Comparative Medicine
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 57, No 2
April 2007
Pages 167–174

Original Research

The Effects of Analgesic Supplements on Neural Activity in the Main Olfactory Bulb of the Mouse

Thomas G Mast[†] and Edwin R Griff[*]

We evaluated ketoprofen, a nonsteroidal anti-inflammatory drug (NSAID), as an antinociceptive supplement to chloral hydrate anesthesia in mouse. Effects of ketoprofen on main olfactory bulb (MOB) neuronal spontaneous activity were investigated using extracellular recordings in mouse in vivo. These effects were compared with those of another nociceptive supplement, the μ-opioid agonist buprenorphine. Ketoprofen (100 or 200 mg/kg) did not significantly alter MOB single-unit spontaneous rates in either ICR or C57BL/6J mice. In contrast, buprenorphine, at doses of 0.02, 0.05, and 0.20 mg/kg, inhibited MOB neuronal spontaneous rates by 19%, 49%, and 57%, respectively. Neither drug altered the temporal patterning of single-unit spike trains, as measured by the interspike interval (ISI) coefficient of variation (CV). We also investigated the ability of ketoprofen and buprenorphine to induce antinociception in the anesthetized mouse. The electroencephalogram (EEG) was used to measure the anesthetic plane. Both ketoprofen and buprenorphine altered the EEG trace and ketoprofen altered the power spectrum in a manner consistent with deepening anesthesia. Lastly, when applied at the time of anesthesia induction, ketoprofen decreased the amount of chloral hydrate necessary to maintain a defined anesthetic plane during the rest of the experiment. These results suggest that ketoprofen induces antinociception under chloral hydrate anesthesia without significantly inhibiting spontaneous activity of MOB neurons. Ketoprofen is therefore suitable as an antinociceptive supplement to chloral hydrate anesthesia during in vivo electrophysiologic recordings of the mouse MOB.

Abbreviations: CV, coefficient of variation; EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; ISI, interspike interval; MCL, mitral cell body layer; MOB, main olfactory bulb; NSAID, nonsteroidal anti-inflammatory drug

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase and thus inhibit prostaglandin synthesis from arachidonic acid. This inhibition produces antinociceptive effects during inflammatory pain.[47] However, inhibition of prostaglandins does not fully explain the antinociceptive activities of NSAIDs, as NSAIDs provide analgesia in acute noninflammatory models of pain.[2,14,28,32,33,34] NSAIDs cross the blood-brain barrier,[30] and therefore activities within the central nervous system have been hypothesized to mediate some of their antinociceptive properties.[2,14,28,32,33,34]

Antinociception in the spinal cord is complex and involves several classes of transmitters and receptors.[12] NSAIDs including aspirin, diclofenac, and ibuprofen inhibit acid-sensing ion channels[48] and acetylsalicylic acid inhibits heat-sensitive currents[14] in dorsal root ganglion neurons. These channels and currents are involved in tissue nociception. Diclofenac can activate the nitric oxide cGMP pathway and suppress tetrodotoxin-sensitive currents in dorsal root ganglion neurons.[21,22] Ketoprofen, another NSAID, may interact with dorsal root adrenergic, cholenergic, or serotinergic pathways.[7,28,32,33,34] In addition, ketoprofen has been shown to provide analgesia similar to μ opioids in a noninflammatory pain model.[27]

The use of antinociceptives such as buprenorphine, a partial μ-opioid agonist,[17,23,31,39] in small-mammal research animals[10,18,23,39] is increasing.[37] However, buprenorphine can alter the activity of spinal cord[7] and olfactory bulb neurons.[26,31] Therefore, during in vivo neurophysiologic recordings, buprenorphine may not be the ideal antinociceptive.

Alternatively, ketoprofen may be useful as an antinociceptive supplement to anesthetic regimens used for small-mammal in vivo neurophysiology. If used during in vivo neurophysiologic experiments, NSAIDs such as ketoprofen could supplant narcotics such as buprenorphine as analgesic supplements. NSAID supplementation also may decrease the amount of anesthesia required to maintain a surgical anesthetic plane. These changes could improve the physiologic conditions during experiments with anesthesia. A potential complication is that anesthetics have been reported to inhibit the analgesic effects of NSAIDs.[3] Another complication is that because ketoprofen crosses the blood-brain barrier and interacts with several neurotransmitter pathways, it might alter neuronal activity.

The present study was part of a larger project to develop an in vivo mouse model to investigate neural circuits in the MOB. Concerns of the veterinary staff about chloral hydrate encouraged us to examine the effects of ketoprofen and buprenorphine on MOB single-unit activity. We also investigated whether ketoprofen would supplement chloral hydrate anesthesia in this model. We report that ketoprofen alters the anesthetic plane without significantly inhibiting spontaneous activity.

Received: 14 Jun 2006. Revision requested: 10 Aug 2006. Accepted: 2 Nov 2006.
Department of Biological Sciences, University of Cincinnati, Cincinnati, OH.
[†]Present address: Program in Neuroscience, Florida State University, Tallahassee, FL.
[*]Corresponding author. Email: edwin.griff@uc.edu

Materials and Methods

General methods. All procedures implemented in this study were in accordance with federal animal care guidelines, were performed at a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, and were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Experiments involved a total of 30 mice (Harlan, Indianapolis, IN): 18 ICR female mice and 12 C57BL6J (B6) male mice; all mice weighed between 19 to 40 g. Unless otherwise specified, experiments were performed on both strains of mice. B6 mice were used to test whether any beneficial results were strain-specific and because the B6 mouse is a common background strain in genetically engineered mice.

Mice were anesthetized with intraperitoneal chloral hydrate at an initial dose of 400 mg/kg; supplemental doses of 30 to 70 mg/kg were administered as needed through an intraperitoneal cannula. Stable-unit recordings require a relatively constant anesthetic plane.[16] The goal was to maintain the anesthetic plane such that a moderate hindpaw pinch did not elicit a withdrawal reflex but did desynchronize the EEG. However, unlike gas anesthesia, chloral hydrate is administered intraperitoneally, and the anesthetic depth cannot be altered immediately. Therefore, the animals were sometimes slightly lighter or deeper than the ideal anesthetic plane. For example, an animal without a withdrawal reflex but with an increasing respiratory rate might receive a small dose of chloral hydrate, even if this dose temporarily put the animal deeper than the desired anesthetic plane.

To monitor the anesthetic plane of the animal, the respiration rate, temperature, withdrawal reflex, and EEG were recorded every 10 to 30 min, more frequently at the beginning of the experiment and less frequently when the animal was stable and did not require supplemental anesthesia frequently. Body temperature was monitored with a rectal thermometer (YSI, Yellow Springs, OH) and maintained between 34.5 and 35.5 °C with a custom-built acrylic water bath connected to a water heater and pump. The water bath was designed to fit in a stereotaxic holder (David Kopf Instruments, Tujunga, CA) underneath the animal.

Surgery. After anesthesia induction, the dorsal and right lateral head and neck were shaved, and 2% lidocaine hydrochloride was administered to incision sites. A silver ground electrode was inserted into the neck muscles. The mouse was placed in a stereotaxic frame, and a 75-µm stainless-steel EEG electrode was inserted into the right parietal cortex by using established stereotaxic coordinates and was secured to the skull with dental acrylic. A 4- to 6-mm^2 window in the frontal bone over the MOB was thinned with a dental bur (diameter, 1.0 mm), and the bone was removed with forceps. The dura was cut with a 30-gauge hypodermic needle and then removed with fine forceps; the underlying MOB surface was kept moist with cotton dampened in artificial cerebral spinal fluid (ACSF).[1,26]

Electrophysiologic recording. The animal and stereotaxic frame were placed within a grounded Faraday cage, and 1 recording and 1 stimulation electrode were positioned. The bipolar stimulation electrode was made of 0.125-mm stainless-steel wire (California Fine Wire, Grover, CA) that was insulated except for cut and polished tips. The electrode was positioned such that the tips contacted the olfactory nerve at the far rostral end of the MOB. The recording electrodes were made from borosilicate glass pipettes ('Dot glass'; outer diameter, 1.0 mm; inner diameter, 0.7 mm; WPI, Sarasota, FL); electrodes were pulled to a tip diameter of 1 to 3 µm, filled with 2% pontamine blue in 0.5 M sodium acetate, and had a final resistance of 5 to 10 MΩ. The depth of the recording electrode from the MOB surface and shape of extracellular field potentials evoked by square-wave stimulation pulses (10 to 100 µs; 70 to 400 µA) were used to position the recording electrode within the various layers of the MOB.[29] Using this technique, we generally targeted units in or very near to the mitral cell body layer (MCL) where the large negative component of the field potential reverses. However, units from other layers were also recorded; the depth and field potential shape were again used to estimate the location of these units in the granule (GCL), external plexiform (EPL) and glomerular (GL) layers.

Extracellular single MOB units were isolated on the basis of a constant shape and constant amplitude of the action potential and an interspike interval (ISI) of at least 2 ms.[49] Action potentials were filtered between 300 and 3000 Hz and stored digitally on magnetic tape (Medical Systems, Greenvale, NY). Action potentials also were detected by using a window discriminator (Dagan, Minneapolis, MN), and each discriminated action potential was stored as an event on the hard drive of a personal computer (µ1401, Spike2 software, CED, Cambridge, UK).

After recording the spontaneous activity from a unit, the location of the microelectrode tip was marked by electrophoretic injection of pontamine blue (10 µA; 7 s on, 3 s off). At the end of the experiment, the animal was given an overdose of anesthetic and was perfused with 10% formalin in phosphate buffered saline; the brain was removed, frozen, and sectioned. The location of all but 1 unit was confirmed histologically; for the remaining unit, the dye mark was not recovered.

EEG recording. The EEG was recorded in order to measure the plane of anesthesia and measure the effect of analgesics. The EEG signal was filtered direct current (DC) to 30 Hz, stored on magnetic tape (Medical Systems, Greenvale, NY), digitized at 100 Hz (µ1401) and stored on the hard drive of a personal computer. Alternatively the EEG was observed during the experiment on an oscilloscope or chart recorder or both.

Experimental design. During single-unit recordings, supplemental doses of chloral hydrate were frequently necessary to maintain the anesthetic plane, and these supplemental doses reduced spontaneous activity (see Results). On 2 occasions, we administered doses larger than would be necessary to maintain the anesthetic plane to test whether chloral hydrate would completely inhibit spontaneous activity. All other doses were the minimal dose necessary to maintain the anesthetic plane. Acute doses are given in milligrams per kilogram. We also examined and compared the cumulative amount of chloral hydrate; for this analysis, doses are reported in milligrams per kilogram per hour.

Buprenorphine and ketoprofen were administered systemically during single-unit recordings. In these experiments, single-units were recorded for approximately 200 s to establish a basal firing rate, after which the analgesic was administered. Buprenorphine (0.02, 0.05, and 0.20 mg/kg) and vehicle (50 mg/ml glucose) were diluted in saline and were injected subcutaneously, whereas ketoprofen (100 and 200 mg/kg) and vehicle (0.7 mg/ml L-arginine), diluted in saline, were administered through an intraperitoneal cannula. After administration of the drugs or vehicles, the unit was maintained for an additional 2100 s. EEGs were recorded before and after administration of analgesics and controls. In these experiments, ketoprofen or buprenorphine injections were given at least 10 min after any supplemental dose of chloral hydrate. Furthermore, in 9 ICR mice and 4 B6, ketoprofen (100 mg/kg) was administered just after anesthesia induction, and the effects

on respiration and supplemental chloral hydrate doses were compared with those of 9 ICR and 4 B6 randomly chosen mice that did not receive ketoprofen.

The recommended dose range for buprenorphine for mice is 0.05 to 2.0 mg/kg.[15] Because preliminary experiments indicated that a dose of 0.2 mg/kg decreased spontaneous activity (data not shown), we also investigated the effects of the minimal recommended dose and a lower dose (0.02 mg/kg). The recommended dose for ketoprofen is 100 to 200 mg/kg, and in most experiments, the lower dose was used. In the B6 mice, 2 of the 6 animals given the higher dose just after anesthesia induction died within 1 h after injection. In 1 experiment, a dose of 1000 mg/kg was administered accidentally, and the animal expired a few minutes afterward.

Some mice were used for more than 1 experiment. In all but 2 experiments examining changes in unit activity in response to chloral hydrate, chloral hydrate was injected to maintain anesthesia, and these animals were used for other experiments. In addition, 5 mice were injected subcutaneously with vehicle only (which did not cause a response), and these animals were used for additional experiments. Further, 5 of the animals injected with ketoprofen at the start of the experiment subsequently were used to examine the effects of buprenorphine. No differences were noted in the response to buprenorphine between these animals and those that did not receive an initial injection of ketoprofen. Mice given an acute injection of either ketoprofen or buprenorphine were never used in additional experiments.

Data analysis and statistics. ***Effects of buprenorphine and ketoprofen.*** The time course of the analgesic and antinociceptive effects of these agents in the mouse are unknown; in larger animals, maximal responses can require as long as 1 h to develop.[11,35] In view of the time course of the effects of these agents on spontaneous activity, we compared a 200-s window centered on the point of analgesic administration with a second 200-s window centered 2000 s later. This procedure is a compromise between allowing sufficient time for the drug to exert its action, our ability to hold a unit reliably, and the need to check anesthesia and add supplemental doses of chloral hydrate. The spontaneous activities of the spike trains in the 'before' and 'after' windows were compared. Using the 100 s before drug administration for the comparison produced similar results.

We chose to compare changes in the mean spontaneous firing rate and the ISI coefficient of variation (CV; defined as the ISI standard deviation divided by the ISI mean[50]). The CV is a relative measure of the dispersion of data about the mean and can be used to measure the temporal patterning of spontaneous activity.[38,43] The CV is dependent on sample size; therefore, we used similar numbers of ISIs for each comparison. Calculations of the CV were limited to the first 1500 ISIs longer than 2 ms, and, in some calculations, shorter than 100 ms. Longer intervals were limited, because spikes separated by more than 100 ms are unlikely to be associated.[38,43] Significant difference was tested with paired *t* tests ($\alpha = 0.05$; Excel, 1985-2003). This practice allowed comparison of units from different layers in the MOB.

Effects of chloral hydrate. Anesthetic effects on single-unit spontaneous activity typically manifest within 5 min.[25] To analyze an expected inhibition of spontaneous activity by chloral hydrate, we compared a 20-s window before chloral hydrate administration to successive 20-s windows after administration. The percentage change in spontaneous firing rate between the 'before' and 'after' windows was calculated and the data were analyzed as a function of the dose of chloral hydrate. Significant differences were tested for with a paired *t* test ($\alpha = 0.05$; Excel, 1985-2003).

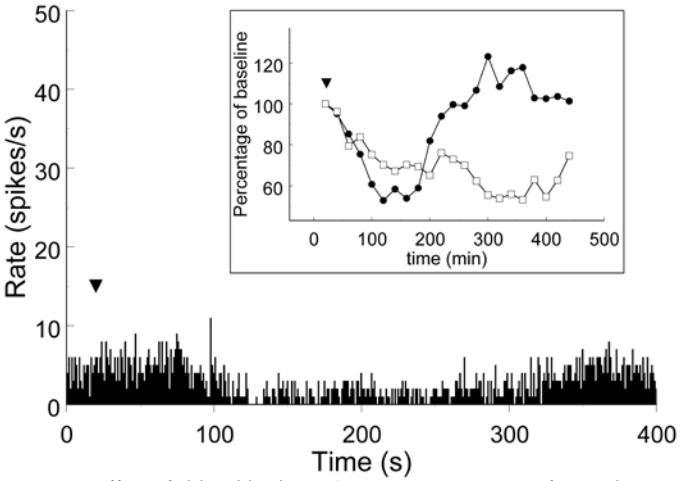

Figure 1. Effect of chloral hydrate. Spontaneous activity of a single-unit is presented as mean rate; a supplemental dose of 33.33 mg/kg chloral hydrate was given intraperitoneally (down arrowhead). In this unit (t80203a), the mean rate decreased from 4.55 to a minimum of 1.15 spikes/s, a decrease of 75%. The inset shows the time course of the decrease in activity evoked by low (<60 mg/kg; filled circles; n = 7) and high (>60 mg/kg; open squares; n = 7) doses of chloral hydrate.

EEG analysis. We captured 60-s recordings of the EEG before and after administration of ketoprofen or vehicle (0.7 mg/ml L-arginine) to 7 ICR and 6 B6 mice. The digitized traces then were analyzed with a fast Fourier transform algorithm to produce a power spectrum (Sudsa22 script, Spike2 software, CED). To produce the power spectra, 24 s of continuous and artifact-free EEG recording was separated into 12 continuous 2-s bins.[24] Each bin was analyzed for absolute power in 5 frequency bandwidths: delta (0 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 14 Hz), beta 1 (14 to 18 Hz), and beta 2 (18 to 30 Hz). The relative power within each bandwidth was calculated by dividing the power in the bandwidth by the total absolute power (0 to 30 Hz).[9] The relative powers in the 12 bins were averaged for each frequency bandwidth in each mouse. The relative power spectra of each mouse then were allocated into 'before' ketoprofen injection (or control) and 'after' groups. The 'before' and 'after' relative power spectra were tested for significant difference with a paired *t* test ($\alpha = 0.05$; Systat 10.2).

Physiologic parameters. The amount of anesthetic, number of anesthetic doses, and respiration rate were recorded. These parameters were compared before and after doses of ketoprofen as well as between mice that received a presurgical dose of ketoprofen and those that did not (control). Differences were tested for significance with 2-sample *t* tests ($\alpha = 0.05$; Systat 10.2).

Results

Effect of chloral hydrate on spontaneous activity. To maintain a surgical anesthetic plane, we administered doses of intraperitoneal chloral hydrate each experiment, and this administration sometimes occurred during recording of single-unit spontaneous activity in ICR mice. Each unit was recorded from animals at or near the anesthetic plane as defined in Methods; each dose of chloral hydrate was the minimum required to maintain the anesthetic plane, except for 2 large doses that were given intention-

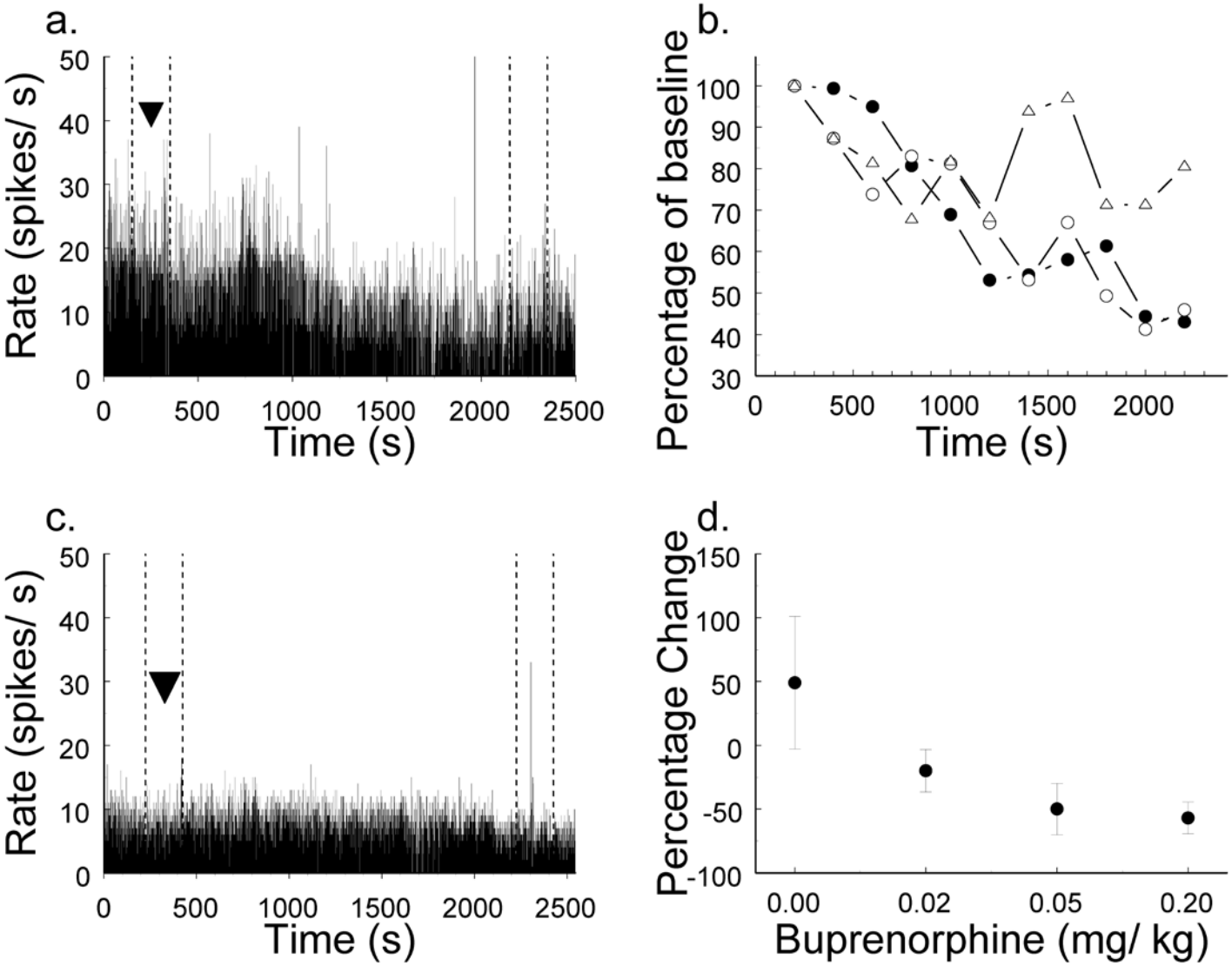

Figure 2. Buprenorphine. (A) An example of a single unit that was recorded during subcutaneous injection of 0.20 mg/kg of buprenorphine (down arrowhead). The vertical broken lines represent the locations of the 200-s periods used to calculate the 'before' and 'after' spontaneous rates. The spontaneous activity in this unit (t21301a) decreased from 15.4 to 9.4 spikes/s, a 39% decrease. (B) Time course of responses to 0.20 (filled circles), 0.02 (open circles), and 0.05 (open triangles) mg/kg buprenorphine. (C) An example of a subcutaneous sham injection; the spontaneous activity in this unit (t80103d) decreased from 7.3 to 5.9 spikes/s (19%). (D) Dose-response curve of buprenorphine on MOB single-unit spontaneous activity. The response is represented by the percentage change calculated by dividing by the spontaneous rate of the unit after vehicle (n = 3); the data point for each dose is the average of 4 units from 4 different animals. The error bars indicate the standard error of the mean.

ally to examine the effects of higher doses of chloral hydrate. As expected, chloral hydrate decreased the MOB single-unit spontaneous rate (Figure 1), and on average, the percentage decrease became significant 80 s after chloral hydrate injection ($P = 0.01$, $t = 2.32$, n = 14). With doses less than 60 mg/kg (n = 7), the decrease was more transient and recovered (was no longer significantly different from baseline) 200 s after the injection; with doses larger than 60 mg/kg (n = 7), the decrease was more prolonged, recovering 440 s after injection. There was no significant difference between the percentage change in activity caused by low and high doses of chloral hydrate. The comparison was done in 2 ways. There was no difference between high and low doses during the transient response evoked by the low dose. There was also no difference in the maximal percentage change in activity between the low dose (measured at 120 s after injection) and the high dose (measured at 360 s after injection). Histologic confirmation of unit location identified 11 units that were in the MCL. There were 3 additional units: 1 from the GL, 1 from GCL, and 1 whose location was not confirmed histologically.

Effect of buprenorphine on spontaneous activity. Preliminary results showed that the spontaneous activity of units in the MCL was inhibited by systemic or topical application of buprenorphine.[26] Here, we report that the inhibition produced by systemic application is dose-sensitive with respect to spontaneous rate but not temporal patterning. A single-unit was isolated and recorded for approximately 200 s to establish a baseline rate of spontaneous activity. After the baseline was established, buprenorphine was administered subcutaneously, and the unit was maintained for another 2100 s. Single-unit spike trains of spontaneous activity 200 s in duration separated by 1800 s were compared before and

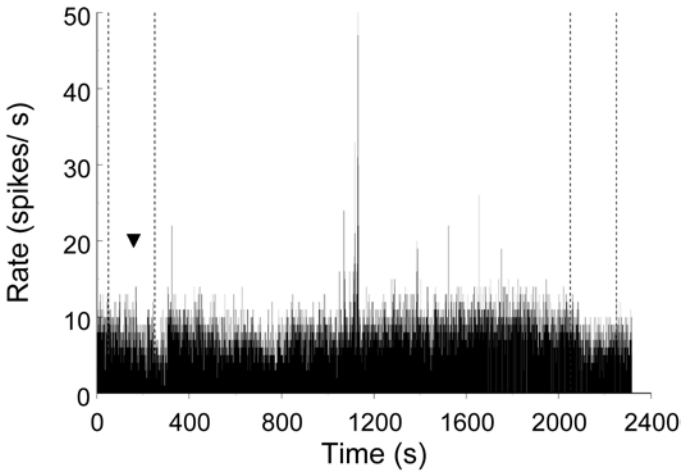

Figure 3. Ketoprofen. An example of a single-unit that was recorded during intraperitoneal injection of 200 mg/kg of ketoprofen (down arrowhead). The vertical broken lines delineate the 200-s time periods used for the 'before' and 'after' spontaneous rate calculations; the spontaneous activity of this unit (t72903e) was 8.3 spikes/s before ketoprofen and 7.8 spikes/s after. The noise at approximately 1100 s was caused by a loose ground connection.

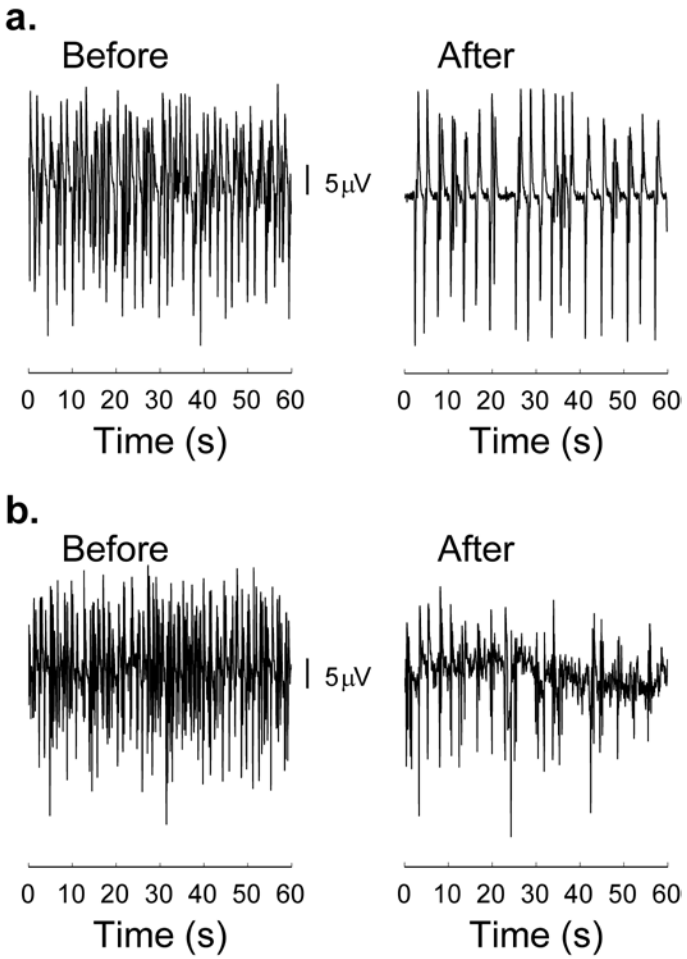

Figure 4. EEG. In each pair of traces, the left-hand trace (Before) was captured at approximately 35 min (2100 s) before the right-hand trace (After). (A) Effect of 0.05 mg/kg of buprenorphine. (B) Effect of 200 mg/kg of ketoprofen. Note that the effect of ketoprofen is similar to that of buprenorphine.

after buprenorphine administration (Figure 2 A). In ICR mice, the rates of 11 of 12 single-units decreased after buprenorphine injection. At doses of 0.02, 0.05, and 0.20 mg/kg, with 4 units per dose, buprenorphine inhibited MOB single-unit spontaneous rates by 19.4%, 49.0%, and 57.4%, respectively (Figure 2 D). Paired *t* test analysis revealed that the effect of buprenorphine was significant for both the pooled samples ($P = 0.002$, $t = 4.185$, n = 12) and the 0.20-mg/kg dose ($P = 0.015$, $t = 5.03$, n = 4). Histological confirmation of unit location identified 10 units in the MCL. The remaining 2 units were located in the GCL and GL. The unit located in the GCL did not respond to buprenorphine. Vehicle did not decrease the spontaneous rate of 3 units, 1 from the GL, 1 from the EPL, and 1 from the MCL (Figure 2 C). In 1 unit, activity increased, thereby causing the average after sham injection to be higher than the average baseline (Figure 2 D).

Although the single-unit spontaneous firing rate was sensitive to buprenorphine, the temporal patterning of the spontaneous activity was not. Buprenorphine did not alter the ISI CV significantly (1.006 versus 0.937, before versus after). Sham injections of vehicle also did not affect the CV of 3 units from 3 separate mice (0.969 versus 1.071, before versus after).

Effect of ketoprofen on spontaneous activity. We tested ketoprofen for an affect on spontaneous rate and temporal patterning. As in the buprenorphine experiments described earlier, a single-unit was recorded for approximately 200 s before ketoprofen was administered by use of an intraperitoneal cannula (Figure 3). The unit was maintained for another 2100 s before the effect of ketoprofen was measured. In contrast to buprenorphine, ketoprofen at doses of either 100 or 200 mg/kg did not affect the single-unit spontaneous rate in either the ICR or B6 strains (paired *t* test for the ICR and B6 strains combining doses: $P = 0.958$, $t = –0.055$, n = 6 and $P = 0.714$, $t = –0.394$, n = 5, respectively; paired t test for the 100 and 200 mg/kg doses combining strains: $P = 0.725$, $t = –0.362$, n = 6 and $P = 0.440$, $t = .813$, n = 5, respectively). Likewise, during the ketoprofen experiments, the ISI CV did not change significantly (0.647 versus 0.642, before versus after). Sham injections of vehicle did not affect the spontaneous activity of 5 units from 5 separate mice. Histologic confirmation of unit location identified that all 11 units were either in or adjacent to the MCL. In addition we recorded from 1 unit in the cerebellum, presumably a Purkinje cell, where 100 mg/kg ketoprofen had little effect on spontaneous rate (61.21 versus 62.78 spikes/s).

Ketoprofen and anesthesia. In these experiments, ketoprofen and buprenorphine were tested for acute effects on the anesthetic plane as monitored by EEG. The EEG was captured before intraperitoneal administration of 100 or 200 mg/kg ketoprofen and captured again 2100 s later. Buprenorphine is a well-known analgesic,[10,18,23,39] and the EEG changed as expected,[17] exhibiting more spindles and fewer high-frequency components (compare top left and top right traces in Figure 4). For 7 ICR and 6 B6 mice, ketoprofen consistently changed the EEG trace in a manner similar to buprenorphine (compare bottom right and top right traces in Figure 4). Accordingly, fast Fourier analysis of the EEG traces revealed a nonsignificant increase in power within the delta band, and significant reduction of power within the theta band (Table 1; $P = 0.026$, $t = 2.518$, n = 13). The EEG was not affected by sham injections of vehicle (n = 5; data not shown). We also monitored the respiration rate as an index of the depth of anesthesia; respiration

Table 1. Effect of ketoprofen on the power spectrum

	Before administration	After administration
Delta	0.790 (0.094)	0.840 (0.089)
Theta	0.120 (0.440)	0.095 (0.049)
Alpha	0.050 (0.032)	0.038 (0.029)
Beta 1	0.017 (0.019)	0.012 (0.012)
Beta 2	0.021 (0.025)	0.013 (0.014)

Power spectrum analysis during chloral hydrate (Before) and chloral hydrate plus ketoprofen (After) anesthesia. Means are shown with standard deviations in parentheses. The majority of the EEG signal is in the delta bandwidth before and after ketoprofen administration. The effect of ketoprofen is to significantly reduce power within the theta bandwidth (n = 14, $t = 2.518$, $P = 0.026$, df = 13), indicative of deeper chloral hydrate anesthesia.

rate increased slightly, but not significantly, after acute ketoprofen administration (126.0 versus 135.6, respectively; $P = 0.14$, $t = 1.616$, n = 10). Acute doses of ketoprofen exceeding 200 mg/kg, including 500 and 1000 mg/kg, could induce death.

We also tested for prolonged effects of ketoprofen on the anesthetic plane by monitoring the amount of chloral hydrate needed to maintain this plane. In these experiments, we compared mice that received ketoprofen intraperitoneally just after induction of anesthesia to control mice. Over the course of the experiment, the group of mice injected with 100 mg/kg ketoprofen required significantly less anesthetic than did control mice (9 ICR and 4 B6 mice per group, 156.6 versus 209.7 mg/kg/h, respectively; $P = 0.025$, $t = 2.392$, n = 26). Each group received a similar number of injections per h (1.97 versus 2.31, respectively). All mice were maintained at an anesthetic plane at which a hindpaw pinch changed the EEG but did not elicit a withdrawal reflex. The mean respiration rate, measured over the entire experiment, was similar between the ketoprofen and control groups (137.6 versus 132.5, respectively; $P = 0.399$, $t = 0.858$, n = 26).

Discussion

Our results indicate that the NSAID ketoprofen deepens the anesthetic plane in chloral hydrate-anesthetized mice. This effect does not significantly alter MOB neuronal spontaneous activity nor the mean respiratory rate. When given at the beginning of an experiment, ketoprofen also decreased the amount of chloral hydrate required to maintain a surgical anesthetic plane during the experiment.

In single-unit recordings, chloral hydrate affected the mean firing rate in a dose-dependent manner; all chloral hydrate doses inhibited spontaneous activity but doses exceeding 60 mg/kg inhibited the mean firing rate of MOB single-units for a longer period of time than did lower doses. This result is not surprising, because the anesthetics chloral hydrate, urethane, pentobarbital, and ketamine have been reported to alter neuronal spontaneous activity in rat.[4,14,41,44] The location of 1 unit in the MCL included in the chloral hydrate analysis was not confirmed histologically; linear regression analysis indicated that this unit was not an outlier and therefore this unit was included in the analysis. We also tested the analgesic buprenorphine for effects on MOB spontaneous activity.

Buprenorphine, a partial μ-opioid receptor agonist, inhibited the mean firing rate of MOB neurons in a dose-dependent manner. The inhibition was evident over a concentration range spanning an order of magnitude (Figure 2 D). However, although buprenorphine decreased the firing rate, it did not alter the temporal patterning of action potentials, measured by the CVs of single-unit ISIs. The buprenorphine experiments were conducted on single units from several MOB layers, and using a paired *t*-test, we combined the results from these layers. Buprenorphine decreased the firing rate from units in the MCL and GL but not the 1 unit in the GCL; our experiments did not specifically address whether some MOB cell types may be more sensitive to buprenorphine than others. Although we did not study the mechanism for buprenorphine-associated inhibition of MOB neurons, this compound likely acts through the many μ-opioid receptors expressed in the MOB,[40,46] and inhibition is consistent with previous results.[28,33]

By contrast, ketoprofen does not significantly alter MOB neuronal spontaneous activity. Neither the spontaneous firing rates nor ISI CVs from single-units changed after systemic injection of ketoprofen. A change in ISI CVs would have revealed a change from more regularly spaced action potentials to a bursting pattern or vise versa. For spontaneous firing rates, we used paired *t*-tests to combine results from several layers; no obvious response to ketoprofen was seen in any layer. Preliminary data indicate that ketoprofen did not alter the rate of Purkinje cells in the cerebellum. That ketoprofen does not alter MOB neuronal activity may seem somewhat surprising in light of the drug's reported pharmacology. Ketoprofen rapidly crosses the blood-brain barrier[30] and provides antinociception through serotonin, acetylcholine, and adrenergic pathways of the dorsal horn and peripheral nerves.[7,28,32,33,34] These neurotransmitters are part of the MOB chemoarchitecture, primarily through afferent pathways.[16,19,20] However, increases in MOB norepinephrine caused by robust stimulation of locus coeruleus produce only a transient (approximately 90 s) decrease in spontaneous activity.[16] In addition, ketoprofen alters the activity of various thalamic nuclei and some areas of the brainstem; however, other regions are not affected.[2,5] Our experiments did not address whether ketoprofen affects the odor-evoked responses of MOB neurons or field potentials evoked by electrical stimulation.

Lastly, we tested ketoprofen for an antinociceptive effect during chloral hydrate anesthesia. For all mice in this study, ketoprofen altered the EEG trace similarly to the analgesic buprenorphine (Figure 4) and deepened anesthesia.[35] In our results, ketoprofen also altered the EEG power spectrum in a manner consistent with deeper chloral hydrate anesthesia. Fourier analysis of the EEG signal revealed loss of power from the losses in theta, alpha, beta 1, and beta 2 bandwidths and a concomitant increase in power in the delta bandwidth (Table 2). The increase in power in the delta bandwidth and significant loss of power in the theta bandwidth are consistent with a deeper anesthetic plane.[42] The lack of significance in the increase in power in the delta bandwidth may reflect variability in the anesthetic plane maintained by chloral hydrate despite efforts to keep it relatively constant. Although the anesthetic plane was deeper with ketoprofen, there was no evidence of oscillations of the spontaneous activity that have been seen in rats under deep anesthesia.[16]

Ketoprofen-injected animals required less chloral hydrate than did control animals. This difference occurred despite the fact that each group was maintained at the same anesthetic plane and received supplemental injections of chloral hydrate at similar frequencies. Therefore, the ketoprofen-injected animals received smaller doses, and these smaller doses affect MOB neuronal ac-

tivity for shorter periods of time. Ketoprofen-injected animals had respiration rates slightly higher than those of control animals, closer to the respiratory rates of awake, unanesthetized animals.[13]

The results we obtained indicate that the NSAID ketoprofen provides antinociception in the chloral hydrate-anesthetized C57BL6J and ICR mice. Given our results and the manufacturer's recommended dose, we recommend a starting dose of 100 mg/kg ketoprofen when used in conjunction with chloral hydrate anesthesia. The B6 mouse is a common genetic background for genetically engineered mice, and future studies are likely to incorporate such animals. The results we obtained in this strain are also important because we previously had noted differential responses to chloral hydrate in B6 and ICR mice, with the B6 mice more prone to mortality from unintentional overdose.[26] Others also have noted potent strain effects on phenotype.[8,45]

Overall, the combination of ketoprofen and chloral hydrate allows the maintenance of mice at a surgical anesthetic plane with a decreased anesthetic dose and without compromising the neurophysiology of MOB neurons. With this mixture, spontaneous activity is maintained, and the animal experiences minimal respiratory depression.

Acknowledgments

This work was performed as a requirement for partial completion of a Master's thesis (TGM) and was supported in part by a Sigma Xi Grant-in-Aid-of-Research (TGM), a University of Cincinnati Department of Biological Sciences Wieman summer fellowship (TGM), and NIH grant 1R15DC04548 (ERG). We also thank Gerald Mast, DVM for the suggestion to use ketoprofen and Gregg Boivin, DVM for critical review of a draft of the manuscript.

References

1. **Aroniadou-Anderjaska V, Ennis M, Shipley MT.** 1997. Glomerular synaptic responses to olfactory nerve input in rat olfactory bulb slices. Neuroscience **79:**425–434.
2. **Braga PC.** 1990. Ketoprofen: ICV injection and electrophysiological aspects of antinociceptive effect. Eur J Pharmacol **184:**273–280.
3. **Buritova J, Besson J-M.** 2001. Urethane anaesthesia could partly mask antinociceptive effects of non-steroidal anti-inflammatory drugs: a spinal c-Fos protein study. Brain Res **891:**281–284.
4. **Chaput MA, Holly A.** 1979. Spontaneous activity of olfactory bulb neurons in awake rabbits, with some observations on the effects of pentobarbital anaesthesia. J Physiologie (Paris) **75:**939–948.
5. **de Beaurepaire R, Suaudeau C, Chait A, Cimetiere C.** 1990. Anatomical mapping of brain sites involved in the antinociceptive effects of ketoprofen. Brain Res **536:**201–206.
6. **Diaz-Reval MI, Ventura-Martinez R, Deciga-Campos M, Terron JA, Cabre F, Lopez-Monoz FJ.** 2004. Evidence for a central mechanism of action of S-(+)-ketoprofen. Eur J Pharamacol **483:**241–248.
7. **Dickenson AH, Sullivan AF, McQuay HJ.** 1990. Intrathecal etorphine, fentanyl and buprenorphine on spinal nociceptive neurons in the rat. Pain **42:**227–234.
8. **Doetschman T.** 1999. Interpretation of phenotype in genetically engineered mice. Lab Anim Sci **49:**137–143.
9. **Filipovic SR, Covickovic-Sternic N, Stojanovic-Svetel , Lecic D, Kostic VS.** 1998. Depression in Parkinson's disease: an EEG frequency analysis study. Parkinsonism Relat Disord **4:**171–179.
10. **Flecknell PA.** 1984. The relief of pain in laboratory animals. Lab Anim **18:**147–160.
11. **Fort Dodge.** 1997. Ketofen product insert. Fort Dodge (IA): Fort Dodge Animal Health.
12. **Furst S.** 1999. Transmitters involved in antinociception in the spinal cord. Brain Res Bull **48:**129–141.
13. **Green EL.** 1966. Biology of the laboratory mouse, 2nd ed. New York: McGraw-Hill. p 342.
14. **Greffrath W, Kirschstein T, Nawrath H, Treede R-D.** 2002. Acetylsalicyclic acid reduces heat responses in rat nociceptive primary sensory neurons-evidence for a new mechanism of action. Neurosci Lett **320:**61–64.
15. **Halk CT, Leary S, Morris T.** 2005. Formulary for laboratory animals, 3rd edition. Ames, Iowa, Blackwell.
16. **Jiang M, Griff ER, Ennis M, Zimmer LA, Shipely MT.** 1996. Activation of locus coeruleus enhances the responses of olfactory bulb mitral cells to weak olfactory nerve input. J Neurosci **16:**6319–6329.
17. **Kareti S, Moreton JE, Khazan N.** 1980. Effects of buprenorphine, a new narcotic agonist-antagonist analgesic on the EEG, power spectrum and behavior of the rat. Neuropharmacology **19:**195–201.
18. **Kogel B, Christoph T, Strassburger W, Friderichs E.** 2005. Interaction of mu-opioid receptor agonists and antagonists with the analgesic effect of buprenorphine in mice. Eur J Pain **9:**599–611.
19. **Kosaka K, Toida K, Aika Y, Kosaka T.** 1998. How simple is the organization of the olfactory glomerulus? The heterogeneity of so-called periglomerular cells. Neurosci Res **30:**101–110.
20. **Kratskin IL.** 1995. Functional anatomy, central connections, and neurochemistry of the mammalian olfactory bulb. In: Doty RL, editor. Handbook of olfaction and gustation. New York: Mercer and Dekker. p 103–119.
21. **Lazaro-Ibanez GG, Torres-Lopez JE, Granados-Soto V.** 2001. Participation of the nitric oxide-cyclic GMP-ATP-sensitive K^+ channel pathway in the antinociceptive action of ketorolac. Eur J Pharmacol **426:**39–44.
22. **Lee HM, Kim HI, Shin YK, Lee CS, Park M, Song J-H.** 2003. Diclofenac inhibition of sodium currents in rat dorsal root ganglion neurons. Brain Res **992:**120–127.
23. **Lutfy K, Eitan S, Bryant CD, Yang YC, Saliminejad N, Walwyn W, Kieffer BL,Takeshima H, Carroll FI, Maidment NT, Evans CJ.** 2003. Buprenorphine-induced antinociception is mediated by mu-opioid receptors and compromised by concomitant activation of opioid receptor-like receptors. J Neurosci **23:**10331–10337.
24. **Martinovic Z, Jovanovic V, Ristanovic D.** 1998. EEG power spectra of normal preadolescent twins. Gender differences of quantitative EEG maturation. Neurophysiol Clin **28:**231–248.
25. **Mast TG.** 2003. Personal observation.
26. **Mast TG, Griff ER.** 2005. In vivo preparation and identification of mitral cells in the main olfactory bulb of the mouse. Brain Res Brain Res Protoc **15:**105–113.
27. **Mazario J, Roza C, Herrero JF.** 1999. The NSAID dexketoprofen trometamol is as potent as μ-opioids in the depression of wind-up and spinal cord nociceptive reflexes in normal rats. Brain Res **816:**512–517.
28. **Miranda HF, Lemus I, Pinardi G.** 2003. Effect of the inhibition of serotonin biosynthesis on the antinociception induced by nonsteroidal anti-inflammatory drugs. Brain Res Bull **61:**417–425.
29. **Nickell WT, Shipley MT.** 1992. Neurophysiology of the olfactory bulb. In: Serby M, Chobor K, editors. The science of olfaction. New York: Springer-Verlag. p 172–212.
30. **Netter P, Lapicque F, Bannwarth B, Tamisier JN, Thomas P, Royer RJ.** 1985. Diffusion of intramuscular ketoprofen into the cerebrospinal fluid. Eur J Clin Pharmacol **29:**319–321.
31. **Perez H, Hernandez A, Inostroza H.** 1989. Mu and kappa opioid modulation of olfactory bulb evoked potentials. Int J Neurosci **49:**329–332.
32. **Pinardi G, Sierralta F, Miranda HF.** 2001. Interaction between the antinociceptive effect of ketoprofen and adrenergic modulatory systems. Inflammation **25:**233–239.
33. **Pinardi G, Sierralta F, Miranda HF.** 2002. Adrenergic mechanisms in antinociceptive effects of non-steroidal anti-inflammatory drugs in acute thermal nociception in mice. Inflamm Res **51:**219–222.

34. **Pinardi G, Sierralta F, Miranda HF.** 2003. Atropine reverses the antinociception of non-steroidal anti-inflammatory drugs in the tail-flick test of mice. Pharmacol Biochem Behav **74:**603–608.
35. **Prior PF.** 1979. Monitoring cerebral function, 1st ed. Toronto: JB Lippincott. p 71–72.
36. **Reckitt-Benckiser.** 2001. Buprenex product insert. Richmond (VA): Reckitt Benckiser Pharmaceuticals Inc.
37. **Richardson CA, Flecknell PA.** 2005. Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress. Altern Lab Anim **33:**119–127.
38. **Rodieck RW.** 1967. Maintained activity of cat retinal ganglion cells. J Neurophysiol **30:**1043–1071.
39. **Roughan JV, Flecknell PAS.** 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. Lab Anim **36:**322–343.
40. **Schulz S, Schreff M, Koch T, Zimprich A, Gramsch C, Elde R, Höllt V.** 1988. Immunolocalization of two mu-opioid receptor isoforms (mor1 and mor2) in the rat central nervous system. Neurosci **82:**613–622.
41. **Scott JW, Stewart WB.** 1979. Mechanisms of augmented field potential responses in the rat olfactory bulb. Brain Res **163:**21–32.
42. **Sisson DF, Siegel J.** 1989. Chloral hydrate anesthesia: EEG power spectrum analysis and effects on VEPs in the rat. Neurotoxicol Teratol **11:**51–56.
43. **Softky WR, Koch C.** 1993. The highly irregular firing of cortical cells is inconsistent with temporal integration of random EPSPs. J Neurosci **13:**334–350.
44. **Stewart WB, Scott JW.** 1979. Anesthetic-dependent field potential interactions in the olfactory bulb. Brain Res **103:**487–499.
45. **Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, Lamantia C, Mourton T, Herrup K, Harris RC, Barnard JA, Yuspa SH, Coffey RJ, Magnuson T.** 1995. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science **269:**230–234.
46. **Tong Y, Chabot J-G, Shen S-H, O'Dowd BF, George SR, Quirion R.** 2000. Ontogenic profile of the expression of the mu opioid receptor gene in the rat telencephalon and diencephalons: an in situ hybridization study. J Chem Neuroanat **18:**209–222.
47. **Vane JR.** 1971. Inhibition of prostiglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol **231:**232–235.
48. **Voilley N, de Weille J, Mame JT, Lazdunski M.** 2001. Nonsteroid anti-inflammatory drugs inhibit both the activity and the inflammation-induced expression of acid-sensing ion channels in nociceptors. J Neurosci **21:**8026–8033.
49. **Young TA, Wilson DA.** 1999. Frequency-dependent modulation of inhibition in the rat olfactory bulb. Neurosci Lett **276:**65–67.
50. **Zar JH.** 1999. Biostatistical analysis, 4th ed. Upper Saddle River (NJ): Prentice Hall. p 40.