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Analgesic Activity of Tramadol and Buprenorphine after Voluntary Ingestion by Rats (*Rattus norvegicus*)

Bryan F Taylor,[1] Harvey E Ramirez,[1,*] August H Battles,[1] Karl A Andrutis,[1] and John K Neubert[2]

Effective pain management for rats and mice is crucial due to the continuing increase in the use of these species in biomedical research. Here we used a recently validated operant orofacial pain assay to determine dose–response curves for buprenorphine and tramadol when mixed in nut paste and administered to male and female rats. Statistically significant analgesic doses of tramadol in nut paste included doses of 20, 30, and 40 mg/kg for female rats but only 40 mg/kg for male rats. For male rats receiving buprenorphine mixed in nut paste, a significant analgesic response was observed at 0.5 and 0.6 mg/kg. None of the doses tested produced a significant analgesic response in female rats. Our results indicate that at the doses tested, tramadol and buprenorphine produced an analgesic response in male rats. In female rats, tramadol shows a higher analgesic effect than buprenorphine. The analgesic effects observed 60 min after administration of the statistically significant oral doses of both drugs were similar to the analgesic effects of 0.03 mg/kg subcutaneous buprenorphine 30 min after administration. The method of voluntary ingestion could be effective, is easy to use, and would minimize stress to the rats during the immediate postoperative period.

Abbreviations: VI, voluntary ingestion; LFR, lick:facial contact ratio; OPAD, orofacial pain assessment device.

Effective pain management for rats and mice is crucial due to the continuing increase in the use of these species in biomedical research. It is necessary not only to satisfy ethical and legal standards, but providing effective pain management may also reduce distress, decrease mortality, and overall eliminate many of the negative postsurgical physiologic consequences that may be confounding factors in research.[52] Effective pain management entails providing analgesics at the optimal dosing regimen (dose, frequency, and duration). Continual refinement of the optimal analgesic dosing regimen is possible due to the availability of new methods for evaluating pain (that is, mouse and rat grimace scales),[47,69] the increasing knowledge of pain mechanisms and pathways,[56,71] and the development of new analgesic formulations.[14,68]

Tramadol is a centrally acting analgesic with both opioid and nonopioid mechanisms of action.[60] Its analgesic activity is due to a high affinity for μ-opioid receptors and both serotonin and norepinephrine reuptake inhibition.[2] In vitro, tramadol has been shown to inhibit the activity of voltage-operated Na^+ channels, delayed rectifier K^+ channels, N-methyl-D-aspartate receptors, and substance P receptors.[27,28,49,75] Tramadol also exhibits relatively few of the adverse effects typically associated with classic opioids, including respiratory depression and ileus.[60] The most commonly reported tramadol-associated adverse events in humans include nausea, dizziness, and drowsiness.[26] In the United States, oral formulations of tramadol are widely used in both human medicine and in companion animals.[59,64] Despite the potential benefits, very few studies have evaluated the efficacy of tramadol after oral administration in laboratory rats.[55]

In comparison, buprenorphine is one of the most common analgesics used for mild to moderate pain in rats, and its use is considered a standard of care for postoperative pain.[14] Buprenorphine acts as a partial μ-opioid receptor agonist, and its slow dissociation kinetics allows for a longer duration of action compared with that of classic μ-opioid agonists, such as morphine.[45,76,79] Additional benefits of buprenorphine include a ceiling effect on respiratory depression and a lack of immunosuppression at doses relevant for analgesia.[30,57,61] Side effects in rats are usually limited but include sedation, cardiovascular depression, decreased appetite, and gastrointestinal distress, which may or may not be accompanied by pica.[15,19,63] Administration of buprenorphine by the oral route in rats is limited by a lack of information regarding its pharmacokinetics and conflicting reports of its efficacy.[1,5,23,24,36,46,74]

Providing analgesics mixed in the food or water of rats and mice is one of the least stressful methods of administration. This method eliminates postoperative manual restraint and parenteral injections, which have been shown to induce stress-like responses in mice and rats.[6,66,67] Providing analgesics by this method has several drawbacks. First, the neophobic behavior of rats and mice may lead to significant underdosing when a period of habituation to the drug is not observed.[70] Second, some drugs are unpalatable and so may not be consumed in sufficient quantities to provide analgesia.[33] Third, animals undergoing surgery typically have reduced food and water intake during the immediate postoperative period, and this behavior may limit the dose administered.[29] Fourth, overdosing may occur when analgesics are provided with a palatable vehicle, such as a cherry-flavored solution.[8] Finally, when opioids are administered in the food or water, tolerance may develop, leading to a decrease in analgesic efficacy.[35]

To mitigate these issues, sufficient dosages of analgesics can be offered for voluntary ingestion (VI) by mixing the drug in

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[1]Animal Care Services and the [2]Department of Orthodontics, University of Florida, Gainesville, Florida
**Corresponding author. Email: hramir@ufl.edu*

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a highly palatable vehicle without needing to add it to food or water. Several recent studies using a sweet nut paste for the administration of buprenorphine have shown its promise as an effective vehicle for administration.[1,23,24] Providing analgesics in this manner allows for a fixed dosage of the drug to be administered consistently, with the added assurance that the animals are consuming an effective dose and are not under- or overdosed.

The goal of the current study was to evaluate the analgesic effects of a range of oral doses of tramadol or buprenorphine mixed with nut paste and provided to rats by VI. Rats were evaluated by using a recently described, nontraumatic, reversible, operant-based, thermal orofacial pain assessment device (OPAD).[53,62] In this model, a low concentration of capsaicin is applied topically to the test area for 5 min and then is removed. Capsaicin sensitizes the transient receptor potential vanilloid 1 receptor to heat.[51] This receptor is a key channel for signaling and modulating heat and inflammatory pain,[12,13,58] and previous studies in mice have documented the importance of the receptor in the development of incisional pain.[40,58] Heat is then applied to the sensitized test area, thereby activating these receptors and eliminating the need for surgical procedures. Our hypothesis was that OPAD evaluation would be effective at establishing clinically relevant doses for tramadol and buprenorphine in both male and female rats.

Materials and Methods

Test subjects and housing conditions. Male and female Sprague–Dawley rats (*Rattus norvegicus*; $n = 20$ per sex; initial weight, 150 to 175 g; Crl:SD, Charles River Laboratories, Portage, MI) were pair-housed in autoclaved polysulfone individually ventilated microisolation cages (39.3 cm × 28.5 cm × 19.4 cm; Allentown Caging, Allentown, NJ) with corncob bedding (7092, Harlan Teklad, Madison, WI). Rats had unrestricted access to irradiated rodent chow (7912, Harlan Teklad) and reverse-osmosis–purified water provided by an automatic watering system. The room was maintained at standard temperature and humidity (21 ± 2 °C, 30% to 70%) and on a 12:12-h light:dark cycle (lights on, 0600). Cages were changed once weekly. Rats were acclimated to these conditions for a minimum of 5 d prior to handling. The rats were antibody-negative for coronavirus (sialodacryoadenitis virus/rat coronavirus), Kilham rat virus, lymphocytic choriomeningitis virus, mouse adenovirus, *Mycoplasma pulmonis*, pneumonia virus of mice, rat minute virus, rat parvovirus, reovirus type 3, Sendai virus, Theiler murine encephalomyelitis virus, and Toolan H-1 virus. In addition, rats were free of external and internal parasites. *Helicobacter* spp. were not part of the pathogen exclusion list and therefore were not tested. Female rats were acquired after completion of the study using the male rats. At the end of the study, all rats were euthanized by carbon dioxide inhalation followed by thoracotomy. The research protocol was approved by the University of Florida Animal Care and Use Committee and was performed in AAALAC-accredited facilities.

Fasting. Food was removed at 0800 on the morning of a planned training or test session. During the initial OPAD box acclimation and training sessions, daily fasting occurred every morning on weekdays. Once task training was achieved, rats were fasted 3 times weekly during alternating testing and training sessions that occurred at 1400 to 1700 (for example, Monday test session, Wednesday training session, Friday test session). Food was available free choice between sessions and on weekends.

Preparation of skin/test area. At 48 h prior to test sessions, rats were anesthetized by placing them into an induction box and delivering isoflurane (5%) and oxygen by a precision vaporizer until the righting reflex was abolished. Rats were then removed from the box and placed on a circulating warm-water blanket to maintain body temperature. Anesthesia was maintained with isoflurane (2% to 2.5%) and oxygen mixture delivered by nose cone. A bland ophthalmic ointment was placed liberally on both eyes to prevent corneal dryness. The hair on the face was removed gently by using clippers followed by depilatory cream (Nair, Church and Dwight, Princeton NJ) over the desired area and allowing a 2-min contact time. Excess cream was removed with a wet cotton facial pad (Cotton Rounds, CVS Pharmacy, Woonsocket, RI) followed by a dry cotton facial pad. Care was taken to retain the whiskers. Rats were then allowed to recover from anesthesia.

Operant box and task training. The OPAD (Stoelting, Wood Dale, IL) used in this study is described elsewhere.[3] Briefly, the test box consisted of acrylic walls (20.3 cm × 20.3 cm × 16.2 cm) with an opening (4 cm × 6 cm) in one wall that was placed directly in front of 2 temperature-controlled vertical metal thermodes. A standard rodent water bottle containing the reward solution of a diluted (1:2 with water) sweetened condensed milk solution (Eagle Brand, El Paso, TX) was mounted outside the box (Figure 1). On the floor of the box was an elevated wire bar grate that served as a ground contact. The box was then connected to a multichannel data acquisition station (ANY-maze, version 4.98, Stoelting).

Training consisted of allowing the rats to comfortably perform the task of placing their face through the opening and having their cheeks maintain contact with the thermodes to access the reward bottle. Fasted, unrestrained animals were placed individually into a test box, and the data acquisition system was activated. The bottle position was adjusted horizontally and vertically to facilitate contact of the thermodes within the same shaved area of the face for each animal. When the rat drank from the bottle, the skin on its shaved face contacted the thermodes and completed an electrical circuit. When the rat's tongue contacted the metal spout of the bottle, a second circuit was completed. The closed circuits registered in the computer, and data were collected at 200 Hz for the entire experiment. Each spout contact was recorded as a 'lick' event, and each face contact was recorded as a 'facial contact' event. A total of 5 training sessions (no capsaicin, thermode temperature set at 37 °C) were necessary for consistent completion of the task. Training and testing sessions lasted for 10 min each and occurred at 1400 to 1700. Each test session was alternated with a training session, with a washout period of at least 4 d. The test room temperature was maintained at 22 ± 1 °C for all behavioral tests.

Syringe training and palatability assessment. After the initial acclimation period, each rat was offered a plastic syringe cap filled with 2 mL of nut paste (Nutella, Ferrero, Somerset, NJ) in its home cage. The rats were then observed to ensure they sampled the nut paste. The caps were removed after 30 min or earlier if the entire amount was consumed. The following day, rats were offered a cap with nut paste for 5 min, after which it was removed, and nut paste at 2 g/kg was immediately offered in a 1-mL syringe through the wire bar lid of the cage (Figure 2). On the following day and thereafter, rats were offered only the nut paste-filled syringe. Rats were considered trained and ready to assess palatability of the drug-nut paste mixture when the entire dose was consumed from the syringe. A total of 5 consecutive once-daily sessions were necessary to train all rats to consume the nut paste from the syringe.

Group assignment and drug mixture preparation. Rats (10 male and 10 female per group) were randomly assigned to 1

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Figure 1. Orofacial pain assessment device. A rat is placed in the test box with access to a reward bottle filled with diluted sweetened condensed milk. To access the reward sipper tube, the rat must make facial contact with 2 parallel vertical metal thermodes which can be adjusted to contact the same shaved area of the face each test session. The temperature of the metal thermodes are controlled by a computer. The number of facial contacts with the metal thermodes and the number of licks to the sipper tube are counted by the computer. The nontraumatic nature of the assay allows the same rat to be evaluated multiple times.

Figure 2. Administration of analgesics to pair-housed rats by voluntary ingestion. Two rats can be effectively medicated simultaneously with analgesics mixed in a palatable vehicle. Voluntary ingestion decreases handling stress and provides assurance that animals obtain adequate doses for effective analgesia.

Table 1. Oral tramadol dosing order.

		Test day				
Rat ID	Sex	1	2	3	4	5
11	M	20	30	40	0	10
12	M	40	0	10	20	30
13	M	10	20	30	40	0
14	M	40	0	10	20	30
15	M	30	40	0	10	20
16	M	0	10	20	30	40
17	M	20	30	40	0	10
18	M	0	10	20	30	40
19	M	30	40	0	10	20
20	M	10	20	30	40	0
31	F	40	0	10	20	30
32	F	10	20	30	40	0
33	F	0	10	20	30	40
34	F	0	10	20	30	40
35	F	40	0	10	20	30
36	F	30	40	0	10	20
37	F	20	30	40	0	10
38	F	30	40	0	10	20
39	F	20	30	40	0	10
40	F	10	20	30	40	0

All doses in mg/kg.

Table 2. Oral buprenorphine dosing order.

		Test day				
Rat ID	Sex	1	2	3	4	5
1	M	0.5	0.6	0	0.3	0.4
2	M	0.4	0.5	0.6	0	0.3
3	M	0.3	0.4	0.5	0.6	0
4	M	0.6	0	0.3	0.4	0.5
5	M	0.6	0	0.3	0.4	0.5
6	M	0	0.3	0.4	0.5	0.6
7	M	0.4	0.5	0.6	0	0.3
8	M	0.3	0.4	0.5	0.6	0
9	M	0	0.3	0.4	0.5	0.6
10	M	0.5	0.6	0	0.3	0.4
21	F	0.3	0.4	0.5	0.6	0
22	F	0.5	0.6	0	0.3	0.4
23	F	0	0.3	0.4	0.5	0.6
24	F	0.6	0	0.3	0.4	0.5
25	F	0	0.3	0.4	0.5	0.6
26	F	0.3	0.4	0.5	0.6	0
27	F	0.4	0.5	0.6	0	0.3
28	F	0.5	0.6	0	0.3	0.4
29	F	0.6	0	0.3	0.4	0.5
30	F	0.5	0.6	0	0.3	0.4

All doses in mg/kg.

of 2 drug groups. Oral doses were randomly assigned prior to the first test session. Two rats received each dose within a test session. All rats in each group received every dose of their assigned drug in a crossover fashion (Tables 1 and 2). For a positive control, both treatment groups were then tested by using buprenorphine HCl (Buprenex injectable, Reckitt Benckiser Pharmaceuticals, Richmond, VA) administered at 0.03 mg/kg SC and diluted with 0.9% saline to obtain an injection volume of 1 mL/kg. All drugs were administered between 1400 and 1600 each test day. Each drug test group received by VI either

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buprenorphine HCl (sublingual tablets, Roxane Laboratories, Columbus, OH) or tramadol HCl (oral tablets, Amneal Pharmaceuticals, Glasgow, KY) mixed in nut paste. All drugs were prepared fresh each test day; the tablets were crushed to a fine powder by using a mortar and pestle and then mixed with nut paste to a uniform consistency. Each stock mixture was made to provide the highest tested dose of each drug (that is, 40 mg/kg tramadol, 0.6 mg/kg buprenorphine) for a total nut-paste mixture amount of 2 g/kg. On test days, rats receiving lower drug doses (and thus less of the total nut-paste mixture) were provided extra nut paste to maintain the total amount at 2 g/kg. By using this method, tramadol was administered at 0, 10, 20, 30, and 40 mg/kg PO and buprenorphine at 0, 0.3, 0.4, 0.5, and 0.6 mg/kg PO. On the last test day, all rats were tested as described after receiving 0.03 mg/kg SC buprenorphine 30 min prior to operant testing.

Thermal pain testing. On test days, rats were brought to the test room at 1330, weighed, and allowed to acclimate to the room for 30 min. Twenty min after ingesting the assigned drug-nut paste mixture, rats were anesthetized as described above. A bland ophthalmic ointment was placed liberally on both eyes to prevent corneal dryness. Capsaicin cream (Capzasin-HP 0.1%, Chattem, Chattanooga, TN) was liberally applied to the hairless areas of the face making sure the cream did not contact the eyes or mouth. After 5 min of contact time, the capsaicin cream was removed with alcohol moistened wipes (BD Alcohol Swabs, Becton Dickinson, Franklin Lakes, NJ), and the face was dried with cotton facial pads. The total anesthesia time for each rat was 10 min. Rats were allowed to recover from anesthesia for 30 min before being placed in the test box as described earlier. The thermode temperature during the testing period was set to 45 °C.

Statistical analysis. Licks and facial contact events were recorded. The primary variable evaluated was the ratio of reward licks to facial contact events (lick:facial contact ratio, LFR). This measure was calculated by dividing the total number of licking events by the total number of facial contact events that were longer than 0.1 s in duration. Groups were analyzed for normality by using Shapiro–Wilk normality test (Prism 5.01, GraphPad Software, San Diego, CA). Data for each drug and sex were analyzed separately by using one-way repeated-measures ANOVA for the dose variable, with posthoc Bonferroni multiple-comparison testing (Prism 5.01, GraphPad Software). *P* values less than 0.05 were considered significant. A 2-tailed paired *t* test was used to compare the LFR of each dose with a statistically significant effect to the LFR obtained from a dose of 0.03 mg/kg buprenorphine given SC (positive analgesic control). All data are presented as the mean ± SEM.

Results

Thermal pain testing after tramadol administration. Comparison of the LFR after VI of tramadol by male rats (Figure 3 A) revealed a significant main effect among all groups ($F_{5, 49}$ = 3.86, P = 0.0099). VI of tramadol provided a mean increase in LFR of –7.5%, 68%, 78%, and 178% for 10, 20, 30, and 40 mg/kg, respectively, with a significant (P < 0.05) difference in dose effect between no drug and 40 mg/kg tramadol. The LFR did not differ between 40 mg/kg tramadol by VI and 0.03 mg/kg SC buprenorphine (t = 0.7610, P = 0.4661).

Comparison of the LFR after VI of tramadol by female rats (Figure 3 B) disclosed a significant main effect among all groups ($F_{5, 49}$ = 7.921, P = 0.001). VI of tramadol provided a mean increase in LFR of 7.6%, 194%, 237%, and 293% for 10, 20, 30, and 40 mg/kg respectively when compared with no drug; these differences were significant for the 20-mg/kg (P < 0.05), 30-mg/kg (P < 0.01), and 40-mg/kg (P < 0.001) doses. Furthermore, the LFR did not differ between buprenorphine at 0.03 mg/kg SC and tramadol at 20 mg/kg (t = 0.7139, P = 0.4934), 30 mg/kg (t = 0.6291, P = 0.5449), or 40 mg/kg (t = 0.01830, P = 0.9858).

Thermal pain testing after buprenorphine administration. Comparison of the LFR after VI of various doses of buprenorphine by male rats (Figure 3 C) demonstrated a significant main effect among all groups ($F_{5,49}$ = 2.762, P = 0.0423). Both the 0.5- and 0.6-mg/kg dose produced greater (P < 0.05 in both comparisons) analgesic responses than did the no-dose treatment. In addition, VI of buprenorphine provided a mean LFR increase of 100%, 103%, 147%, and 135% for the 0.3, 0.4, 0.5, and 0.6 mg/kg buprenorphine, respectively, when compared with no drug. The LFR did not differ between 0.03 mg/kg buprenorphine SC and 0.5 mg/kg VI (t = 0.1488, P = 0.8850) or 0.6 mg/kg VI (t = 0.7649, P = 0.4639).

Comparison of the LFR after VI of various doses of buprenorphine by female rats (Figure 3 D) revealed a significant main effect among all groups ($F_{5, 49}$ = 2.181, P = 0.0908). VI of buprenorphine provided a mean increase in LFR of 48%, 101%, 94%, and 105% for 0.3, 0.4, 0.5, and 0.6 mg/kg respectively when compared with no drug. None of these differences was significant.

Discussion

Refinements in the availability of analgesic options with proven efficacy, effective methods of administration, and duration of action of analgesics are necessary to better alleviate pain and distress in laboratory rats. However, the limited availability of a model system capable of assessing clinical analgesic efficacy has slowed the pace with which these data are published. In the current study, an innovative orofacial pain assay was used to evaluate the analgesic efficacy of 2 commonly available analgesics, tramadol and buprenorphine, when given by VI to male and female rats.

Our main goal was to find optimal doses of tramadol and buprenorphine by evaluating the analgesic effects of these drugs over selected dose ranges and when mixed in nut paste and administered to rats by VI. At the doses tested, no adverse effects were observed for either drug. Tramadol has a broad therapeutic profile and low cost, making it a good candidate for use in laboratory rats.[26] One potential drawback is that it is only commercially available in tablet form in the United States, making the oral route the only viable option for clinical use. Currently few data are available regarding the use of tramadol in rats. In one study, the efficacy of oral tramadol was evaluated in male Sprague–Dawley rats by using the hot-plate and tail-flick assays.[11] Rats in the cited study received various doses of tramadol mixed in flavored gelatin cubes 60 min prior to nociception testing. Oral doses as high as 25 mg/kg lacked significant analgesic effect in both assays. In addition, the 50-mg/kg doses were not consistently ingested by rats and so could not be evaluated. The study concluded that either the window of efficacy after oral administration was less than 60 min or that the plasma tramadol concentration was too low after first-pass hepatic metabolism to produce an analgesic effect.[11] The current study found similar results in that oral tramadol does not provide significant analgesic effect at doses between 20 and 30 mg/kg for male rats. In contrast, male rats given an oral dose of 40 mg/kg and tested at 60 min after administration showed significant increases in LFR, indicating that this dose is effective at providing analgesia at 60 min after ingestion. In addition, the analgesic effect produced by 40 mg/kg tramadol

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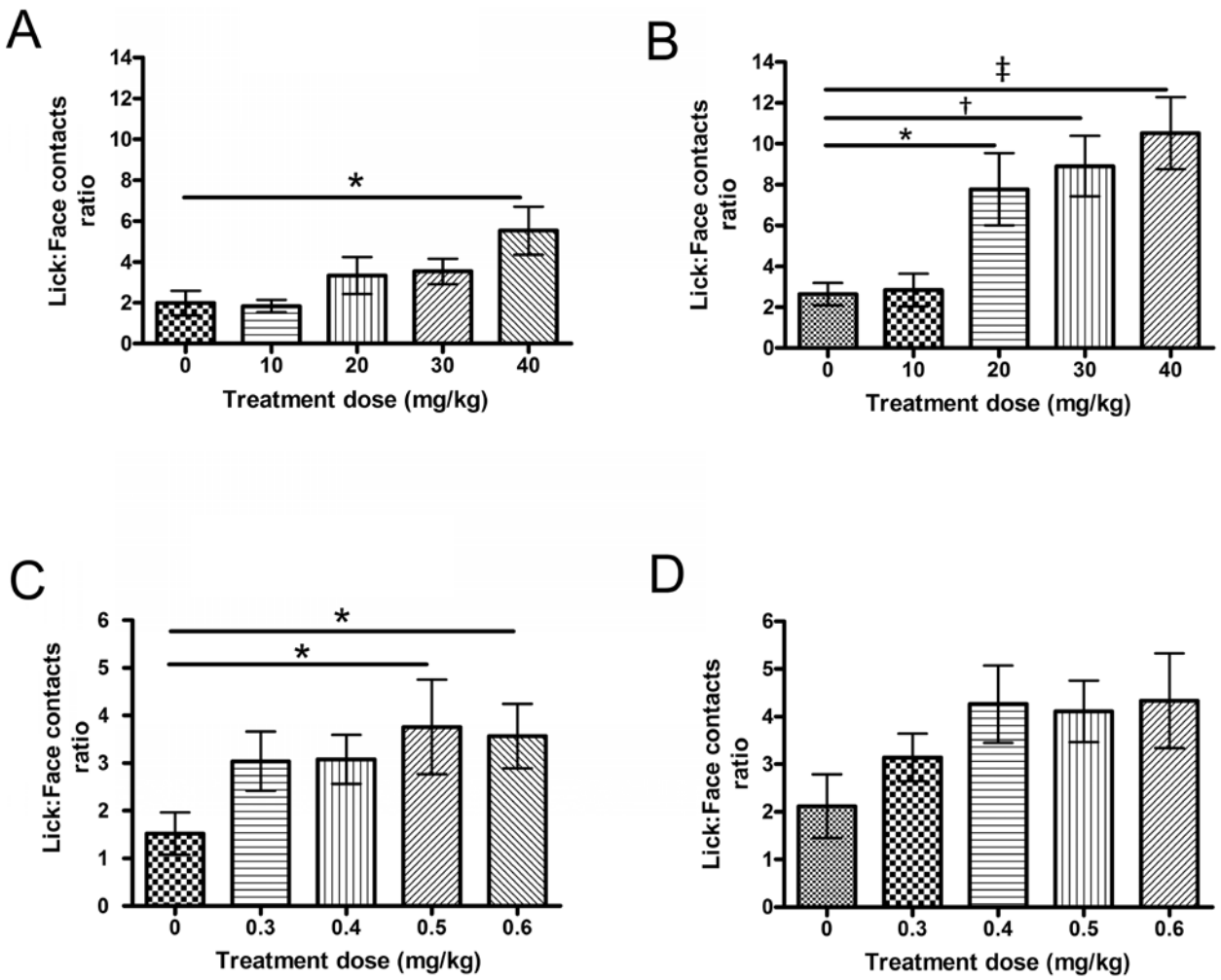

Figure 3. Mean lick:face-contact ratios (mean ± SEM) after voluntary ingestion of various doses of tramadol by (A) male and (B) female rats and of buprenorphine by (C) male and female rats (D). Values differed (*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$) from those for no-dose controls.

VI did not differ from that produced by buprenorphine at 0.03 mg/kg SC. This finding suggests that 40 mg/kg VI tramadol is as effective in producing an analgesic effect at 60 min after administration as is the current standard of postoperative care.

To our knowledge, our current study is the first to evaluate the analgesic efficacy of tramadol after VI in female rats. Tramadol doses as low as 20 mg/kg produced significant increases in LFR in female rats and therefore an increased analgesic effect at 60 min after ingestion. Additional significant increases in LFR were seen at 30 and 40 mg/kg VI tramadol, and the increase in LFR at these doses did not differ from that produced by buprenorphine at 0.03 mg/kg SC. This result suggests that tramadol given at doses between 20 and 40 mg/kg are as effective in producing an analgesic effect in female rats as is the most commonly used opioid analgesic. These results are interesting but not necessarily unexpected. Sex-associated differences in the pharmacokinetics of tramadol and its metabolites after oral administration have been reported in human and animal studies.[31,42,43,48] In the animal studies, female rats showed higher concentration of (+)-*trans*-tramadol, (–)-*trans*-tramadol, and (+)-*trans*-*O*-demethyltramadol.[42] In another study, (+)-*trans*-*O*-demethyltramadol was significantly higher in the plasma of female Sprague–Dawley rats than in male rats, for as long as 6 h after oral administration.[42] This sex-associated difference in dose response may be explained, at least in part, by the hepatic metabolism of tramadol. In rats, tramadol is metabolized in the liver by the hepatic enzyme CYP2D1 to its only pharmacologically active metabolite, *O*-demethyltramadol.[43] This active metabolite has 2 enantiomers, (+)-*O*-demethyltramadol and (–)-*O*-demethyltramadol; (+)-*O*-demethyltramadol has a high affinity for μ-opioid receptors, and (–)-*O*-demethyltramadol inhibits monoamine reuptake.[42] The increased in LFR noted with lower doses of tramadol in female rats are thought to be due to the higher plasma levels of (+)-*O*-demethyltramadol after hepatic metabolism in female rats, therefore yielding a dramatic sex-associated difference in analgesic response when compared with male rats of the same stock and age.

Despite buprenorphine being one of the most commonly used analgesics for rodents, few studies evaluating the efficacy of buprenorphine after oral administration are available, with most providing conflicting results. The use of pain assays with low clinical relevance (for example, hot-water tail flick and paw-withdrawal reflex) combined with variations in the testing paradigm, dosing, and drug preparation likely have contributed to many of these conflicting results.[1,5,23,24,36,41,47,74] For example, buprenorphine doses of 0.4 to 0.5 mg/kg most often are reported as being effective in male rats. However, in one study using the hot-water tail-flick assay, buprenorphine administered to male Long–Evans rats at 0.5 mg/kg in flavored gelatin did not cause significant differences in the pain threshold between before and

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after administration.[46] Although a 10-fold higher dose (100-fold higher than the parenteral dose) provided a significant increase in tail-flick latency, this higher dose had to be administered by gavage, and the authors did not provide a rational for needing such a high dose. In our study using the more clinically relevant OPAD, we showed that oral buprenorphine doses of 0.5 and 0.6 mg/kg significantly increased the LFR in male rats at 60 min after ingestion and therefore can be considered to provide effective analgesia at this time point and in the stock studied (Sprague–Dawley).

In contrast to the male rats in our study, female rats did not achieve a significant increase in LFR at the doses tested. This lack of a significant effect of oral buprenorphine in female rats might be attributed to several factors, including sex-associated differences in analgesic responses and in pain mechanisms and pathways. These sex-associated biases have been well documented in mice and rats.[7,10,16,18,39,44,50,73,77] In addition, μ-opioid agonists are more potent in male than female rats,[44,72] with increasing differences noted between the sexes as the efficacy at μ-opioid receptors decreases.[16] The difference in the analgesic responses observed is less likely to be reflective of the estrous cycle, given that this effect is considered to be weak in rodents.[25] As described previously for tramadol, sex-associated differences in the absorption and metabolism of oral buprenorphine may alter its analgesic effect. Additional studies are needed to confirm this hypothesis.

Voluntary ingestion of analgesics by rodents is considered a preferred method of administration because it eliminates manual restraint as it achieves for accurate and reliable dosage. Voluntary ingestion minimizes handling stress during the immediate postoperative period, potentially minimizing overall postprocedural morbidity. Several studies have evaluated the effect of buprenorphine–nut paste mixtures on corticosterone levels and clinical parameters, including body weight and activity level.[23,24,36] One study found that male Wistar rats receiving a single dose of buprenorphine (0.4 mg/kg) mixed with nut paste had significantly lower plasma corticosterone levels for 18 h after jugular vein catheterization than did rats given buprenorphine subcutaneously.[24] In addition, the plasma buprenorphine levels after ingestion of the buprenorphine–nut paste mixture were equivalent to those achieved after the same treatment duration of subcutaneous buprenorphine.[24] A similar study by the same group found that rats receiving buprenorphine–nut paste mixtures consumed more water and maintained body weight better than did control rats that received only local anesthetics for surgical arterial catheterization.[23] Another study found that male rats given 0.6 mg/kg buprenorphine in nut paste had lower levels of and smaller interindividual differences in fecal corticosterone concentrations after surgically induced cerebral ischemia than did control rats.[36]

In our current studies using VI as a method for administering analgesics, buprenorphine mixtures were placed on a piece of tape, which was then placed on the interior cage wall for rats to ingest. Our initial attempts at this failed with our pair-housed rats, because both rats tended to lick the mixture from the same piece of tape, confounding the dose for both animals. In addition, when separated for a short time, the paired rats appeared uninterested in the mixture. These problems were overcome by training the rats to ingest the drug-nut paste mixture from a syringe. Syringe training facilitated pair-housing, thus providing social interactions as described in the *Guide*.[34] We easily trained all of the rats to ingest the nut paste from a syringe over a period of 5 d, by committing about 30 min each day for training 20 pair-housed rats at a time. At a room temperature of 21 °C, both the nut paste alone and the drug–nut paste mixtures were easily aspirated into a 1-mL syringe. After syringe training, we assessed palatability by using the highest chosen dose of each drug (40 mg/kg tramadol, 0.6 mg/kg buprenorphine). To this end, all rats in their respective groups received one dose of either tramadol or buprenorphine mixed with nut paste in a total amount of 2 g/kg. Only one male rat initially failed to consume the entire 40 mg/kg tramadol–nut paste mixture during the first exposure; this rat successfully consumed the entire amount during the second training period the following day. There were no further issues associated with palatability in any rats for the remainder of the study.

The current study shows that the orofacial operant thermal pain assay described here can become an integral test that can dramatically optimize the preclinical evaluation of effective analgesics for use in rats. The automated, investigator-independent nature of the assay allows for improved assessment and sensitivity of analgesic efficacy. This advantage makes this assay useful for establishing effective analgesic doses that are tailored to each sex.

Our study had several limitations. First, the nut paste used as a vehicle contains high levels of fat, and food items high in fat have been reported to alter the bioavailability of medications.[26,78] Administering tramadol or buprenorphine mixed with the nut paste might have altered their absorption due to the high lipophilicity of these drugs.[17,32] One study in humans found that tramadol administered orally after a high-fat meal did not increase the maximal concentration or AUC compared with those in fasted volunteers.[26] Although some change in the bioavailability of both drugs is likely in our study, these changes are unlikely to be clinically significant. Similarly, sucrose induces naloxone-reversible analgesia in rats, but this effect occurred only after continuous access to sucrose solutions for 2 to 3 wk.[9,65] Sucrose also has been shown to provide analgesia in both thermal and mechanical assays and to modulate the effects of some opioid drugs, including spiradoline.[4,20,21,37,38] These effects were observed either in preweaning pups only or after chronic unlimited administration of sucrose in the drinking water. When a single sucrose dose was infused orally over a period of several minutes, the analgesic effects were not observed in adult rats.[4] In addition, the sweetened condensed milk solution used as the reward in our study did not induce analgesia in a similar study using the OPAD model.[54] Therefore, it is unlikely the sucrose in the nut paste or sweetened condensed milk significantly affected the LFR for any of the rats in the current study. Due to the high fat and sucrose contents, nut paste may not be suitable to use in specific studies, such as in those studying diabetes mellitus.

Second, few pharmacokinetic studies of oral tramadol in rats are available. One study in Wistar rats indicated that oral tramadol administered at 30 mg/kg has a time at maximal concentration of 0.7 ± 0.3 and 0.5 h, and a $t_{1/2}$ of 3.0 ± 1.5 and 3.9 ± 0.6 h, for male and female rats, respectively.[48] However, the pharmacokinetics of *O*-demethyltramadol metabolites were not reported. Another study using Sprague–Dawley rats indicated a time at maximal concentration of 22 ± 11 and 45 ± 14 min and a t1/2 of 105 ± 50 and 218 ± 21 min, for male and female rats respectively, for (+)-transtramadol.[42] For the (+)-*O*-demethyltramadol metabolite, the same study reported a time at maximal concentration of 34 ± 21 and 49 ± 11 min and a half-life of 78.0 ± 28 and 163 ± 98 min in male and female rats, respectively. The current study evaluated the efficacy of oral tramadol at one time point only (60 min after oral administration), and we might have missed the analgesic assessment at

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the peak analgesic effect. Similarly, whether the maximal effect of buprenorphine occurs 60 min after oral administration is unknown. The pharmacokinetics of oral buprenorphine in rats are poorly characterized, and the maximal effect of buprenorphine might have been missed in the current study. After a single dose of tramadol in humans, the extent of oral absorption is nearly 100%, and its bioavailability is 70%;[26] repeated oral dosing of tramadol leads to saturation of first-pass hepatic metabolism and an increased bioavailability to nearly 100%.[26] In rats, a single oral dose of tramadol at 40 mg/kg may saturate first-pass hepatic metabolism, but to our knowledge no such study has yet been performed. Alternatively, repeated dosing of tramadol in rats may lead to saturation of first-pass metabolism and increased bioavailability and thus increased analgesic efficacy at lower doses.

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