities after simian immunodeficiency virus infection. Proc Natl Acad Sci U S A 1998;95:15072–7.

Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001;24:97–129.

Marchant EG, Mistlberger RE. Morphine phase-shifts circadian rhythms in mice: role of behavioural activation. NeuroReport 1995;7:209–12.

McDougal JN, Marques PR, Burks TF. Restraint alters the thermic response to morphine by postural interference. Pharmacol Biochem Behav 1983;18:495–9.

Mordenti J, Chappell W. The use of interspecies scaling in toxicokinetics. In: Y. A., K. J., B. V., Y. A., K. J., B. Vs., editors. Toxicokinetics and new drug development. New York: Pergamon Press; 1989. p. 42–96.

Patti CL, Frussa-Filho R, Silva RH, Carvalho RC, Kameda SR, Takatsu-Coleman AL, et al. Behavioral characterization of morphine effects on motor activity in mice. Pharmacol Biochem Behav 2005;81:923–7.

Robert C, Stinus L, Limoge A. Sleep impairments in rats implanted with morphine pellets. Neuropsychobiology 1999;40:214–7.

Shaw IR, Lavigne G, Mayer P, Choiniere M. Acute intravenous administration of morphine perturbs sleep architecture in healthy pain-free young adults: a preliminary study. Sleep 2005;28:677–82.

Solomon RL, Corbit JD. An opponent-process theory of motivation: I. Temporal dynamics of affect. Psychol Rev 1974;81:119–45.

Spencer RL, Hruby VJ, Burks TF. Body temperature response profiles for selective mu, delta and kappa opioid agonists in restrained and unrestrained rats. J Pharmacol Exp Ther 1988;246:92–101.

Swerdlow NR, Vaccarino FJ, Amalric M, Koob GF. The neural substrates for the motor-activating properties of psychostimulants: a review of recent findings. Pharmacol Biochem Behav 1986;25:233–48.

Tahsili-Fahadan P, Yahyavi-Firouz-Abadi N, Ghahremani MH, Dehpour AR. Effect of light/dark cycle alteration on morphine-induced conditioned place preference. NeuroReport 2005;16:2051–6.

Uhl GR, Hall FS, Sora I. Cocaine, reward, movement and monoamine transporters. Mol Psychiatry 2002;7:21–6.

Wasielewski JA, Holloway FA. Alcohol's interactions with circadian rhythms. A focus on body temperature. Alcohol Res Health 2001;25:94–100.

Winget CM, Fryer TB. Telemetry system for the acquisition of circadian rhythm data. Aerosp Med 1966;37:800–3.

Wright BE, Katovich MJ. Effect of restraint on drug-induced changes in skin and core temperature in biotelemetered rats. Pharmacol Biochem Behav 1996;55:219–25.

Yoshida M, Ohdo S, Takane H, Tomiyoshi Y, Matsuo A, Yukawa E, et al. Chronopharmacology of analgesic effect and its tolerance induced by morphine in mice. J Pharmacol Exp Ther 2003;305:1200–5.