Buprenorphine Disrupts Sleep and Decreases Adenosine Concentrations in Sleep-regulating Brain Regions of Sprague Dawley Rat

Elizabeth A. Gauthier, B.S.,* Sarah E. Guzick, B.S.,† Chad M. Brummett, M.D.,‡
Helen A. Baghdoyan, Ph.D.,§ Ralph Lydic, Ph.D.§

ABSTRACT

Background: Buprenorphine, a partial μ-opioid receptor agonist and κ-opioid receptor antagonist, is an effective analgesic. The effects of buprenorphine on sleep have not been well characterized. This study tested the hypothesis that an antinociceptive dose of buprenorphine decreases sleep and decreases adenosine concentrations in regions of the basal forebrain and pontine brainstem that regulate sleep.

Methods: Male Sprague Dawley rats were implanted with intravenous catheters and electrodes for recording states of wakefulness and sleep. Buprenorphine (1 mg/kg) was administered systemically *via* an indwelling catheter and sleep–wake states were recorded for 24 h. In additional rats, buprenorphine was delivered by microdialysis to the pontine reticular formation and substantia innominata of the basal forebrain while adenosine was simultaneously measured.

Results: An antinociceptive dose of buprenorphine caused a significant increase in wakefulness (25.2%) and a decrease in nonrapid eye movement sleep (-22.1%) and rapid eye movement sleep (-3.1%). Buprenorphine also increased electroencephalographic delta power during nonrapid eye movement sleep. Coadministration of the sedative-hypnotic eszopiclone diminished the buprenorphine-induced decrease in sleep. Dialysis delivery of buprenorphine significantly decreased adenosine concentrations in the pontine reticular

What We Already Know about This Topic

- Opiate-induced sleep disruption can contribute to hyperalgesia
- Endogenous adenosine promotes sleep and decreases nociception

What This Article Tells Us That Is New

- Antinociceptive doses of buprenorphine disrupted normal sleep architecture in rats, an effect that was attenuated by the sedative-hypnotic eszopiclone
- Buprenorphine also decreased adenosine levels in brain regions known to modulate sleep and nociception

formation (-14.6%) and substantia innominata (-36.7%). Intravenous administration of buprenorphine significantly decreased (-20%) adenosine in the substantia innominata.

Conclusions: Buprenorphine significantly increased time spent awake, decreased nonrapid eye movement sleep, and increased latency to sleep onset. These disruptions in sleep architecture were mitigated by coadministration of the nonbenzodiazepine sedative-hypnotic eszopiclone. The buprenorphine-induced decrease in adenosine concentrations in basal forebrain and pontine reticular formation is consistent with the interpretation that decreasing adenosine in sleep-regulating brain regions is one mechanism by which opioids disrupt sleep.

* Medical Student, † Graduate Student, ‡ Assistant Professor, § Professor, Department of Anesthesiology, University of Michigan, Ann Arbor, Michigan.

Address correspondence to Dr. Lydic: 7433 Medical Science I, 1150 West Medical Center Drive, Ann Arbor, Michigan 48109. rlydic@umich.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

OPIOIDS are used effectively in the treatment of chronic and acute pain, and the extensive use of opioids encourages efforts to develop countermeasures to combat unwanted side effects.[1,2] Opioids disrupt sleep,[3–7] and sleep disruption can contribute to hyperalgesia,[8–16] impaired immune function,[17] and postoperative cognitive impairment.[18,19]

Adenosine is an endogenous neuromodulator that significantly enhances sleep[20] and diminishes nociception.[21] Sleep is increased by increasing adenosine in the pontine reticular formation (PnO)[22–24] and in the substantia innominata (SI) area of the basal forebrain.[20,25] Adenosine concentrations in the PnO and SI are decreased by the μ-opioid receptor agonists morphine and fentanyl.[26]

Buprenorphine, a partial μ-opioid receptor agonist and κ-opioid receptor antagonist, is an effective analgesic, but no previous studies have quantified the effects of buprenorphine on sleep architecture[7,27,28] or on adenosine concentrations in

the PnO and SI. This study was designed to test the hypothesis that buprenorphine decreases sleep and adenosine concentrations in PnO and SI, brain regions known to modulate sleep and nociception.

Materials and Methods

Animals
Adult, male Crl:CD(SD) (Sprague Dawley) rats (n = 26) purchased from Charles River Laboratories (Wilmington, MA) were used for all studies. Rats weighing 250–350 g were used because brains from rats in this weight range are known to fit the rat stereotaxic atlas.[29] Male rats were chosen to facilitate comparison of the current results to previous data obtained from males.[26,30–32] Rats were housed in a 12:12-h light–dark cycle (lights on from 8:00 to 20:00) with access to food and water *ad libitum*. Procedures were reviewed and approved by the University of Michigan Committee on the Use and Care of Animals. Every phase of this study adhered to the *Guide for the Care and Use of Laboratory Animals* (Eighth Edition, National Academy of Sciences Press, Washington DC, 2011).‖

Surgical Procedures
Rats were anesthetized with 3% isoflurane (Hospira, Inc., Lake Forest, IL). The jugular vein was exposed, and a catheter (12 cm of Micro-Renathane tubing [MRE–040], Braintree Scientific, Braintree, MA) was inserted in the direction of the heart. The other end of the catheter was tunneled subcutaneously and implanted between the scapulas. A back-mounted flange guide cannula (8I 1000BM10; Plastics One, Roanoke, VA) and dummy cannula (8IC313DCCACC; Plastics One) were secured with the catheter in the midscapular position. This procedure provided subsequent venous access.

Implantation of the jugular vein catheter was followed immediately by implantation of electrodes for recording sleep. Rats were moved to a Kopf Model 962 small animal stereotaxic instrument fitted with a Model 906 rat anesthesia mask (David Kopf Instruments, Tujunga, CA), and anesthesia was maintained with isoflurane (2.0%). Three electrodes (8IE36320SPCE, Plastics One) for recording cortical electroencephalogram were placed 2.0 mm posterior and 1.3 mm lateral to bregma, 2.0 mm posterior and 1.5 mm lateral to bregma, and 1.0 mm anterior and 1.5 mm lateral to bregma.[29] Two electrodes (4 cm of AG 7/40T Medwire, Mt. Vernon, NY) for electromyogram recordings were placed in the dorsal neck muscle, and a third electrode was placed under the skin of the neck muscle as a reference. The nonimplanted ends of the electroencephalogram and electromyogram electrodes were soldered to electrical contact pins (E363/0; Plastics One) that were plugged into a plastic pedestal (8K00022980IF; Plastics One). Three stainless steel

‖ http://www.nap.edu/catalog.php?record_id=12910. Last accessed May 31, 2011.

anchor screws (MPX-0080–02P-C; Small Parts Inc., Miami Lakes, FL) were placed in the skull to secure the electrodes. Dental acrylic was used to construct a head cap covering the electrodes and to anchor the electrical connector and electrodes to the skull. Rats were then removed from the stereotaxic frame and monitored during recovery from anesthesia. Once ambulatory, animals were returned to their home cages.

Behavioral Conditioning for Sleep Recordings
Rats were given 1 week for surgical recovery and then conditioned for an additional week to 10 days to sleeping in a Raturn (Bioanalytical Systems, West Lafayette, IN) recording chamber. During conditioning, the implanted electrodes were attached by a cable (363–441/six 80CM 6TCM; Plastics One) to amplifiers and a computer for digital recording of electroencephalogram and electromyogram signals. Rats had free access to food and water while in the recording chambers.

Nociceptive Testing
An initial series of experiments was conducted to confirm that the 1 mg/kg dose of buprenorphine produced antinociception as reported previously.[33] Procedures for thermal nociceptive testing have been described in detail.[34,35] Briefly, rats were conditioned to being placed in the plexiglass chamber of a Hargreaves Paw Withdrawal unit (Model 336T; IITC Life Science, Woodland Hills, CA) 1 h each day for the week before data collection. The Model 400 (IITC Life Science) heated glass stand and base was set to 30°C for the last 10 min of each conditioning session, and both hind paws of the rat were exposed to the heat stimulus.[36] Five baseline measurements were taken after the habituation time. As soon as baseline measurements were recorded, saline or buprenorphine hydrochloride (Sigma–Aldrich, Saint Louis, MO; 1 mg/kg) was administered *via* the jugular vein catheter. The injection volume was 1 ml. Measures of paw withdrawal latency (PWL) were taken at 10, 20, 30, 60, 90, and 120 min after saline or buprenorphine administration. A cutoff time of 15 s was set to prevent tissue damage of the hind paw.

Drug Administration and Recordings of Sleep–Wake States
A second series of experiments was designed to quantify the effect of intravenously administered buprenorphine on states of sleep and wakefulness. Buprenorphine was dissolved in sterile saline (pH 5.8 ± 0.2) and administered intravenously in a 1-ml volume at a dose of 1 mg/kg. Saline injection provided a negative control condition.

Recordings of sleep and wakefulness began at 08:00 at the initiation of the light phase of the light–dark cycle. Rats are nocturnal and light onset corresponds to the rat subjective night. To determine whether buprenorphine caused sleep disruption, as do other opioids,[26,37] this study was designed to deliver buprenorphine at light onset. Rats were placed in

the recording chamber, and the electromyogram and electroencephalogram electrodes were attached *via* swivel cable to the amplifiers and computer. All injections were administered during a 4-min interval. The data acquisition software was started when drug or vehicle administration began. The electroencephalogram signals were filtered between 0.3 and 30 Hz and amplified. Each rat (n = 7) received one injection of buprenorphine and one injection of saline separated by at least 1 week. The rats were then allowed to sleep and wake spontaneously for the remainder of the 24-h recording. At the end of the recording interval, rats were returned to the vivarium. Every 10 s of the 24-h recording was scored as wakefulness, nonrapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep. All sleep recordings were also scored by a second individual who was blinded to the injection condition. There was 93% agreement between the two sleep scorers.

A third series of experiments was designed to coadminister the nonbenzodiazepine sedative-hypnotic eszopiclone (Toronto Research Chemicals, Toronto, Canada) with buprenorphine to quantify the effect on sleep and wakefulness. Eszopiclone is a benzodiazepine receptor agonist with a nonbenzodiazepine structure, marketed as Lunesta® (Sunovion Pharmaceuticals, Marlborough, MA). Eszopiclone is the (S)-isomer of the cyclopyrrolone zopiclone and is indicated for the treatment of insomnia.[38] As discussed in detail elsewhere,[26] patients who experience pain often report poor sleep. Clinically used doses of opioids significantly disrupt sleep,[37] and disordered sleep exacerbates pain.[26,39,40] These data raise the question of whether enhancement of sleep by a sedative-hypnotic would have a beneficial effect of diminishing opioid-induced sleep disruption. If so, this would encourage future studies aiming to determine whether combining opioid and sedative-hypnotic therapy could diminish pain. Eszopiclone was dissolved in sterile saline and dimethyl sulfoxide (1%; pH 6.0 ± 0.2) and administered intravenously (3 mg/kg). Buprenorphine (1 mg/kg) was then delivered *via* the same IV cannula. For these studies, rats (n = 4) received an injection of eszopiclone followed immediately by an injection of saline or buprenorphine.

Measurement of Brain Adenosine Concentrations during Microdialysis Delivery of Buprenorphine

A fourth set of experiments sought to identify brain regions through which buprenorphine decreased sleep. Normal cholinergic neurotransmission is essential for maintaining wakefulness, and opioids disrupt cholinergic neurotransmission in the SI region of the basal forebrain.[30] Adenosine is known to promote sleep, and previous studies have shown that adenosine concentrations in the PnO are decreased by fentanyl and morphine.[26] Both fentanyl and morphine cause sleep disruption. Therefore, the current experiments also used *in vivo* microdialysis and high-performance liquid chromatography to measure adenosine concentrations in the PnO and SI during dialysis delivery of buprenorphine.

Buprenorphine (100 μM) was prepared the morning of each experiment. The drug was dissolved in Ringer's solution (pH 5.8–6.2) comprised of 146 nM NaCl, 4.0 mM KCl, 2.4 mM CaCl$_2$, and 10 μM erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA; Sigma–Aldrich), which is an adenosine deaminase inhibitor. Each rat was placed in an induction chamber and anesthetized with 4% isoflurane (Hospira, Inc.) in 100% oxygen. After 5 min, the rat was moved from the chamber into a stereotaxic frame and fitted with a rat anesthesia mask, as described. The isoflurane concentration was reduced to 2.5%. A midline scalp incision was then made to expose lambda and bregma. A rotary tool (Dremel, Racine, WI) was used to make a small craniotomy, through which a dialysis probe was placed in the brain. A rat brain atlas[29] was used to position a CMA-11 microdialysis probe (Cuprophane membrane: 1 mm long, 0.24 mm in diameter, 6-kDa cutoff; CMA Microdialysis, North Chelmsford, MA) in the PnO or in the SI. Stereotaxic coordinates for the PnO were 8.4 mm posterior to bregma, 1.0 mm lateral to the midline, and 9.2 mm below bregma. The coordinates for the SI were 1.6 mm posterior to bregma, 2.5 mm lateral to the midline, and 8.7 mm below bregma. The delivered isoflurane concentration was held at 1.5% and measured continuously throughout the duration of the experiment. A water blanket and recirculating heat pump (Gaymar Industries, Orchard Park, NY) were used to maintain body temperature at 37°C throughout data collection and recovery.

The dialysis probe was perfused with Ringer's solution at a constant flow rate of 2 μl/min using a CMA/400 pump. Dialysis intervals of 15 min produced 30-μl samples, which were injected into an high-performance liquid chromatography system coupled to a UV-Vis detector (wavelength of 254 nm). This system made it possible to express measured adenosine concentrations as nanomolar (nM). The digitized chromatographs were quantified against a standard curve using ChromGraph software (Bioanalytical Systems). Adenosine concentrations were allowed to stabilize for 2 h before beginning data collection. A control sample was collected every 15 min for 1 h during dialysis with Ringer's solution. At the end of the fourth control sample, a liquid switch was activated to begin dialysis with Ringer's solution containing buprenorphine (100 μM). As noted elsewhere,[26,41,42] the characteristics of the dialysis membrane are such that only approximately 5% of the 100 μM buprenorphine was delivered to the brain. After the final dialysis sample was collected, the probe was removed from the brain and the scalp incision was closed. The delivery of isoflurane was discontinued, and the animal was removed from the stereotaxic frame. The rats were returned to their cages and monitored until they were ambulatory.

Histologic Localization of Dialysis Sites

Four to 5 days after the microdialysis experiment, each rat was deeply anesthetized and decapitated. Brains were removed, cut into 40-μm thick coronal sections with a cryostat

(Leica Microsystems, Nussloch, Germany), and slide-mounted serially. Slides with brain sections containing the SI and PnO were fixed in paraformaldehyde vapor (80°C) and stained with cresyl violet. All sections were then digitized using a Nikon Super Coolscan 4000 scanner (Tokyo, Japan). Coronal sections were compared with plates in a rat brain atlas,[29] and the dialysis sites were localized to either the PnO or the SI.

Statistical Analysis

Statistical programs used for data analysis included Prism 5 (Graph Pad Software, Inc., La Jolla, CA) and SAS v9.2 (SAS Institute Inc., Cary, NC). All data were tested to ensure they met the assumptions of the underlying statistical model. PWL was converted to percent maximum possible effect (% MPE) using the following equation: % MPE = (PWL experimental − PWL baseline)/(15 s − PWL baseline) × 100. Repeated measures, two-way analysis of variance (ANOVA) was used to analyze results for changes over time and changes caused by the drug, and Bonferroni *post hoc* tests were used to detect differences at specific time points.

Every 10 s of the 24-h sleep and wakefulness recording was scored as wakefulness, NREM sleep, or REM sleep. Dependent measures included percent of time spent in each state, latency to onset of the first episode of NREM sleep and REM sleep, number of episodes, average episode duration, and number of transitions between states. To avoid the problem of inflated degrees of freedom resulting from the large number of 10-s epochs analyzed, the sleep–wake data were averaged for each rat. Dependent measures of sleep and wakefulness were analyzed by repeated measures two-way ANOVA and paired *t* tests using Bonferroni correction.

As described in detail elsewhere,[24,43,44] fast Fourier transform of the electroencephalogram was performed to determine whether the electroencephalogram was altered by buprenorphine. Electroencephalographic power was analyzed by repeated measures two-way ANOVA and *post hoc* tests for comparison at every 0.5-Hz frequency band (wakefulness and REM sleep 5.0–10.0 Hz; NREM sleep 0.5–5.0 Hz).

For each experiment, adenosine measures during dialysis with Ringer's solution (control) were compared with adenosine concentrations during dialysis delivery of buprenorphine. This design allowed each experiment to contribute one mean adenosine value derived from four control (Ringer's solution) samples and one mean adenosine value derived from four measures obtained during administration of buprenorphine. These values were then averaged across multiple experiments and analyzed individually for PnO and SI brain regions using paired *t* tests. A probability value of $P < 0.05$ was considered statistically significant.

Results

Buprenorphine was Antinociceptive

Figure 1 depicts % MPE for PWL as a function of time after IV administration of saline and buprenorphine. ANOVA
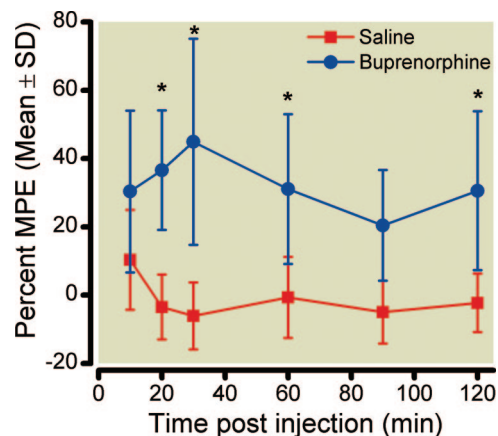

Fig. 1. Buprenorphine increased paw withdrawal latency (PWL) after thermal stimulus. The ordinate shows PWL expressed as percent maximum possible effect (% MPE). The abscissa shows time course of % MPE relative to injection at time zero. Asterisks (*) indicate a significant increase in % MPE after intravenous administration of buprenorphine (1 mg/kg) compared with saline (vehicle control).

revealed that buprenorphine caused significant ($P = 0.0072$) antinociception. Bonferroni *post hoc* comparisons indicated that buprenorphine significantly ($P < 0.05$) increased % MPE at 20, 30, 60, and 120 min after injection. This antinociceptive dose of buprenorphine was used for subsequent studies of sleep and wakefulness.

Buprenorphine Altered the Temporal Organization of Sleep and Wakefulness

Figure 2 illustrates the temporal distribution of wakefulness, NREM sleep, and REM sleep for 24 h after IV administration of saline (control) and buprenorphine. Figure 3 summarizes group data for the light phase (first 12 h after injection) showing buprenorphine-induced changes in the temporal organization of sleep and wakefulness. ANOVA indicated a significant ($P < 0.01$) effect of buprenorphine on percent of time spent in states of wakefulness, NREM sleep, and REM sleep, as well as a significant ($P < 0.0001$) drug-by-state interaction (fig. 3A). Paired *t* tests with Bonferroni correction showed that buprenorphine significantly ($P < 0.001$) increased the percentage of time spent in waking (25.2%) and significantly decreased the amount of time spent in NREM sleep (−22.1%) and REM sleep (−3.1%). Buprenorphine significantly delayed the onset of NREM sleep and REM sleep (fig. 3C).

There was a significant ($P < 0.0001$) drug main-effect and state-by-drug interaction ($P < 0.0001$) for the number of sleep–wake episodes (fig. 3E). Buprenorphine decreased the number of episodes of wakefulness (−88.2%), NREM sleep (−89.5%), and REM sleep (−90.8%). Figure 3G shows that buprenorphine significantly ($P < 0.0001$) altered the duration of sleep–wake episodes. The average duration of wakefulness was significantly increased (529.6%), and the duration of sleep epochs was decreased for both NREM sleep
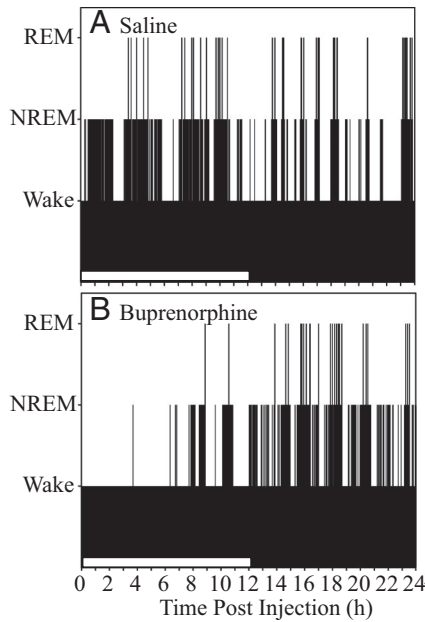
Fig. 2. Representative 24-h plots from one rat illustrating the buprenorphine-induced disruption of sleep architecture. The ordinate plots the temporal distribution of wakefulness (Wake), nonrapid eye movement sleep (NREM), and rapid eye movement sleep (REM) for 24 h after intravenous administration of saline (*A*) and 1 mg/kg buprenorphine (*B*). The injections and recordings of sleep and wakefulness began at 08:00 (time 0 on ordinate) at the initiation of the light phase (white horizontal rectangle) of a 12:12 h light–dark cycle. During the 12-h light phase (abscissa 0–12), *A* illustrates extended blocks of NREM sleep typically displayed by these nocturnal animals. In contrast, *B* shows that during this same interval buprenorphine decreased NREM sleep and REM sleep.

Fig. 3. Buprenorphine altered the temporal organization of sleep and wakefulness, and eszopiclone countered buprenorphine-induced sleep disruption. Panels *A, C, E, G,* and *I* summarize the effect of buprenorphine relative to saline (control) on five dependent measures averaged for seven rats recorded during the light portion (rat sleep phase) of the light–dark cycle. Panels *B, D, F, H,* and *J* summarize the temporal changes in sleep and wakefulness after coadministration of buprenorphine and eszopiclone (n = 4). Eszopiclone counteracted most of the sleep disruption caused by buprenorphine. Asterisks (*) indicate significant differences compared with saline across states of wakefulness (Wake), nonrapid eye movement sleep (NREM), and rapid eye movement sleep (REM).

(−30.8%) and REM sleep (−87.5%). Figure 3I shows that buprenorphine also significantly ($P < 0.0001$) decreased the number of transitions (−89.8%) between states.

Figure 4 plots the percentage state for each drug condition during the 12-h dark phase (rat subjective day) of the light–dark cycle that followed the 12-h light phase depicted by figure 3. Within the dark phase, when rats are normally awake and active, the time spent awake was significantly ($P = 0.012$) decreased by buprenorphine. The buprenorphine condition within the dark phase revealed significantly ($P = 0.0019$) more NREM sleep and a nonsignificant decrease in REM sleep compared with the saline condition.

The effect of buprenorphine on states of sleep and wakefulness can also be visualized by comparing the light phase (fig. 3A) and dark phase (fig. 4) results. NREM sleep after buprenorphine increased significantly ($P = 0.0003$) from an average of 5.5% in the light phase (fig. 3A) to 27.4% in the dark phase (fig. 4). There was also a significant ($P = 0.003$) rebound increase in REM sleep, from an average of 0.33% after buprenorphine during the light phase (fig. 3A) to approximately 4% after buprenorphine during the dark phase (fig. 4).
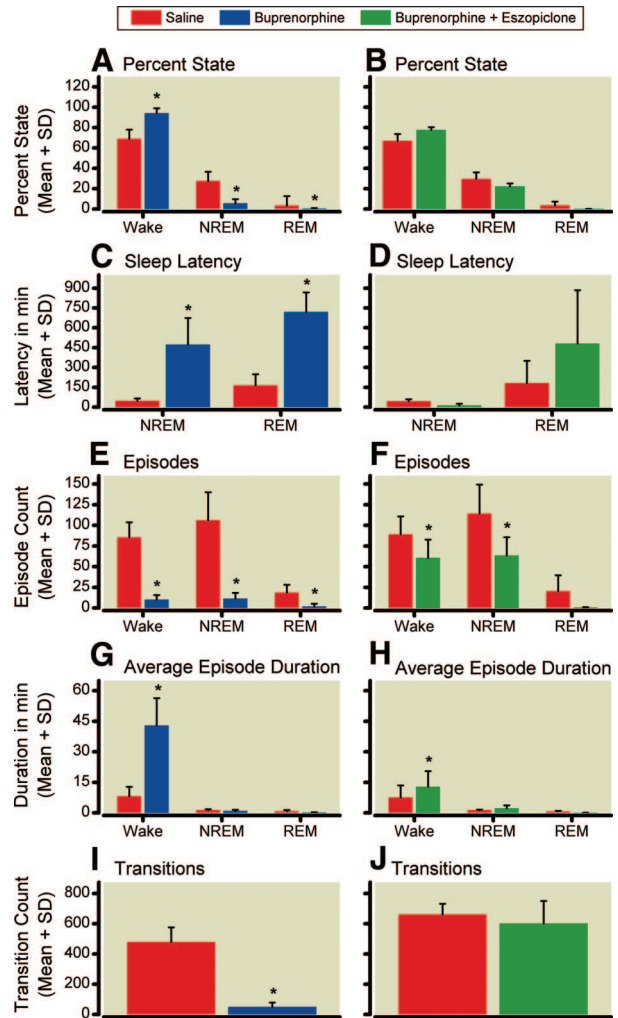
Eszopiclone Decreased the Sleep Disruption Caused by Buprenorphine

The five illustrations in the right column of figure 3 summarize the results of experiments designed to determine whether the sedative-hypnotic eszopiclone countered the buprenorphine-induced inhibition of sleep. Eszopiclone when coadministered with buprenorphine prevented the significant increase in wakefulness (fig. 3B) caused by buprenorphine alone (fig. 3A). Similarly, the significant buprenorphine-induced decrease in NREM sleep and REM sleep (fig. 3A) was
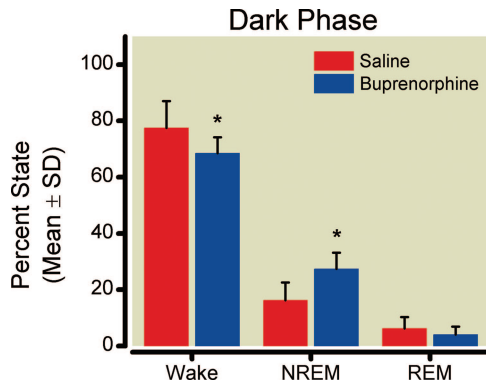
Fig. 4. The percentage of time during the 12-h dark phase that rats (n = 7) spent in states of wakefulness (Wake), nonrapid eye movement sleep (NREM), and rapid eye movement sleep (REM). These recordings were obtained during hours 12 through 24 after administration of either saline (control) or buprenorphine. Asterisks (*) indicate significant differences compared with saline. These results illustrate that after the buprenorphine-induced inhibition of sleep during the light phase (Fig. 3A) there was a significant rebound increase in NREM sleep and REM sleep during the dark phase, when these rats normally would be awake.

prevented by coadministration of eszopiclone (fig. 3B). Eszopiclone blocked the significant increase in latency to sleep onset (compare fig. 3C and D). Eszopiclone partially reversed the buprenorphine-induced decrease in the number of wakefulness and NREM sleep episodes (compare fig. 3E and F). The 530% increase in average duration of waking episodes caused by buprenorphine (fig. 3G) was reduced to a 171% increase by coadministration of eszopiclone (fig. 3H). Eszopiclone blocked the significant decrease in number of state transitions caused by buprenorphine (compare fig. 3I and J).

Buprenorphine Increased Electroencephalogram Delta Power during NREM Sleep

Figure 5A–C illustrates electroencephalogram power recorded across states of sleep and wakefulness after IV administration of buprenorphine to awake, freely moving rats. Buprenorphine did not alter electroencephalogram power during wakefulness or REM sleep (fig. 5, A and C) but did increase electroencephalogram power in the delta frequency range during NREM sleep (fig. 5B). ANOVA revealed a significant ($P = 0.007$) buprenorphine main effect on electroencephalogram frequency bands ranging from 0.5 to 5.0 Hz in 0.5-Hz increments (fig. 5B). The fast Fourier transform analyses were conducted for electroencephalogram measures obtained during the 12-h light period (*i.e.*, rat's subjective night) that immediately followed buprenorphine administration. As figures 2 and 3 show, buprenorphine depressed NREM sleep for 6–8 h. Measurement of the increase in electroencephalogram delta power was conducted for as long as 12-h after buprenorphine administration. A future study will be needed to determine whether, and for how long beyond 12-h, electroencephalogram delta power is increased by buprenorphine.
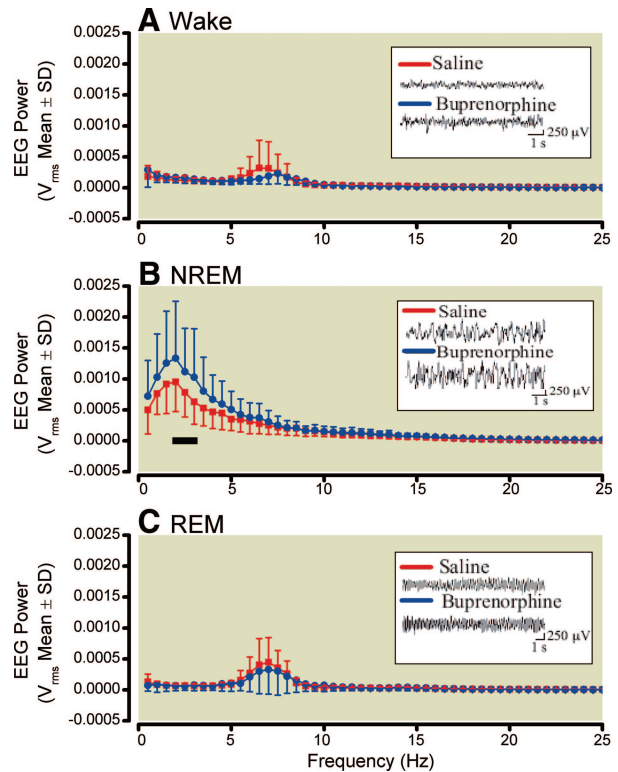

Fig. 5. Electroencephalographic power (ordinate) plotted as a function of frequency (abscissa) averaged for five rats across states of sleep and wakefulness. During wakefulness (*A*) and rapid eye movement (REM) sleep (*C*), buprenorphine did not change electroencephalographic power. In contrast, during NREM sleep that occurred after buprenorphine administration (*B*) there was a significant increase in electroencephalogram delta power (*black horizontal bar* above 2.0–3.0 Hz). EEG = electroencephalogram; NREM = nonrapid eye movement.

Buprenorphine Decreased Adenosine Concentrations in PnO and SI

Histologic analyses confirmed that all microdialysis sites were localized to the PnO or SI (fig. 6A). Figure 6B shows the results of one representative experiment. Adenosine concentrations in the SI are plotted as a function of time during dialysis with Ringer's solution (121–180 min after probe placement) followed by dialysis delivery of buprenorphine (181–240 min after probe placement). Figure 6, C and D, confirms chromatographic identification of adenosine. Figure 6C illustrates chromatograms produced by five known concentrations of adenosine. Figure 6D shows chromatograms reflecting brain adenosine (dialyzed Ringer's solution), a negative control (nondialyzed Ringer's solution), a positive control (brain adenosine sampled during dialysis delivery of the adenosine deaminase inhibitor EHNA), and an adenosine standard.

Figure 7A summarizes a final set of experiments that quantified adenosine concentrations in SI and PnO as a function of route of buprenorphine administration. Microdialysis delivery of buprenorphine significantly ($P = 0.03$) decreased adenosine concentrations in PnO
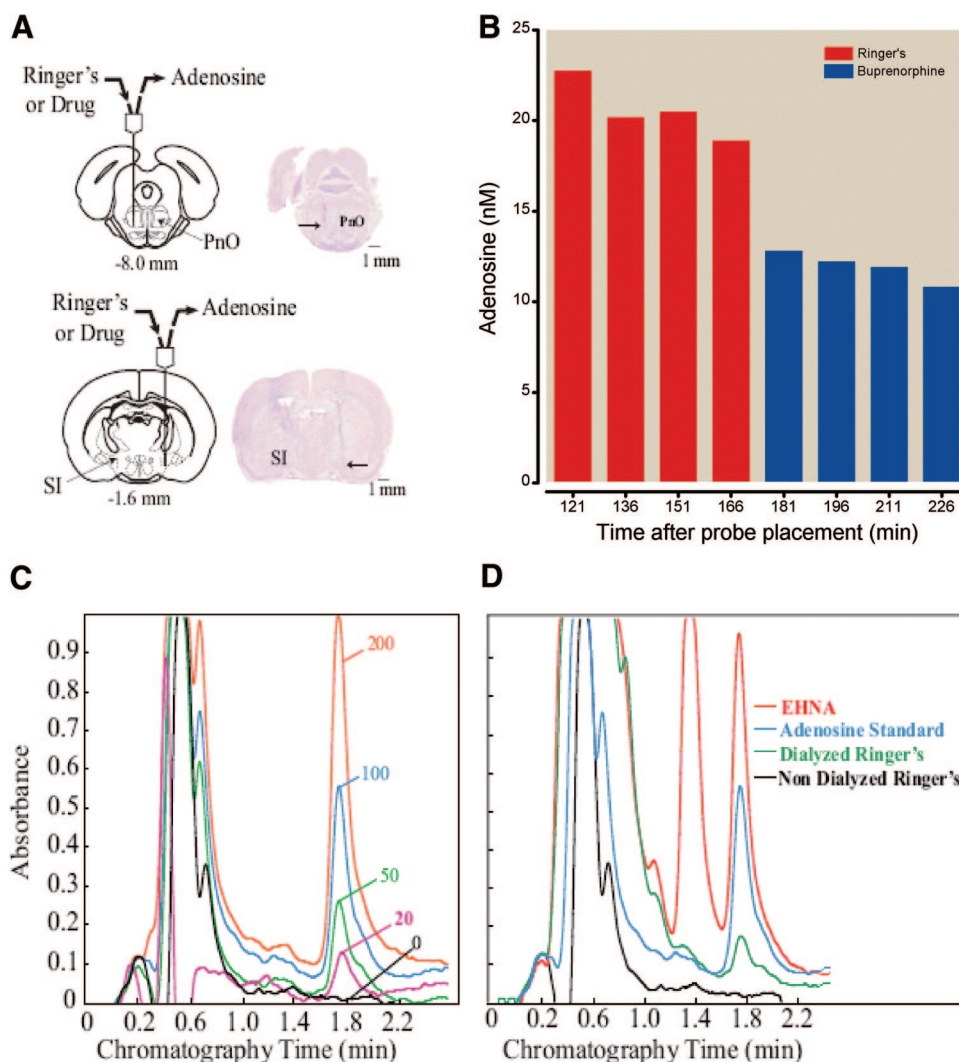
Fig. 6. Microdialysis delivery of buprenorphine decreased adenosine concentrations in the basal forebrain and pontine reticular formation oral part (PnO). (*A*) Cresyl-violet stained coronal sections show representative dialysis sites in the PnO and in the substantia innominata (SI) region of the basal forebrain. Arrows indicate the location of each microdialysis membrane. (*B*) Time course of a representative experiment showing adenosine concentrations in the SI before (Ringer's solution) and during dialysis delivery of buprenorphine. Each bar represents one sample (30 μl) acquired during 15 min of dialysis. (*C*) Chromatograms produced by ultraviolet measurement during the detection of adenosine concentrations ranging from 0 to 200 nM. Each microdialysis experiment began by creating a 5-point standard curve. The standard curve was used to convert ultraviolet absorbance values to concentration of brain adenosine for each microdialysis sample. (*D*) Chromatograms resulting from four different measurement conditions. The *black trace* illustrates the chromatogram produced when the instrument was injected with Ringer's solution that did not pass through the microdialysis probe (negative control). The *green trace* shows a chromatogram reflecting brain adenosine measured during brain microdialysis with Ringer's solution. The *blue trace* is a chromatogram produced by measurement of a 100-nM adenosine standard. The *red trace* represents a brain sample obtained during dialysis with Ringer's solution + 1 mM erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an adenosine deaminase inhibitor that increased adenosine (positive control). The key point is that all chromatograms showed the same elution time for adenosine. Thus, the magnitude of the standards (*C*) and the elution times (*D*) confirm the presence of adenosine in the brain samples.

(-14.8%) and significantly ($P = 0.0004$) decreased adenosine concentrations in the SI region of the basal forebrain (-36.7%). Figure 7B plots adenosine concentrations in the SI before and after IV administration of buprenorphine to isoflurane-anesthetized rat. Buprenorphine significantly ($P < 0.0001$) decreased (-20.3%) adenosine concentrations in the SI.

Discussion

Buprenorphine can be efficacious in the treatment of opioid and heroin addiction,[45–47] and there is increasing interest in the use of buprenorphine for pain management.[27,28] The analgesic effects of buprenorphine are mediated, in part, *via* agonist actions at the μ-opioid receptor.[48] This is the first
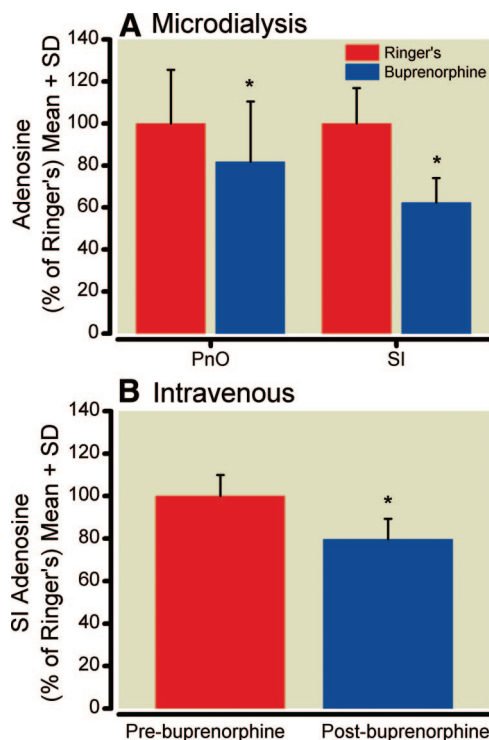
Fig. 7. Central and systemic delivery of buprenorphine decreased brain adenosine concentrations. Microdialysis delivery of buprenorphine to the pontine reticular formation (PnO) oral part (n = 5 rats) or to the substantia innominata (SI) (n = 5 rats) decreased adenosine concentrations in the PnO or SI, respectively (*A*). When administered systemically (n = 5), buprenorphine also decreased adenosine concentrations in the SI (*B*). Asterisks (*) in *A* indicate a significant decrease in adenosine concentrations during microdialysis delivery of buprenorphine compared with Ringer's solution (control). Asterisk (*) in *B* indicates a significant decrease in adenosine concentrations in the SI caused by intravenous (IV) delivery of buprenorphine. Thus, SI adenosine concentrations were decreased by microdialysis delivery of buprenorphine to the SI and by IV buprenorphine (*B*).

study presenting electrographic data that demonstrate significant sleep disturbance (fig. 3) caused by an antinociceptive dose of buprenorphine (fig. 1). The current finding that an antinociceptive dose of buprenorphine disrupts sleep is discussed relative to the relationship between sleep and nociception, the potential for developing countermeasures for opioid-induced sleep disruption, and the underlying mechanisms.

Buprenorphine Disrupted Sleep

Some data suggest that buprenorphine is superior to traditional opioids for the treatment of pain because of its reported analgesic and antihyperalgesic effects with fewer side effects (low incidence of respiratory depression and less constipation).[28] Buprenorphine shares some similar pharmacodynamic properties with traditional opioids, and patient-report data indicate benefits from buprenorphine therapy. Freye *et al.*[27] found that self-report sleep quality rated as "good" or "very good" increased from 14% to 74% when

patient regimens were transitioned from high-dose oral morphine to transdermal buprenorphine. Transdermal buprenorphine has been compared with placebo for ability to decrease pain and promote sleep, and patients randomized to receive buprenorphine report less pain and improved sleep.[49] Specifically, subjects who received buprenorphine reported less trouble falling asleep, decreased requirement for sleeping medication, and decreased awakening at night because of pain. Another study found a nonsignificant trend of improved sleep favoring transdermal buprenorphine over extended-release tramadol tablets for the treatment of osteoarthritis.[50] A known limitation of such studies is that self-assessment of sleep quality may not show faithful concordance with objective, electrographic measures of sleep.[51]

There is a growing appreciation for the interrelationship between sleep and pain.[39] Sleep deprivation in healthy normal individuals decreases pain perception thresholds.[52] The chronic effects of μ-opioid receptor agonists on sleep in patients with pain are not understood completely.[39] Opioids cause sleep disturbance[4,37,53] and the current results demonstrate that buprenorphine increases wakefulness and disrupts the temporal organization of sleep (figs. 2–4). Sleep, like breathing, is an endogenously generated biologic rhythm. Just as rhythmic switching from inspiration to expiration is essential for gas exchange, the ability of sleep to produce reports of rest and recovery requires a normal temporal organization. Buprenorphine caused a decrease in the number (fig. 3E) and an increase in the duration (fig. 3G) of wakefulness episodes. The decreased number of state transitions (fig. 3I) reflects the buprenorphine-induced disruption of sleep continuity. Figure 4 summarizes the percentage of time spent in states of sleep and wakefulness during the 12-h dark phase when rats are normally active. These dark-phase recordings were continuous with the figure 3 data during the 12-h light phase. Thus, the figure 4 data show that for 12–24 h after administration of buprenorphine, there was a rebound increase in sleep at a time when nocturnal rodents are normally most active. The potential clinical relevance of buprenorphine-induced disruption of sleep continuity derives from repeated sleep disruption negatively affecting neurocognitive function as severely as does total sleep deprivation.[54]

Opioid-induced sleep disruption has the potential to negatively affect patient care because sleep deprivation is known to decrease pain threshold.[15,16,52] This study did not address the impact of pain or the treatment of pain on sleep disturbance. Some believe that medications from the agonist-antagonist class, such as buprenorphine, may be less associated with the adverse effects of traditional μ-agonists; however, the current data indicate that the sleep-disrupting effects of buprenorphine are similar to those of other opioids.[26,30,37,53] The current results encourage studies designed to objectively quantify the effects of buprenorphine on sleep in humans.

The U.S. Food and Drug Administration approved buprenorphine for the treatment of opioid addiction. Subox-

one® (buprenorphine-naloxone sublingual tablet, Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA), Subutex® (buprenorphine sublingual tablet, Reckitt Benckiser Pharmaceuticals Inc.), and transdermal buprenorphine (not available in the United States) are increasingly used to treat pain. The current finding of buprenorphine-induced sleep disruption also is relevant to evidence indicating that sleep disturbance can lead to a higher potential for addiction relapse.[55–57]

Electroencephalogram delta waves (0.5–4 Hz) are one of three rhythmic waveforms characteristic of NREM sleep.[58] Delta waves provide an index of sleep intensity,[59,60] and electroencephalogram power in the delta frequency increases during recovery sleep that follows sleep deprivation.[61,62] The finding that buprenorphine caused an increase in electroencephalogram delta power during NREM sleep (fig. 5B) is consistent with subjective reports that buprenorphine improves sleep[7,27] and with evidence that opioids increase delta power in humans.[63] A search of the Medline database from 2001 to 2011 revealed no polysomnographic data characterizing the effect of buprenorphine on human sleep.

Eszopiclone Was an Effective Countermeasure that Prevented Sleep Disruption by Buprenorphine
Buprenorphine significantly disrupted the temporal organization of sleep (fig. 3, panels A, C, E, G, and I). Most aspects of sleep disturbance were prevented by concomitant treatment with the nonbenzodiazepine hypnotic eszopiclone (fig. 3, panels B, D, F, H, and J). Sedative-hypnotics are a standard treatment for insomnia, but their effects in the treatment of pain- and opioid-induced sleep disturbance remain poorly understood. The use of sedative-hypnotics may not be a mainstay of addiction therapy, but the current results (fig. 3, panels B, D, F, H, and J) indicate their potential to prevent buprenorphine-induced sleep disturbance. Eszopiclone as an adjunctive agent coadministered with the antidepressant fluoxetine resulted in a faster onset and greater magnitude of the desired antidepressant effect.[64] An exciting area open to future study is to determine whether hypnotics can be used as an effective countermeasure for opioid-induced sleep disruption.

Limitations, Potential Clinical Relevance, and Conclusions
The current study was designed to quantify the effects of buprenorphine on sleep and adenosine concentrations. The results are limited to documenting that buprenorphine, similar to morphine and fentanyl, disrupted sleep and decreased adenosine concentrations in sleep-related brain regions. The results do not imply that the effects of buprenorphine were mediated only by μ-opioid receptors. Buprenorphine may have disrupted sleep and decreased adenosine, in part, by acting as a κ antagonist.

The analgesic[21] and sleep-promoting[65] effects of adenosine are well known and suggest adenosine as a molecule of potential clinical relevance for anesthesiology. There is good agreement between preclinical and clinical data that opioids disrupt sleep,[37] a finding confirmed by administering opioids to pain-free humans.[53] The restorative effects of sleep require normal temporal organization of sleep. Unfortunately, morphine and fentanyl slow the electroencephalogram during wakefulness, increase lighter stage 2 NREM sleep, decrease stage 3 and 4 NREM sleep, and decrease or eliminate REM sleep. Disruption of normal sleep impairs immune function, exacerbates pain,[39] and, particularly in older patients, can be a precipitating factor for postoperative delirium.[66]

In conclusion, the results show for the first time that buprenorphine disrupted normal sleep architecture and decreased adenosine concentrations in sleep-regulating regions of the basal forebrain and PnO (figs. 6 and 7). The buprenorphine results are consistent with the discovery that fentanyl and morphine decrease adenosine concentrations in basal forebrain and PnO.[26] The current study extends the previous findings by providing mechanistic insights into a brain site and a molecule by which buprenorphine disrupts sleep. Novel insights were obtained by holding the site of adenosine measurement constant within the SI region of the basal forebrain while varying the route of buprenorphine delivery. The results show that both microdialysis delivery to the SI and systemic administration of buprenorphine caused a significant decrease in adenosine in the SI. As demonstrated elsewhere,[30,44] when effects caused by drug delivery to a specific brain region replicate the effects caused by systemic delivery, it is logical to infer that the actions of systemically administered drugs are mediated, in part, by that brain region and by the neurotransmitter molecule being measured. Thus, the neurochemical results, combined with the sleep-disrupting effect of buprenorphine, support the interpretation that one mechanism by which buprenorphine disrupts sleep is decreasing adenosine concentrations in the SI region of the basal forebrain.

References

1. Furlan AD, Sandoval JA, Mailis-Gagnon A, Tunks E: Opioids for chronic noncancer pain: A meta-analysis of effectiveness and side effects. CMAJ 2006; 174:1589–94
2. Noble M, Tregear SJ, Treadwell JR, Schoelles K: Long-term opioid therapy for chronic noncancer pain: A systematic review and meta-analysis of efficacy and safety. J Pain Symptom Manage 2008; 35:214–28
3. Cronin A, Keifer JC, Baghdoyan HA, Lydic R: Opioid inhibition of rapid eye movement sleep by a specific mu receptor agonist. Br J Anaesth 1995; 74:188–92
4. Dimsdale JE, Norman D, DeJardin D, Wallace MS: The effect of opioids on sleep architecture. J Clin Sleep Med 2007; 3:33–6
5. Keifer JC, Baghdoyan HA, Lydic R: Sleep disruption and increased apneas after pontine microinjection of morphine. ANESTHESIOLOGY 1992; 77:973–82

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

7. Sittl R, Griessinger N, Likar R: Analgesic efficacy and tolerability of transdermal buprenorphine in patients with inadequately controlled chronic pain related to cancer and other disorders: A multicenter, randomized, double-blind, placebo-controlled trial. Clin Ther 2003; 25:150–68

8. Baghdoyan HA: Hyperalgesia induced by REM sleep loss: A phenomenon in search of a mechanism. Sleep 2006; 29: 137–9

9. Haack M, Mullington JM: Sustained sleep restriction reduces emotional and physical well-being. Pain 2005; 119:56–64

10. Kundermann B, Krieg JC, Schreiber W, Lautenbacher S: The effect of sleep deprivation on pain. Pain Res Manag 2004; 9:25–32

11. Menefee LA, Cohen MJ, Anderson WR, Doghramji K, Frank ED, Lee H: Sleep disturbance and nonmalignant chronic pain: A comprehensive review of the literature. Pain Med 2000; 1:156–72

12. Naughton F, Ashworth P, Skevington SM: Does sleep quality predict pain-related disability in chronic pain patients? The mediating roles of depression and pain severity. Pain 2007; 127:243–52

13. Hakki Onen S, Alloui A, Jourdan D, Eschalier A, Dubray C: Effects of rapid eye movement (REM) sleep deprivation on pain sensitivity in the rat. Brain Res 2001; 900:261–7

14. Roehrs T, Roth T: Sleep and pain: Interaction of two vital functions. Semin Neurol 2005; 25:106–16

15. Roehrs T, Hyde M, Blaisdell B, Greenwald M, Roth T: Sleep loss and REM sleep loss are hyperalgesic. Sleep 2006; 29: 145–51

16. Smith MT, Edwards RR, McCann UD, Haythornthwaite JA: The effects of sleep deprivation on pain inhibition and spontaneous pain in women. Sleep 2007; 30:494–505

17. Rogers NL, Szuba MP, Staab JP, Evans DL, Dinges DF: Neuroimmunologic aspects of sleep and sleep loss. Semin Clin Neuropsychiatry 2001; 6:295–307

18. Clemons M, Regnard C, Appleton T: Alertness, cognition and morphine in patients with advanced cancer. Cancer Treat Rev 1996; 22:451–68

19. Sjøgren P: Psychomotor and cognitive functioning in cancer patients. Acta Anaesthesiol Scand 1997; 41:159–61

20. Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW: Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. Science 1997; 276:1265–8

21. Gan TJ, Habib AS: Adenosine as a non-opioid analgesic in the perioperative setting. Anesth Analg 2007; 105:487–94

22. Coleman CG, Baghdoyan HA, Lydic R: Dialysis delivery of an adenosine A2A agonist into the pontine reticular formation of C57BL/6J mouse increases pontine acetylcholine release and sleep. J Neurochem 2006; 96:1750–9

23. Marks GA, Shaffery JP, Speciale SG, Birabil CG: Enhancement of rapid eye movement sleep in the rat by actions at A1 and A2a adenosine receptor subtypes with a differential sensitivity to atropine. Neuroscience 2003; 116:913–20

24. Van Dort CJ, Baghdoyan HA, Lydic R: Adenosine A(1) and A(2A) receptors in mouse prefrontal cortex modulate acetylcholine release and behavioral arousal. J Neurosci 2009; 29:871–81

25. Kalinchuk AV, Urrila AS, Alanko L, Heiskanen S, Wigren HK, Suomela M, Stenberg D, Porkka-Heiskanen T: Local energy depletion in the basal forebrain increases sleep. Eur J Neurosci 2003; 17:863–9

26. Nelson AM, Battersby AS, Baghdoyan HA, Lydic R: Opioid-induced decreases in rat brain adenosine levels are reversed by inhibiting adenosine deaminase. ANESTHESIOLOGY 2009; 111:1327–33

27. Freye E, Anderson-Hillemacher A, Ritzdorf I, Levy JV: Opioid rotation from high-dose morphine to transdermal buprenorphine (Transtec) in chronic pain patients. Pain Pract 2007; 7:123–9

28. Kress HG: Clinical update on the pharmacology, efficacy and safety of transdermal buprenorphine. Eur J Pain 2009; 13: 219–30

29. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates, 6th edition. London, Elsevier, 2007

30. Osman NI, Baghdoyan HA, Lydic R: Morphine inhibits acetylcholine release in rat prefrontal cortex when delivered systemically or by microdialysis to basal forebrain. ANESTHESIOLOGY 2005; 103:779–87

31. Tanase D, Martin WA, Baghdoyan HA, Lydic R: G protein activation in rat ponto-mesencephalic nuclei is enhanced by combined treatment with a mu opioid and an adenosine A_1 receptor agonist. Sleep 2001; 24:52–62

32. Watson CJ, Lydic R, Baghdoyan HA: Sleep and GABA levels in the oral part of rat pontine reticular formation are decreased by local and systemic administration of morphine. Neuroscience 2007; 144:375–86

33. Gades NM, Danneman PJ, Wixson SK, Tolley EA: The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. Contemp Top Lab Anim Sci 2000; 39:8–13

34. Wang W, Baghdoyan HA, Lydic R: Leptin replacement restores supraspinal cholinergic antinociception in leptin-deficient obese mice. J Pain 2009; 10:836–43

35. Watson SL, Watson CJ, Baghdoyan HA, Lydic R: Thermal nociception is decreased by hypocretin-1 and an adenosine A1 receptor agonist microinjected into the pontine reticular formation of Sprague Dawley rat. J Pain 2010; 11:535–44

36. Dirig DM, Salami A, Rathbun ML, Ozaki GT, Yaksh TL: Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli. J Neurosci Methods 1997; 76:183–91

37. Lydic R, Baghdoyan HA: Neurochemical mechanisms mediating opioid-induced REM sleep disruption, Sleep and Pain. Edited by Lavigne G, Sessle BJ, Choinière M, Soja PJ. Seattle, WA, International Association for the Study of Pain (IASP) Press, 2007, pp 99–122

38. Krystal AD, Walsh JK, Laska E, Caron J, Amato DA, Wessel TC, Roth T: Sustained efficacy of eszopiclone over 6 months of nightly treatment: Results of a randomized, double-blind, placebo-controlled study in adults with chronic insomnia. Sleep 2003; 26:793–9

39. Lavigne G, Sessle BJ, Choiniére M, Soja PJ, editors: Sleep and Pain. Seattle, WA, International Association for the Study of Pain (IASP) Press, 2007

40. Lavigne GJ: Effect of sleep restriction on pain perception: Towards greater attention! Pain 2010; 148:6–7

41. Watson CJ, Soto-Calderon H, Lydic R, Baghdoyan HA: Pontine reticular formation (PnO) administration of hypocretin-1 increases PnO GABA levels and wakefulness. Sleep 2008; 31:453–64

42. Zhu Z, Bowman HR, Baghdoyan HA, Lydic R: Morphine increases acetylcholine release in the trigeminal nuclear complex. Sleep 2008; 31:1629–37

43. Vanini G, Watson CJ, Lydic R, Baghdoyan HA: Gamma-aminobutyric acid-mediated neurotransmission in the pontine reticular formation modulates hypnosis, immobility, and breathing during isoflurane anesthesia. ANESTHESIOLOGY 2008; 109:978–88

44. Hambrecht-Wiedbusch VS, Gauthier EA, Baghdoyan HA, Lydic R: Benzodiazepine receptor agonists cause drug-specific and state-specific alterations in EEG power and acetylcholine

release in rat pontine reticular formation. Sleep 2010; 33: 909–18

45. Fudala PJ, Jaffe JH, Dax EM, Johnson RE: Use of buprenorphine in the treatment of opioid addiction. II. Physiologic and behavioral effects of daily and alternate-day administration and abrupt withdrawal. Clin Pharmacol Ther 1990; 47:525–34

46. Orman JS, Keating GM: Buprenorphine/naloxone: A review of its use in the treatment of opioid dependence. Drugs 2009; 69:577–607

47. Ponizovsky AM, Margolis A, Heled L, Rosca P, Radomislensky I, Grinshpoon A: Improved quality of life, clinical, and psychosocial outcomes among heroin-dependent patients on ambulatory buprenorphine maintenance. Subst Use Misuse 2010; 45:288–313

48. Yamamoto T, Shono K, Tanabe S: Buprenorphine activates mu and opioid receptor like-1 receptors simultaneously, but the analgesic effect is mainly mediated by mu receptor activation in the rat formalin test. J Pharmacol Exp Ther 2006; 318:206–13

49. Gordon A, Callaghan D, Spink D, Cloutier C, Dzongowski P, O'Mahony W, Sinclair D, Rashiq S, Buckley N, Cohen G, Kim J, Boulanger A, Piraino PS, Eisenhoffer J, Harsanyi Z, Darke AC, Michalko KJ: Buprenorphine transdermal system in adults with chronic low back pain: A randomized, double-blind, placebo-controlled crossover study, followed by an open-label extension phase. Clin Ther 2010; 32:844–60

50. Karlsson M, Berggren AC: Efficacy and safety of low-dose transdermal buprenorphine patches (5, 10, and 20 microg/h) versus prolonged-release tramadol tablets (75, 100, 150, and 200 mg) in patients with chronic osteoarthritis pain: A 12-week, randomized, open-label, controlled, parallel-group noninferiority study. Clin Ther 2009; 31:503–13

51. Baker FC, Maloney S, Driver HS: A comparison of subjective estimates of sleep with objective polysomnographic data in healthy men and women. J Psychosom Res 1999; 47:335–41

52. Chhangani BS, Roehrs TA, Harris EJ, Hyde M, Drake C, Hudgel DW, Roth T: Pain sensitivity in sleepy pain-free normals. Sleep 2009; 32:1011–7

53. Bonafide CP, Aucutt-Walter N, Divittore N, King T, Bixler EO, Cronin AJ: Remifentanil inhibits rapid eye movement sleep but not the nocturnal melatonin surge in humans. ANESTHESIOLOGY 2008; 108:627–33

54. Van Dongen HP, Maislin G, Mullington JM, Dinges DF: The cumulative cost of additional wakefulness: Dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 2003; 26:117–26

55. Arnedt JT, Conroy DA, Brower KJ: Treatment options for sleep disturbances during alcohol recovery. J Addict Dis 2007; 26:41–54

56. Brower KJ, Perron BE: Sleep disturbance as a universal risk factor for relapse in addictions to psychoactive substances. Med Hypotheses 2010; 74:928–33

57. Mahfoud Y, Talih F, Streem D, Budur K: Sleep disorders in substance abusers: How common are they? Psychiatry 2009; 6:38–42

58. Steriade M, McCarley RW: Brain Control of Wakefulness and Sleep, 2nd edition. New York, Plenum Press, 2005

59. Friedman L, Bergmann BM, Rechtschaffen A: Effects of sleep deprivation on sleepiness, sleep intensity, and subsequent sleep in the rat. Sleep 1979; 1:369–91

60. Brunner DP, Dijk DJ, Borbély AA: Repeated partial sleep deprivation progressively changes in EEG during sleep and wakefulness. Sleep 1993; 16:100–13

61. Tobler I, Borbély AA: Sleep EEG in the rat as a function of prior waking. Electroencephalogr Clin Neurophysiol 1986; 64:74–6

62. Feinberg I, Floyd TC, March JD: Effects of sleep loss on delta (0.3–3 Hz) EEG and eye movement density: New observations and hypotheses. Electroencephalogr Clin Neurophysiol 1987; 67:217–21

63. Greenwald MK, Roehrs TA: Mu-opioid self-administration vs passive administration in heroin abusers produces differential EEG activation. Neuropsychopharmacology 2005; 30: 212–21

64. Fava M, McCall WV, Krystal A, Wessel T, Rubens R, Caron J, Amato D, Roth T: Eszopiclone co-administered with fluoxetine in patients with insomnia coexisting with major depressive disorder. Biol Psychiatry 2006; 59:1052–60

65. Porkka-Heiskanen T, Alanko L, Kalinchuk A, Stenberg D: Adenosine and sleep. Sleep Med Rev 2002; 6:321–32

66. Rudolph JL, Marcantonio ER: Postoperative delirium: Acute change with long-term implications. Anesth Analg 2011; 112: 1201–11