

# Acute Oxycodone Induces the Pro-Emetic Pica Response in Rats<sup>S</sup>

Vinita R. Batra and Lisa M. Schrott

Department of Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, Louisiana

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## ABSTRACT

Oxycodone, a semisynthetic opioid analgesic, is frequently prescribed for the management of pain. Side effects of nausea and emesis affect patient compliance and limit its therapeutic use. The present study established that an antinociceptive dose of oxycodone (15 mg/kg; oral) induces the pica response. We found sex differences in the temporal course of pica, with females having a longer duration. Opioid receptors mediated the pica response, as 1.0 mg/kg naloxone transiently attenuated and 2.0 mg/kg naloxone blocked pica. A  $\kappa$ -selective antagonist failed to block the response, suggesting mediation by  $\mu$  opioid receptor. For further validation, we used the well established kaolin intake model to assess pica with the chemotherapeutic drug cisplatin as a positive control. Oxycodone

and cisplatin significantly increased kaolin intake 4- to 7-fold, and the wet weight of stomach was elevated 2- to 3-fold. To examine the underlying neural circuitry, we investigated c-fos activation in the area postrema and nucleus of solitary tract (NTS). Oxycodone treatment significantly increased the number of c-fos-positive neurons in the area postrema and NTS compared with water controls. As expected, cisplatin also increased the number of c-fos-positive cells in these regions. In the area postrema, the oxycodone effect was greater than cisplatin, especially at 2 h. These results indicate that an antinociceptive dose of oxycodone is associated with the expression of pica, a pro-emetic response.

## Introduction

The prescription opioid analgesic oxycodone is a potent  $\mu$ -opioid receptor agonist frequently prescribed for the management of acute and chronic pain (Coluzzi and Mattia, 2005). The therapeutic efficacy of opioids is often limited by severe adverse effects of nausea and emesis (Wheeler et al., 2002). These debilitating side effects compromise patient compliance, leading to discontinuation of opioids and subsequently inadequate analgesia (Redmond and Glass, 2005; Miaskowski, 2009); thus, there is the need to further explore mechanisms that underlie opioid-induced nausea/emesis. Dogs, cats, house musk shrew, and ferrets have been the traditional model for emesis research (Florczyk et al., 1982). However, these animals are difficult to work with in a laboratory setting, and in recent years, the rat model of nausea/emesis has emerged as an important tool for investigating

the mechanisms underlying drug-induced emesis (Takeda et al., 1993).

Rats, mice, and guinea pigs lack a motoric reflex of emesis but display atypical feeding behavior termed the pica response (Takeda et al., 1993; Yamamoto et al., 2004). Pica is an aversive/illness response that results in consumption of non-nutritive substances, such as kaolin, after an emetic stimulus (Mitchell et al., 1976). Pro-emetic agents such as cisplatin and apomorphine have been shown to induce the pica response in rats (Takeda et al., 1993). A study by Yamamoto et al. (2007) indicated that the magnitude of pica in rats was directly related to their "emetogenic potential" in humans. Cisplatin and cyclophosphamide lead to high and sustained levels of pica, actinomycin D and 5-fluorouracil lead to moderate levels, and vincristine induced low levels of the pica response. In addition to these pro-emetic agents, opioids such as morphine and buprenorphine induce the pica response in rats (Bender, 1998; Aung et al., 2004). For instance, Aung et al. (2004) showed that morphine-induced pica was attenuated by opioid antagonist methylnaltrexone, suggesting an opioid receptor-mediated effect.

In addition to this consummatory behavior, other measures of pica, such as impaired gastric function (Liu et al.,

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**ABBREVIATIONS:** NTS, nucleus of the solitary tract; Nor-BNI, norbinaltorphimine; PBS, phosphate-buffered saline; BSA, bovine serum albumin; ANOVA, analysis of variance.

2005) and activation of the neural emetic circuitry, have been investigated. Neuroanatomical regions of the area postrema and nucleus of the tractus solitarius (NTS) in the brainstem underlie emetic responding. Vagal afferents from the gastrointestinal tract innervate these areas, which lie outside the blood-brain barrier, to mediate the emetic response (Hornby, 2001; Saito et al., 2003). Using pharmacological magnetic resonance imaging in live rats, Chin et al. (2006) found that apomorphine activated the area postrema and NTS at plasma concentrations of drug known to induce emesis. Additional studies demonstrated that cisplatin selectively increased the c-fos mRNA in the NTS and area postrema and increased the labeling of c-fos-immunoreactive positive neurons in these nuclei (Endo et al., 2004; Horn et al., 2007).

The objective of the present study was to investigate whether oxycodone induces the behavioral response of pica and activates the emetic circuitry underlying the pica expression. A novel consummatory behavioral response of bedding intake and the well established kaolin intake paradigm were assessed to examine pro-emetic effects. In addition, wet weight analysis of the stomach was used as another measure of pica. A separate group of rats was used to determine whether oxycodone induces the expression of c-fos in the brainstem nuclei of area postrema and NTS.

In addition to the above objective, we assessed sex differences and opioid receptor mediation of pica. The former was important because opioid-induced sex differences have been well documented for measures of analgesia, locomotion, rewarding, and aversive effects. Striking sex differences have been observed for responses to the prototypical opioid morphine. Clinical and preclinical evidence indicate that males are more sensitive to the analgesic and sedative effects of morphine. In contrast, females are more sensitive to nausea, reinforcing, and the locomotor stimulant effects of morphine compared with males (Craft, 2008). We recognize that oxycodone has a different pharmacology than morphine, which may lead to other sexual dimorphisms. For instance, clinical data indicate that under conditions of adjusted body weight, women demonstrate 25% slower clearance of oxycodone compared with men (Kalso, 2005). Female rats are more sensitive than males to the antinociceptive response to oxycodone after subcutaneous administration (Holtman and Wala, 2006), an effect opposite to that seen with morphine. Because the route of administration affects pharmacokinetics (Chan et al., 2008) and can have profound effects on the functional effects, it was also important for us to examine sex differences in the antinociceptive and pro-emetic effects after oral administration.

## Materials and Methods

### Subjects

Nulliparous adult female or male Sprague-Dawley rats (2–4 months of age; Harlan, Indianapolis, IN) were adapted to an oral gavage or intraperitoneal injection procedure using vehicle. The experimental subjects were group-housed in standard flat-bottom plastic cages containing hardwood bedding (SaniChip; PJ Murphy Forest Products, Montville, NJ). Temperature ( $22 \pm 1^\circ\text{C}$ ), humidity (40–50%), and a 12-h light/dark cycle with lights on at 6:00 AM remained constant throughout the experimentation. Food (Teklad; Harlan) and water were available ad libitum throughout the experiment except as noted. The experimental protocol and animal husbandry procedures were approved by the Institutional Animal Care

and Use Committee and comply with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996). All experimental assessments were conducted between 1:00 and 5:00 PM.

### Drug Treatments

Oxycodone HCl (dihydrohydroxycodone, 15 mg/kg; Mallinckrodt, St. Louis, MO) or vehicle (water or saline) was administered orally or intraperitoneally in a volume of 1 ml/kg. In preliminary studies, this dose of oxycodone was found to be antinociceptive (see Supplemental Fig. 1) with no overt toxicity. Oral gavage was done using an 18-gauge, 7.6-cm-long gavage tube (Popper and Sons, New Hyde Park, NY). Naloxone (0.5, 1.0, or 2.0 mg/kg; Sigma-Aldrich, St. Louis, MO) was administered subcutaneously 30 min before oxycodone, and the  $\kappa$  receptor antagonist norbinaltorphimine (Nor-BNI; 7.5 or 15 mg/kg s.c.; Tocris Bioscience, Ellisville, MO) was administered 2 h before oxycodone. The timing of the naloxone and Nor-BNI pretreatments was based on preliminary studies and the work of Takemori et al. (1988), respectively. Cisplatin (Sigma-Aldrich) at 5 mg/kg was injected in a volume of 3 ml/kg i.p. Saline served as the vehicle control for naloxone, Nor-BNI, and cisplatin.

### Behavioral Expression of Pica

Increased consumption of non-nutritive substances is termed pica behavior. Two measures of pica were used in the present study, ingestion of cage bedding (experiment 1) and kaolin intake (experiment 2). The advantage of the bedding ingestion is that temporal analysis of the data is feasible, although the magnitude of the pica response is more difficult to quantify (see supplemental data for a video clip of the bedding ingestion response). The kaolin consumption measure provides quantitative analysis of the data (i.e., kaolin consumption in grams). However, this response is a summation of a 3-h session, which limits temporal analyses.

**Bedding Intake.** Assessment was conducted by observation of rats in clean, transparent rat cages that were the same dimensions as the home cage. The cages contained equal amounts of standard bedding. Ingestion of bedding into the mouth was scored as a quantal (all-or-none) response. Paw gnawing and rigidity were also scored at 15-min intervals for 4 h.

**Kaolin Intake.** Kaolin was made using a standard protocol from literature (Mitchell et al., 1976; Aung et al., 2004). In brief, 1 g of acacia (gum Arabic; Thermo Fisher Scientific, Waltham, MA) was mixed with 99 g of kaolin powder (hydrated aluminum silicate; Thermo Fisher Scientific) in a 99:1 ratio, with distilled water, to form a thick paste. The paste was rolled on a stainless-steel tray, passed through a 5-ml syringe, and cut into pieces in a shape and size similar to that of regular rat chow pellets. The pellets were placed on trays and completely dried at room temperature for 72 h.

The assessment method for kaolin intake was as described previously (Aung et al., 2004) with slight modifications. The procedure consisted of three phases.

**Adaptation.** Rats were acclimatized to the presence of preweighed kaolin and chow in the cages for 3 h each during the 3 days of the adaptation phase.

**Baseline.** Rats were administered the vehicle and baseline measures of kaolin, and chow intake was recorded. Kaolin and food pellets were placed in separate areas in the rat cage.

**Test days.** Cisplatin has an extended (>48 h) pro-emetic action and thus was administered only on test day 1, but the rats were assessed on test days 1 and 2. Oxycodone's duration of action is much shorter and thus was administered on both test days 1 and 2. Kaolin pellets and food pellets were weighed to the nearest 0.1 g before and at the conclusion of the 3-h test session on each day. The difference was recorded as the intake, and the 2-day data were summed and analyzed for treatment effects.

## Stomach Wet Weight

Increase in the wet weight of the stomach has also been observed in rats expressing the pica response. The wet weight of the stomach was measured by a procedure described previously (Malik et al., 2007). In brief, rats were sacrificed at the end of a 3-h session on test day 2. An incision was made proximal to the gastroesophageal junction and distal to the pyloric sphincter to isolate the stomach. After isolation, the stomach was blotted dry, and the wet weight was recorded to the nearest gram.

## Immunohistochemistry for c-fos

**Tissue Collection.** The procedure for tissue collection was performed as described previously (Bennett and Semba, 1998) with slight modifications. In brief, rats were deprived of food or water 3 h before sacrifice to reduce the potential effect of feeding on neural c-fos expression (Horn et al., 2007). Different groups of rats received either 15 mg/kg oxycodone or water via oral gavage and were sacrificed 1 or 2 h after treatment. Separate groups of rats received intraperitoneal injection of cisplatin or saline and were sacrificed 6 h after treatment. The timing for cisplatin treatment was based on optimal results obtained for c-fos activation in the hindbrain region (Horn et al., 2007).

During sacrifice, rats were deeply anesthetized by an injection of sodium pentobarbital (50 mg/kg). The subjects were transcardially perfused with phosphate-buffered saline (PBS) to exsanguinate followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After fixation, brains were dissected from the skull and stored in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 24 h. The brains were then passed through 15% sucrose for 24 h for cryoprotection followed by preservation in 30% sucrose. Three days after cryopreservation, brains were blocked to isolate the forebrain and brainstem. The isolated brainstem was further sectioned at a thickness of 40  $\mu$ m using a cryostat, and serial sections were collected and stored in 24-well plates filled with PBS. Sections were processed for c-fos analysis within 1 week.

**Immunohistochemistry Procedures.** On the basis of the bregma coordinates in the atlas, brain sections were sampled between the coordinates of  $-13.56$  to  $-14.40$  mm (Paxinos and Watson, 2007). Three sections per rat were selected for histochemical analysis, which represented the following regions: nucleus of solitary tract rostral to area postrema, nucleus of solitary tract medial to area postrema, and nucleus of solitary tract caudal to area postrema. These sections were also used to survey the area postrema and determine whether there were any regional differences in the area postrema across the rostrocaudal axis. Another set of sections adjacent to the above were stained with cresyl violet using standard procedures. This was done to specifically match coordinates from c-fos processed tissue and the plate from the rat atlas.

Brain sections were washed in PBS. The sections were then incubated in 3% hydrogen peroxide (30 min) and then in 0.5% BSA (20 min), followed by 1% normal goat serum containing 0.1% Triton X-100 in PBS for 1 h. Sections were rinsed in PBS followed by an overnight incubation at 4°C with a primary c-fos rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a dilution of 1:500. Sections were rinsed in PBS and incubated in 0.5% BSA for 20 min. They were then incubated in a biotinylated goat anti-rabbit secondary antibody (1:2000; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at room temperature for 2.5 h. Sections were subjected to PBS rinses followed by incubation in 0.5% BSA (20 min) and peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories Inc.) at a dilution of 1:7500 for 30 min. After PBS rinses, sections were developed and visualized using an ImmPACT DAB kit (Vector Laboratories, Burlingame, CA). Finally, sections were mounted, dehydrated, counterstained with hematoxylin, and coverslipped.

**Cell Counting.** Pictures of the area of interest on each slide were taken on a microscope equipped with a digital camera. The number

of c-fos-positive cells was quantified by the presence of positively stained brown cells in the region of interest. Cells with punctate dark brown staining above background were scored positive. The c-fos-positive cells were counted manually using NIH ImageJ software (National Institutes of Health, Bethesda, MD). For data analysis, the sum of c-fos-positive cells counted across three sections is presented because there were no regional differences.

## Statistical Analyses

For the experiments assessing pica as bedding intake, paw gnawing, and rigidity, we had quantal responses (yes or no). None of these behaviors were displayed by the water-treated controls. To avoid inflating our rates of significance by including groups that did not display the behavior at any time point, we used a single 0-h baseline control (predrug) for the measures in experiment 1. The number of positive responses within each hour across all subjects for a treatment group was summed, and graphs depicting the percentage of positive responses across each hour were created. Each sex was analyzed separately. An initial  $\chi^2$  contingency analysis was done comparing the responses across the five time points (0, 1, 2, 3, and 4 h). Fisher's exact tests were then conducted to compare each group to the 0-h baseline. For the portion of experiment 1 that examined receptor mediation, the percentage of positive responses was analyzed via repeated-measures ANOVA. We used a percentage of positive responses compared with a summation of the positive responses because there were unequal numbers across the treatment groups. An ANOVA was used as opposed to a  $\chi^2$ , because all of the groups had received oxycodone treatment and floor effects were not present. The saline-oxycodone group served as a control in this experiment, and the naloxone-oxycodone groups were compared with the control by Tukey's post hoc contrasts.

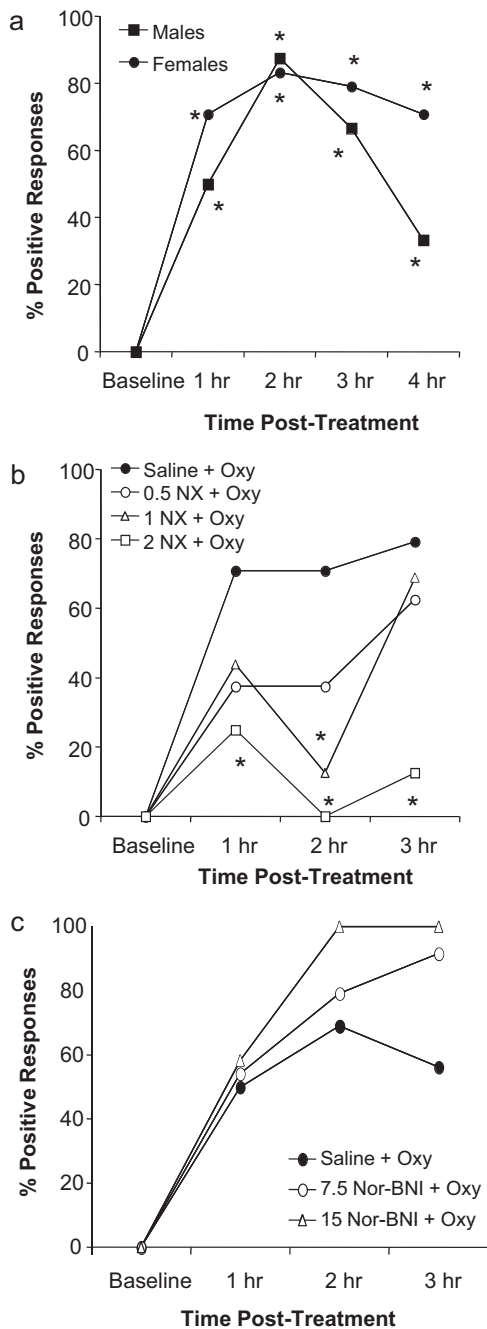
For experiments 2 and 3, the data were analyzed by a one-way ANOVA followed by a post hoc Tukey's test. In experiment 2, drug treatment was the between-subjects measure, and in experiment 3, the between-subjects measure was condition (control, cisplatin, 1 h after oxycodone or 2 h after oxycodone).

## Results

### Experiment 1: Does Oxycodone Induce the Pica Response of Bedding Intake?

**Expression of the Pica Response.** Water-treated control rats did not exhibit pica behavior, whereas the oxycodone-treated rats did (Fig. 1a). A  $\chi^2$  analysis was done within each sex to examine differences between the baseline predrug response and the response after drug. The overall analysis for both males and females was significant ( $\chi^2 = 42.64$  and  $47.78$ , respectively;  $df = 4$ ,  $p < 0.0001$  for both). Subsequent analyses were conducted to determine the time points when the oxycodone-treated subjects differed from baseline using a Fisher's exact test. For males, significant effects were found at all times (1 h:  $\chi^2 = 16.00$ ,  $p < 0.0001$ ; 2 h:  $\chi^2 = 37.33$ ,  $p < 0.0001$ ; 3 h:  $\chi^2 = 24.00$ ,  $p < 0.0001$ ; 4 h:  $\chi^2 = 9.60$ ,  $p < 0.004$ ;  $df = 1$  for all). Likewise for females, significant effects were found across the 4 h (1 h:  $\chi^2 = 26.32$ ,  $p < 0.0001$ ; 2 h:  $\chi^2 = 34.29$ ,  $p < 0.0001$ ; 3 h:  $\chi^2 = 31.45$ ,  $p < 0.0001$ ; 4 h:  $\chi^2 = 26.32$ ,  $p < 0.004$ ;  $df = 1$  for all). A second set of analyses was conducted to compare males with females for the pica response. There was an overall effect of sex ( $\chi^2 = 6.10$ ,  $p = 0.02$ ) with females displaying a greater degree of pica than males.

To ensure that the expression of pica was not solely a consequence of the oral route of administration, the experiment was repeated with an intraperitoneal injection of oxycodone at two doses, 10 and 15 mg/kg. As shown in Supple-



**Fig. 1.** a, male and female rats ( $n = 6$  per group) have increased intake of cage bedding after oral administration of oxycodone (15 mg/kg). The graph depicts the percentage of responses that were positive for bedding intake collapsed across each hour. This intake is sustained for 3 to 4 h. No bedding intake was found before drug treatment or after vehicle administration. Overall, females displayed a greater pica response than males (\*,  $p < 0.05$  versus baseline within each sex;  $\chi^2$  analysis). b, naloxone blocked or attenuated the pica response to oxycodone in female rats. The 2 mg/kg dose decreased the bedding intake across the 3-h session, whereas the 1 mg/kg dose transiently attenuated the response at 2 h (\*,  $p < 0.05$  versus oxycodone; Tukey's post hoc test).  $n = 6$  per group. c, the selective  $\kappa$  antagonist Nor-BNI did not alter the oxycodone-induced pica response in female rats.  $n = 4$  for saline + oxycodone (Saline + Oxy), and  $n = 6$  for the Nor-BNI + oxycodone (NX + Oxy) groups.

mental Fig. 2, dose-related induction in the expression of pica behavior was also robust. Because similar effects were found with oral and intraperitoneal administration and our objective was to mimic the route of administration used in hu-

mans, subsequent studies were conducted with oral administration of oxycodone only. In addition, the temporal pattern of pica expression did not differ significantly during the third and the fourth hour of treatment when the drug was administered orally. Therefore, in all our future studies, we assessed pica behavior for 3 h only.

**Receptor Mediation in the Temporal Expression of Pica.** We first determined that the three doses of naloxone blocked the antinociceptive effect of oxycodone in female rats (see Supplemental Fig. 3). These doses of naloxone were then tested to determine whether they block the expression of oxycodone-induced pica behavior. As shown in Fig. 1b, naloxone attenuated oxycodone-induced pica behavior in a dose-dependent manner in females, revealing the involvement of opioid receptors in pica behavior. Repeated-measures ANOVA revealed a significant treatment effect ( $F_{3,14} = 4.37$ ,  $p < 0.03$ ) but no treatment  $\times$  time interaction. A post hoc Tukey's test was done comparing the various treatments. There was a significant blockade of the oxycodone-induced pica response in the group receiving 2 mg/kg naloxone ( $p < 0.05$  for all time points; Tukey's post hoc test).

There was also a marginal effect of time in the repeated-measures ANOVA ( $F_{2,28} = 3.30$ ,  $p = 0.052$ ). Because naloxone is known to have a short half-life, we did a subsequent Bonferroni post hoc test on the various naloxone doses at each time point. This analysis revealed that naloxone at 1.0 mg/kg transiently attenuated oxycodone-induced pica at 2 h ( $p < 0.05$ ). In preliminary studies, we administered naloxone alone, and we failed to see evidence of a pica response (data not shown).

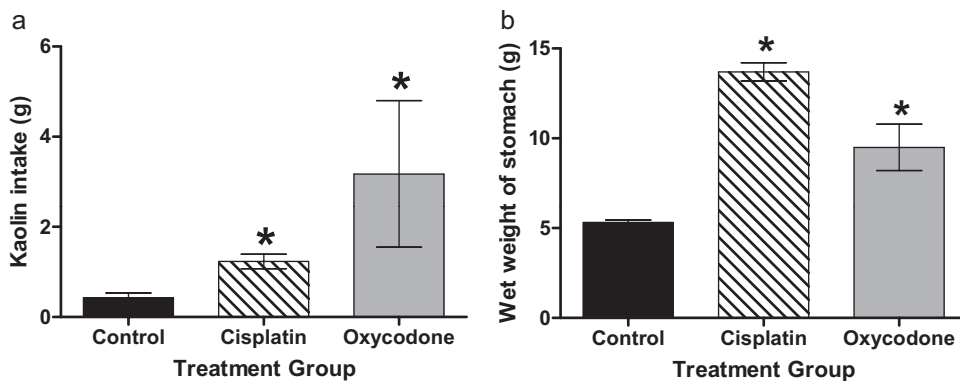
A second set of female rats was analyzed for  $\kappa$  receptor involvement in the pica response. The rats were treated with 7.5 or 15 mg/kg Nor-BNI before oxycodone administration. There was a significant time effect ( $F_{3,39} = 36.48$ ,  $p < 0.0001$ ). However, as can be seen in Fig. 1c, there was no effect of treatment or a treatment  $\times$  time interaction. Nor-BNI pretreatment did not affect the expression of the pica response, suggesting that the effect is not mediated by  $\kappa$ -opioid receptors.

## Experiment 2: Does Oxycodone Induce the Pica Response of Kaolin Intake?

**Kaolin Intake and Oxycodone.** We assessed whether oxycodone induces the pica response of kaolin intake, which is a standard model of nausea/emesis. Initial analyses compared baseline chow and kaolin intake. We did not find any statistical significant differences in the baseline consumption of chow across all the treatment groups. In addition, the rats across all the groups consumed kaolin in minimal quantities on the baseline days (0.6–0.9 g).

Oxycodone treatment induced a significant increase in the consumption of kaolin ( $F_{2,16} = 3.71$ ,  $p < 0.05$ ; one-way ANOVA). Post hoc Tukey's tests on the data revealed a significant difference for oxycodone compared with control ( $p < 0.05$ ), as shown in Fig. 2a. Cisplatin, which served as a positive control in our study, also increased kaolin intake ( $p < 0.05$ ).

**Wet Weight of Stomach as a Measure of Pica.** After the above assessment, rats were sacrificed, and the wet weight of stomach was recorded. There was an overall effect of treatment ( $F_{2,11} = 23.58$ ,  $p < 0.0001$ ; Fig. 2b). As expected, the pro-emetic agent cisplatin increased the wet weight of the



**Fig. 2.** a, oxycodone-induced pica manifests as increased kaolin intake in female rats ( $n = 4-6$ ). Kaolin intake was monitored over two 3-h sessions across 2 days. Data are represented as kaolin intake in grams. Cisplatin also increased kaolin intake compared with controls. b, cisplatin and oxycodone significantly increased the wet weight (in grams) of the stomach compared with the control group in these female rats. \*,  $p < 0.05$  compared with control; post hoc Tukey's test.  $n = 5$  to 7 per group.

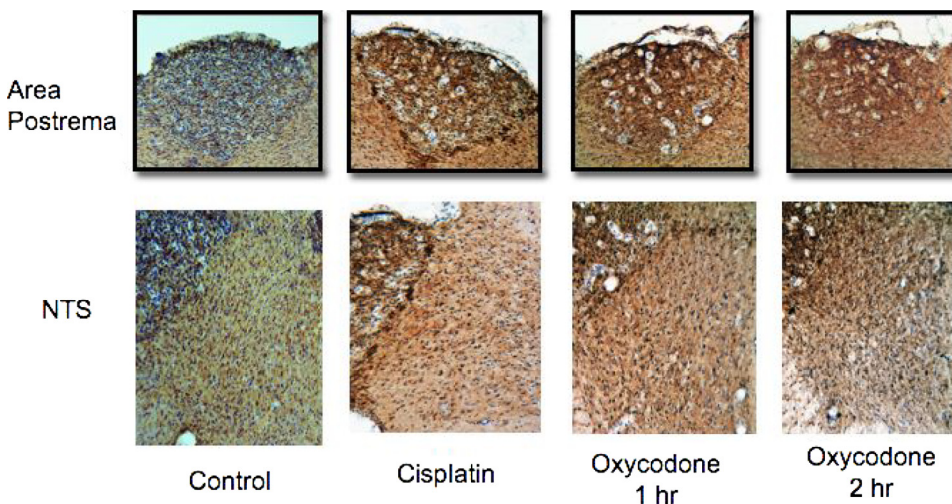
stomach by almost 3-fold compared with the control group ( $p < 0.05$ ; Tukey's test). Oxycodone also significantly increased the wet weight of the stomach by 2-fold compared with the water-treated controls ( $p < 0.05$ ; Tukey's test).

### Experiment 3: Does Oxycodone Activate Pica-Related Brain Regions?

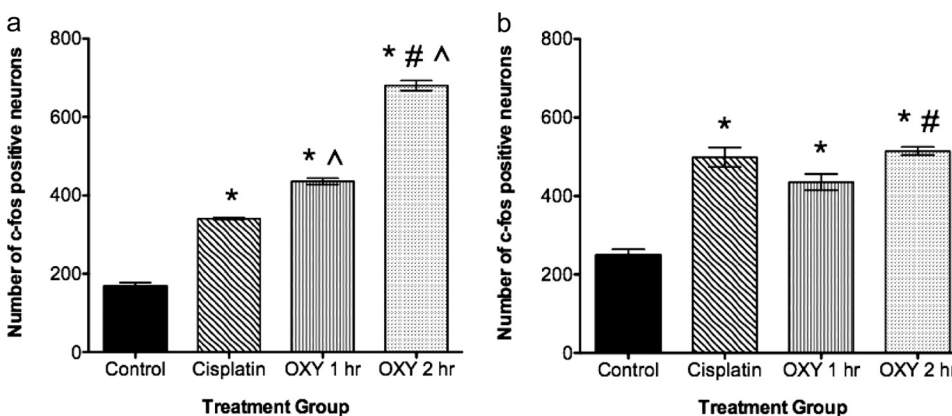
**c-fos Immunostaining and Oxycodone.** We assessed whether oxycodone treatment activated the pro-emetic neuroanatomical regions during the time course of pica behavior. Two controls were used in this study, oral water gavage for the oxycodone treatment and intraperitoneal saline for the cisplatin-treated subjects. There was no difference between these controls, so they were combined to form a common control group. There also were no regional differences (ante-

rior, medial, and posterior) across the rostrocaudal axis for area postrema or NTS, and thus the sum of all the regions is reported. Figure 3 shows the pattern of c-fos staining in the area postrema and NTS.

One-way ANOVA revealed a significant treatment effect ( $F_{3,21} = 509.5$ ,  $p < 0.0001$ ) in the area postrema (Fig. 4a). As expected, the positive control, cisplatin, increased the number of c-fos-positive cells in the area postrema compared with controls ( $p < 0.05$ , post hoc Tukey's test). It is worth noting that oxycodone at 1 and 2 h led to strong induction of c-fos, increasing the c-fos immunostaining 2.5- to 4-fold over controls ( $p < 0.05$ , post hoc Tukey's test for both). Oxycodone increased c-fos protein expression to a greater extent than did cisplatin ( $p < 0.05$ , post hoc Tukey's test), and this effect



**Fig. 3.** Immunohistochemical staining for oxycodone-induced c-fos activation in emetic circuitry after oxycodone or cisplatin administration. The area postrema (top) and the NTS (bottom) are shown. Compared with control, increased c-fos-positive cells were observed in cisplatin- and oxycodone-treated rats 1 and 2 h after treatment. Magnification,  $10\times$ .



**Fig. 4.** Quantification of c-fos in emesis-related circuitry after cisplatin or oxycodone (OXY) treatment. Controls were collapsed across saline- and water-treated groups. Depicted are the mean  $\pm$  S.E.M. for the number of c-fos-positive cells summed across the anterior, medial, and posterior regions. a, in the area postrema, cisplatin and both oxycodone time points increased c-fos expression, with the greatest effect 2 h after treatment. b, similar effects were found in the NTS, where oxycodone and cisplatin increased the number of c-fos-positive cells. \*,  $p < 0.05$  versus control; ^,  $p < 0.05$  versus cisplatin; #,  $p < 0.05$  versus 1-h oxycodone.  $n = 10$  for controls;  $n = 4$  for cisplatin;  $n = 5$  to 6 for oxycodone treated.

was particularly pronounced at 2 h, where there were elevated c-fos levels compared with 1 h after treatment.

Oxycodone treatment also increased the number of c-fos-positive cells in the NTS at 1 and 2 h after oxycodone treatment compared with control (Fig. 4b). One-way ANOVA revealed a significant treatment effect ( $F_{4,21} = 54.55$ ,  $p < 0.0001$ ). Cisplatin and oxycodone at both time points significantly increased c-fos immunoreactivity compared with the combined controls ( $p < 0.05$ , post hoc Tukey's test for all comparisons). Although the effect was less pronounced in the NTS than in the area postrema, there were more c-fos positive cells after 2 h of oxycodone treatment than after 1 h.

## Discussion

Prescription opioid-induced emesis during postoperative pain management is a major clinical concern. This is the first report describing that an analgesic dose of oxycodone is able to produce the pro-emetic response of pica in the rat, as assessed by non-nutritive consummatory behavior. The pica was accompanied by gastrointestinal dysfunction and activation of emetic regions in the brain. We used two models to assess the pica response, bedding intake and kaolin consumption. The bedding intake is robust, is easy to score, and permits a temporal analysis. But the data are categorical in nature. The second measure used kaolin consumption, a well established model of pica. We found that oxycodone induced a significant increase in kaolin consumption similar to that observed after administration of pro-emetic agents such as cisplatin, apomorphine, and copper sulfate (Takeda et al., 1993). We used cisplatin as a positive pro-emetic control in this study. In general, cisplatin-induced pica is monitored over a 24-h period after administration because of the long duration of cisplatin's effects. We recorded the kaolin consumption in 3-h sessions across 2 days. This change was made to match the shorter duration of action of oxycodone. We acknowledge that cisplatin is an imperfect control for opioids because of this difference in duration; however, it does permit us to gauge the magnitude of our effects with a benchmark from the literature. Another challenge in validating pica as a pro-emetic response is whether emesis is viewed as an all-or-none response or whether there are gradations. In dogs, researchers establish an emetic threshold, the dose of a drug that induces emesis (Burkman, 1982). In rodents that lack the emetic motoric reflex, research has viewed the pica response as more of a continuous measure and possibly more analogous to nausea.

In experiment 1, we found that an oral route of oxycodone administration induced the expression of pica. This route was chosen because oxycodone has high oral bioavailability and it is the route most typically used in humans (Kalso, 2005). More importantly, as seen in the supplemental data, intraperitoneal administration also induced a robust pica response, indicating that it is not a route of administration effect. We found strong sex differences in the expression of pica after oral oxycodone. Female rats demonstrated a higher magnitude and prolonged duration of pica compared with male rats. Males demonstrated a sharp onset and offset in the expression pattern, whereas females displayed a steadily elevated and prolonged expression. This sex difference may be a result of pharmacokinetic differences. Chan et al. (2008) found sex differences in the plasma concentration of oxy-

codone after oral administration. The levels of the parent drug oxycodone were higher in females, whereas the males had higher levels of an inactive metabolite of oxycodone (noroxycodone) under similar experimental conditions. Aside from pharmacokinetic effects, there are sex differences in the response to emetic stimuli. Women are more likely to develop nausea and vomiting after acute opiate administration than are men (Cepeda et al., 2003), including oxycodone treatment (Campora et al., 1991).

Opioids, including oxycodone, generally exert their pharmacological action via interaction with the opioid receptor, which can be demonstrated by blockade of opioid actions by the antagonist naloxone (Inturrisi, 2002; Lemberg et al., 2006). We first found that naloxone effectively blocked or attenuated oxycodone-induced antinociception in a dose-related manner (supplemental data) and then found that the higher doses (1–2 mg/kg) of naloxone attenuated oxycodone-induced pica behavior. The lower dose of naloxone failed to block the pica response. A prominent rebound emergence of pica behavior was observed after 2 h for the 1 mg/kg naloxone-treated group, which clearly delineated the short-acting nature of this opioid antagonist. It is important to note that the rats were not undergoing opioid withdrawal during the pica assessment because the naloxone was administered before the oxycodone. Nausea and emesis can accompany opioid withdrawal in humans, although we have failed to see pica when we have precipitated withdrawal or permitted rats to undergo spontaneous withdrawal after chronic oxycodone exposure (data not shown).

The present data indicate that oxycodone-induced pica behavior is mediated via the opioid receptor. It is worth noting that pica responses from nonopioid drugs may also be mediated by opioid receptors, as naloxone was shown to block a ritonavir-induced pica response (Yuan et al., 2009). Many oxycodone effects, including analgesia and gastrointestinal distress, are thought to be mediated by the  $\mu$  opioid receptor. Naloxone binds to the  $\mu$ ,  $\kappa$ , and  $\delta$  receptors, but it does have higher affinity for the  $\mu$  receptor. However, dysphoric effects have been related to the  $\kappa$  opioid receptor (Knoll and Carlezon, 2010). In addition, Tsukamoto et al. (2007) found that cisplatin increased the immunoreactivity of endogenous  $\kappa$ -opioid receptor ligand dynorphin A in area postrema neurons. Thus, we also investigated the role of  $\kappa$  receptors by administering the  $\kappa$ -selective antagonist Nor-BNI. Nor-BNI was not able to block or attenuate the pica response, consistent with an interpretation of  $\mu$  opioid receptor involvement.

In addition to nausea and vomiting, opioids produce other types of gastrointestinal distress. We observed an increase in the wet weight of stomach after administration of cisplatin or oxycodone. The magnitude of the pica effect and the stomach wet weight differed between the treatment groups. Oxycodone-treated rats had a greater pica response with respect to kaolin intake than did cisplatin-treated rats. However, for the stomach wet weight, the effect was reversed, and the cisplatin-treated rats showed a greater effect. This pattern of data indicates that the wet weight of the stomach was reflecting more than just the pro-emetic pica response. Opioids and cisplatin have different pharmacological actions but can trigger common mechanisms to modulate the gastrointestinal tract. Common effects include delayed gastric emptying, increased intestinal transit time, impaired gastrointestinal motility, and constipation (Rudd et al., 2002; Bates et al.,

2004; Liu et al., 2005; Cabezos et al., 2008). It is possible that opioids or cisplatin can trigger these processes individually or act in concert to increase stomach wet weight. Association of emetic events with abnormal patterns of gastrointestinal motility has been reported previously (Takahashi et al., 2007). The goal of the present study was to demonstrate gross alterations in the gastrointestinal events that occur along with central effects of pro-emesis.

In the final experiment, we observed that oxycodone increased the number of neurons that were positive for the protein product from the immediate early gene c-fos in the area postrema and the NTS. These brainstem regions are well studied in regards to the emetic potential of various drugs (Horn et al., 2007). As expected, our positive control, cisplatin, also increased the number of c-fos-positive cells. However, oxycodone induced more robust c-fos expression than did cisplatin in the area postrema, matching the data for the kaolin intake. There are a few caveats that are important to mention with regard to these data. First, all of the rats in the study were anesthetized with sodium pentobarbital before removal of the brain for immunohistochemical processing. We do not think that interfered with assessment of c-fos activation because Morgan et al. (1987) reported that doses up to 80 mg/kg pentobarbital did not alter c-fos and our dose was below this threshold. Second, it is possible that opiate-induced gastric dysfunction as described above may be activating the brainstem. There are vagal connections that carry information from the gut to the brainstem and stimulate emetic brain regions (Danzer et al., 2004; Coda, 2006). Thus, we cannot discount that some of the c-fos staining was a consequence of gastric feedback signaling. This may be especially true for the area postrema, where there was a greater effect at 2 h than at 1 h. The pica behavior itself may be a feedback stimulus that activates the area postrema and the NTS. It will be important for us to further investigate this possibility by pharmacological blockade of the pica response using opioid (e.g., naloxone) and nonopioid (e.g., serotonin antagonists) manipulations. Nonetheless, the data in the present study indicate that brainstem nuclei that underlie processing of pro-emetic stimuli and gastrointestinal distress were activated by oxycodone.

In conclusion, we provide data that indicate an analgesic dose of oxycodone induces the pro-emetic pica response in rats. Future studies will explore mechanisms in the emetic centers of the brain that underlie the expression of oxycodone-induced pica behavior. In particular, a more fine-tuned analysis of the timing and circuitry that underlie nausea and emesis compared with gastric distress is warranted. These symptoms are a major reason for discontinuation of analgesic therapy and noncompliance with drug-taking regimens.

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#### Authorship Contributions

Participated in research design: Batra and Schrott.

Conducted experiments: Batra.

Performed data analysis: Batra and Schrott.

Wrote or contributed to the writing of the manuscript: Batra and Schrott.

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**Address correspondence to.** Dr. Lisa M. Schrott, Department of Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center—Shreveport, 1501 Kings Highway, P.O. Box 33932, Shreveport, LA 71130-3932. E-mail: lschro@lsuhsc.edu

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