

Acetaminophen as a Postsurgical Analgesic in Rats: A Practical Solution to Neophobia

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Acetaminophen administration is gaining popularity as a postsurgical analgesic in many rodent labs despite reports that animals may consume suboptimal doses as a result of taste neophobia. The present study evaluated the presence, duration, and extent of acetaminophen neophobia in adult male and female rats (Long Evans) with the intent of developing a protocol for administration of this analgesic in the rodent surgery lab. After a 7-day baseline period in which average water consumption, food consumption, and body weight were established for 32 rats (20 females and 12 males), cherry-flavored acetaminophen was administered (6 mg/ml) in the animals' water bottles for an additional 7 days. Fluid consumption, food consumption, and weight were monitored during this period of drug exposure. Male rats displayed a transient period (1 day) of reduced fluid consumption followed by elevated fluid consumption on subsequent days. Female animals displayed normal to elevated fluid consumption on all days of drug exposure. Both male and female animals, however, decreased their food intake after drug exposure and subsequently lost weight. Recommendations for the oral administration of acetaminophen as a postsurgical analgesic are discussed.

Public Law 89-544 (Animal Welfare Act) and its amendments mandate the use of appropriate analgesics in most research animals following major survival surgery. Although this Act does not apply specifically to rodents, most agencies (see the NIH *Guide for the Care and Use of Laboratory Animals*) strongly recommend that postoperative analgesics be used for rodents. Recommended analgesic agents include opiates, barbiturates, and nonsteroidal antiinflammatory drugs (NSAIDs). The NSAIDs (aspirin, acetaminophen, ibuprofen, carprofen, ketoprofen, and others), in particular, have received considerable attention recently because of their effective analgesic properties in rats (1-4), ease of use as a solute in drinking water or in gelatin versus injection (5, 6), low toxicity relative to opiates or barbiturates, ready availability, and need for minimal record-keeping as it is not classified as a controlled substance. Of concern, however, is the possible neophobic response of rodents to the novel taste of the NSAIDs.

Neophobia ("fear of the new") is commonly observed in rodents, especially to novel tastes (7-13). A common ethological perspective holds that neophobia is a defense mechanism in which the rodents will only consume small amounts of a novel food or fluid source until they determine if they are likely to become ill as a result of consumption. Although the neophobic response typically is considered instinctual, it can be enhanced and generalized (14).

Recently, Speth and colleagues (6) documented a neophobic response by rats to acetaminophen in water. In their study, the food and water intake of two groups of five female rats was monitored for 4 days. On the fifth day, the acetaminophen solutions were administered for 24 h. One group of rats received a cherry-flavored acetaminophen solution, while the other received an alcohol-containing acetaminophen solution. Following the day of drug administration, the rats were again given water, and their intake was monitored for 5 days. Speth et al. (6) found that the rats drank significantly less water-plus-acetaminophen during the day of drug exposure and that they drank significantly more water the day after drug exposure. These researchers also reported that the rats that consumed the cherry-flavored solution consumed significantly less food during the testing day than during the baseline period or the day after acetaminophen exposure.

The purpose of the present study was to determine the length of time necessary for extinction of any neophobic response to acetaminophen in adult Long Evans rats and to determine whether the patterns of neophobic acquisition and extinction differed according to the sex of the animal. Our rationale was that exposure to an acetaminophen solution prior to surgery is a safe and effective method of analgesic administration that can be continued postsurgery without concomitant neophobic behaviors and stress to the animals. We predicted that rats would show a mild neophobic effect that would extinguish within 7 days of continued exposure to the drug.

Materials and Methods

Subjects in this study (N = 32) consisted of 20 female adult Long Evans rats weighing 287.45 ± 16.46 g (mean \pm 1 standard deviation) and 12 male adult Long Evans rats weighing 520.18 ± 38.39 g (mean \pm 1 standard deviation). All rats were procured from Harlan (Indianapolis, Ind.) and were murine pathogen-free. The rats were run in two separate experiments, the first consisting of the female rats, and the second consisting of the male animals. All rats were housed in standard hanging cages in a temperature-controlled (20 to 22°C) room isolated from other animals with a 12:12-h light-dark cycle beginning at 0700 and with access to food (Teklad Rodent Lab Chow, Harlan Teklad, Madison, Wis.) and water ad libitum. All animal procedures were approved and overseen by the University of Wisconsin at Milwaukee Institutional Animal Care and Use Committee.

Baseline measures of fluid consumption, food consumption, and weight were established via daily monitoring over a 7-day period. Fluid consumption was measured by weighing the water bottles, and thus is reported in grams. Daily food consumption was determined by weighing any food remaining in the cage after 24 h (plus any waste food collected in a container underneath the cage) and subtracting that amount from the starting value. The averages for each variable over the baseline week constituted the daily baseline averages used in the analyses.

After the baseline week of regular tap water, the rats' fluid was replaced with a novel cherry-flavored acetaminophen solution (Children's Tylenol; 32 mg/ml; Johnson and Johnson, New Brunswick, N.J.; additional ingredients included: butylparaben, cellulose, citric acid, corn syrup, FD&C Red#40, flavors, glycerin, propylene glycol, purified water, sodium benzoate, sorbitol, and

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xanthan gum). The solution was diluted in tap water to 6 mg/ml, yielding a dose of approximately 700 mg/kg in a 350-g rat if it were to drink 40 ml of solution. The dosage is identical to that in the Speth et al. (6) study and is body-size-equivalent to approximately twice the recommended maximum daily dose for analgesics in adult humans (4 g daily for a 70-kg adult). Speth et al. (6) rationalized that the higher dose was necessary "because fluid intake during the light period is considerably less than that during the dark period" (p. 16).

The rats drank the acetaminophen solution for a period of 1 week, during which time fluid consumption, food consumption, and weight were monitored on a daily basis. During that time, the acetaminophen solution was the only fluid available to the rats and was available ad libitum. Food continued to be available ad libitum.

Analyses were performed using SPSS software (SPSS, Inc., Chicago, Ill.). All initial analyses consisted of repeated measures analysis of variance. Post-hoc analyses were performed using paired samples *t* tests. Results for which $P \leq 0.10$ are reported; results with $P \leq 0.05$ were considered significant.

Results

All results are presented in both graphical and in tabular form in Figs. 1 through 3 and Tables 1 through 3, respectively. Fluid consumption of the rats was significantly increased after administration of the acetaminophen solution ($F_{7,24} = 3.923$, $P = 0.005$). Although overall the rats drank approximately 5.8% less than the baseline value on the first day of drug exposure, the difference was not statistically significant. The downward trend immediately reversed on the second day, when rats drank 7.35% more than baseline quantities, a difference that approached significance ($t_{31} = -1.752$, $P = .090$). Rats drank significantly more on days 3 through 7 of testing (all $t_{31} \leq -2.884$, all $P \leq 0.007$), with respective consumption rates of 11.17%, 16.55%, 18.87%, 13.20%, and 18.35% higher than baseline values (Fig. 1, Table 1).

Although there was no gender-by-fluid-consumption interaction, male and female rats demonstrated slightly different patterns of intake during the testing period. Male rats did not demonstrate a significant overall difference in fluid consumption ($F_{7,5} = 1.251$, $P = 0.417$) but did drink significantly less (15.55%) than the baseline amount on the first day of testing ($t_{11} = 2.210$, $P = 0.049$). Afterwards, they consumed higher-than-baseline levels on days 2 through 7. None of these differences were significant, but fluid consumption on days 2 ($t_{11} = -2.097$, $P = 0.060$; +10.09%) and 7 ($t_{11} = -1.962$, $P = 0.076$; +11.79%) approached significance. In contrast, the female rats did demonstrate an overall significant difference in fluid consumption ($F_{7,13} = 5.989$, $P = 0.003$) and did not drink less than baseline quantities on any day of testing. In fact, they drank significantly more than baseline on days 3 through 7 of testing (all $t_{19} \leq -2.396$, all $P \leq 0.027$), with respective consumption rates of 13.87%, 21.69%, 27.24%, 17.99%, and 22.44% higher than the baseline value.

Food consumption of the rats was significantly lower after acetaminophen administration ($F_{7,24} = 46.324$, $P < 0.001$). In fact, food consumption was lower on every day of testing as compared to baseline amounts (all $t_{31} \geq 3.872$, all $P \leq 0.001$), ranging between 16.52% to 31.97% lower than the baseline quantity (Fig. 2, Table 2).

A gender-by-food-consumption interaction was found ($F_{7,24} = 5.370$, $P = 0.001$), indicating that the male rats ate significantly more than did the female rats. Within-gender overall differences in food consumption also were found for both male ($F_{7,5} = 11.324$, $P = 0.008$) and female ($F_{7,13} = 63.560$, $P < 0.001$) rats. Males ate significantly less food on every day of testing as compared to baseline (all $t_{11} \geq 6.436$, all $P \leq 0.001$), ranging between 19.19% to 31.87% lower than baseline levels. Female animals exhibited

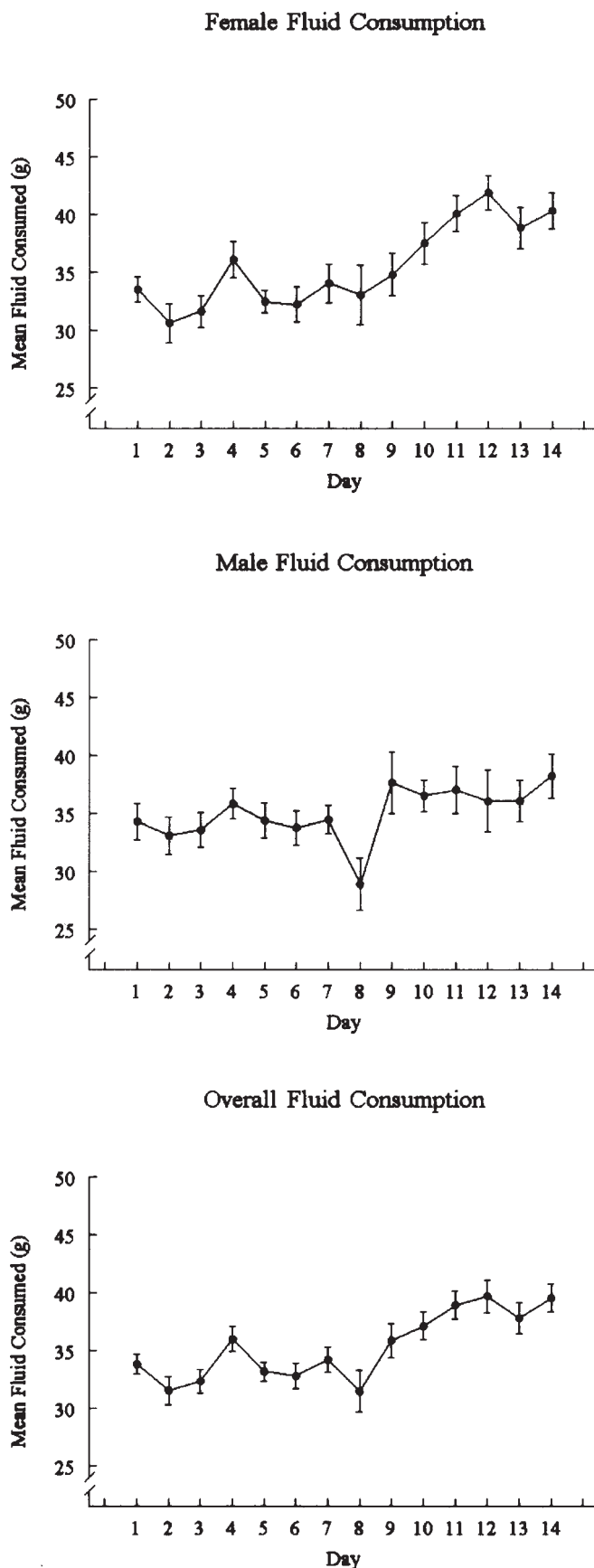
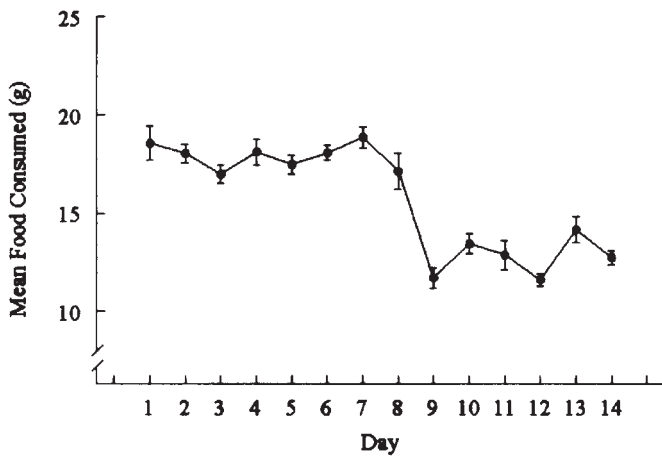


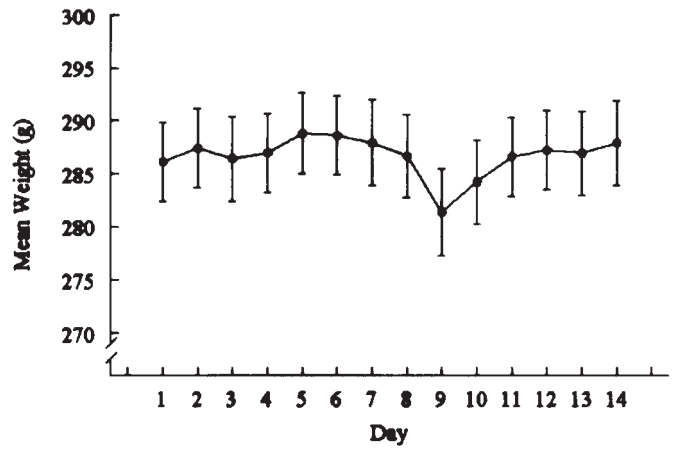
Figure 1. Fluid consumption for female rats (top), male rats (middle), and overall (bottom). Values represent the fluid consumed daily (in g; mean \pm standard error of the mean). Days 1 through 7, baseline period; days 8 through 14, testing with acetaminophen in water (6 mg/ml).

a similar, but not identical, pattern: females consumed significantly less food on every day of testing except day 1, as compared

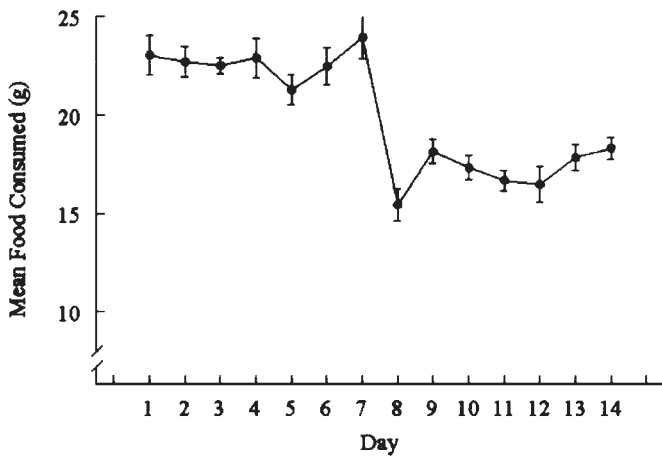
Female Food Consumption



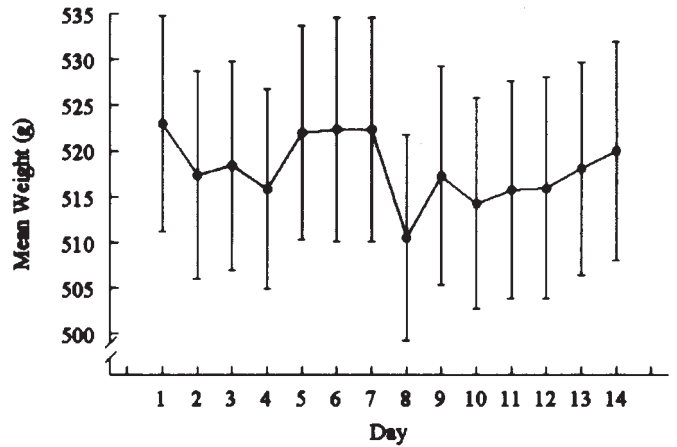
Female Weight



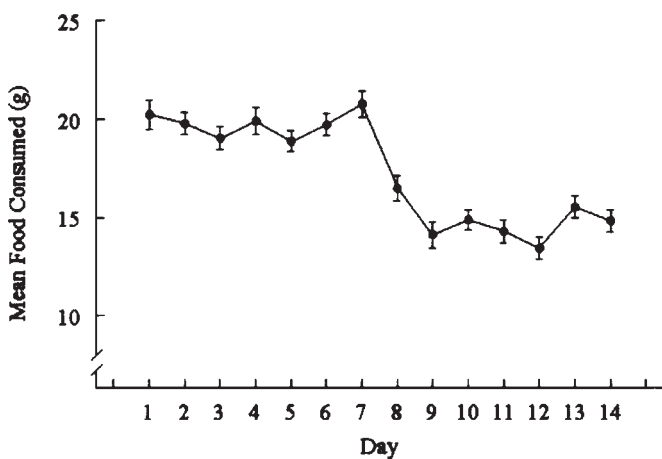
Male Food Consumption



Male Weight



Overall Food Consumption



Overall Weight

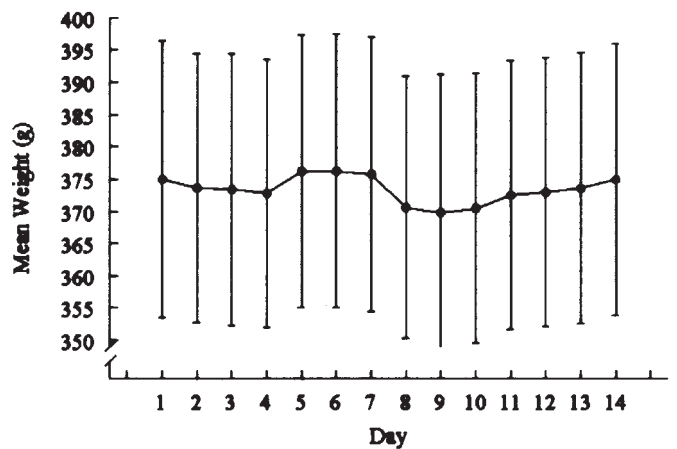


Figure 2. Food consumption for female rats (top), male rats (middle), and overall (bottom panel). Values represent amount of lab chow consumed daily (in g; mean \pm standard error of the mean). Days 1 through 7, baseline period; days 8 through 14, testing with acetaminophen in water (6 mg/ml).

to baseline values (days 2 through 7: all $t_{19} \geq 6.782$, all $P < 0.001$). During this period, females consumed between 21.33% and

Figure 3. Body weight for female rats (top), male rats (middle), and overall (bottom). Values represent average daily body weight (in g; mean \pm standard error of the mean). Days 1 through 7, baseline period; days 8 through 14, testing with acetaminophen in water (6 mg/ml).

34.94% less food, as compared to baseline levels.

A significant overall difference in weight was found ($F_{7,24} = 16.619$, $P < 0.001$) after acetaminophen administration. The rats weighed

Table 1. Fluid consumption (mean \pm standard error of the mean) for overall (female + male), female, and male rats during the baseline period and the 7 days of acetaminophen exposure

	Fluid Consumption (g)		
	Overall	Female	Male
Baseline	33.39 \pm 0.85	32.91 \pm 1.12	34.19 \pm 1.37
Test Day 1	31.46 \pm 1.81	33.01 \pm 2.58	28.88 \pm 2.28*
Test Day 2	35.85 \pm 1.49	34.77 \pm 1.84	37.64 \pm 2.62
Test Day 3	37.12 \pm 1.19**	37.48 \pm 1.76*	36.53 \pm 1.38
Test Day 4	38.92 \pm 1.22***	40.06 \pm 1.55***	37.03 \pm 2.05
Test Day 5	39.69 \pm 1.41***	41.88 \pm 1.49***	36.05 \pm 1.78
Test Day 6	37.80 \pm 1.30**	38.84 \pm 1.81**	36.08 \pm 1.78
Test Day 7	39.52 \pm 1.20***	40.30 \pm 1.59***	38.23 \pm 1.91

* $P \leq 0.05$ versus baseline
 ** $P \leq 0.01$ versus baseline
 *** $P \leq 0.001$ versus baseline

Table 2. Food consumption (mean \pm standard error of the mean) for overall (female + male), female, and male rats during the baseline period and the 7 days of acetaminophen exposure

	Food Consumption (g)		
	Overall	Female	Male
Baseline	19.75 \pm 0.52	18.00 \pm 0.32	22.67 \pm 0.69
Test Day 1	16.49 \pm 0.65***	17.12 \pm 0.93	15.44 \pm 0.80***
Test Day 2	14.12 \pm 0.68***	11.71 \pm 0.50***	18.13 \pm 0.62***
Test Day 3	14.88 \pm 0.51***	13.43 \pm 0.51***	17.32 \pm 0.63***
Test Day 4	14.29 \pm 0.59***	12.86 \pm 0.74***	16.67 \pm 0.53***
Test Day 5	13.43 \pm 0.56***	11.62 \pm 0.31***	16.47 \pm 0.89***
Test Day 6	15.54 \pm 0.56***	14.16 \pm 0.64***	17.83 \pm 0.68***
Test Day 7	14.83 \pm 0.58***	12.74 \pm 0.36***	18.32 \pm 0.56***

* $P \leq 0.05$ versus baseline
 ** $P \leq 0.01$ versus baseline
 *** $P \leq 0.001$ versus baseline

significantly less on days 1 through 4 of the testing period as compared to baseline weight (all $t_{31} \geq 3.017$, all $P \leq 0.005$), with the day 5 value approaching significance ($t_{31} = 1.819$, $P = 0.079$). Weights on days 1 through 5 were decreased from the baseline by 1.10%, 1.31%, 1.14%, 0.58%, and 0.47%, respectively (Fig. 3, Table 3).

A gender-by-weight interaction was found ($F_{7,24} = 7.164$, $P < 0.001$), indicating that the male rats weighed significantly more than the female rats. Within-gender overall differences in weight also were found in both male ($F_{7,5} = 11.304$, $P = 0.008$) and female ($F_{7,13} = 3.969$, $P = 0.015$) animals. However, the genders varied considerably in their patterns of weight loss and gain. Female rats weighed significantly differently from their baseline level only on days 2 ($t_{19} = 4.186$, $P = 0.001$) and 3 ($t_{19} = 2.737$, $P = 0.013$) of testing, with decreases of 2.12% and 1.13%, respectively. In contrast, male rats displayed significant weight loss on day 1 of testing ($t_{11} = 4.543$, $P = 0.001$), a 1.86% decrease from baseline. By the second day of testing, they had gained weight back to a baseline level, and their weight again was decreased significantly from baseline on days 3 ($t_{11} = 5.134$, $P < 0.001$; -1.14%) and 4 ($t_{11} = 2.930$, $P = 0.014$; -0.85%). Males approached a significantly decreased weight as compared to baseline on day 5 of testing ($t_{11} = 2.116$, $P = 0.058$; -0.82%).

Discussion

Exposure to cherry-flavored acetaminophen in water provoked only mild neophobia in male animals in our study and was not universal in scope. Only 75% of the males exhibited this response. In all cases, however, the neophobia extinguished within 24 h. Female animals lacked any neophobic response. Within 2 days of drug exposure, resumption of normal drinking levels, even though the water still contained the analgesic, was evident in both sexes. Within 3 days, male and female consumption of the analgesic had increased to 37.48 \pm 7.69 ml, a 13.87% increase from baseline. These results indicate that neophobia to oral ad-

Table 3. Weight (mean \pm standard error of the mean) for overall (female + male), female, and male rats during the baseline period and the 7 days of acetaminophen exposure

	Weight (g)		
	Overall	Female	Male
Baseline	374.72 \pm 21.09	287.45 \pm 3.78	520.18 \pm 11.58
Test Day 1	370.59 \pm 20.31**	286.65 \pm 3.90***	510.50 \pm 11.25***
Test Day 2	369.81 \pm 21.41***	281.35 \pm 4.08*	517.25 \pm 11.95
Test Day 3	370.47 \pm 20.87***	284.20 \pm 3.92	514.25 \pm 11.51***
Test Day 4	372.53 \pm 20.81**	286.60 \pm 3.74	515.75 \pm 11.92*
Test Day 5	372.97 \pm 20.78	287.20 \pm 3.74	515.92 \pm 12.10
Test Day 6	373.59 \pm 20.98	286.90 \pm 3.97	518.08 \pm 11.63
Test Day 7	374.94 \pm 21.08	287.90 \pm 4.01	520.00 \pm 11.93

* $P \leq 0.05$ versus baseline
 ** $P \leq 0.01$ versus baseline
 *** $P \leq 0.001$ versus baseline

ministration of flavored acetaminophen in the animals' drinking water is short-lived and can easily be accommodated within the context of the rodent surgery lab by exposing the animals to the drug for a period of time as short as 1 or 2 days preceding the day of surgery. The observation that the animals steadily increased their drinking levels within 3 days of exposure indicates that the likelihood of the animals consuming therapeutic doses of the drug is enhanced (Fig. 1, Table 1).

One potential caveat to the acceptability of this form of analgesic administration is the significant decline in food consumption and body weight in response to drug exposure. Food consumption was lower in both sexes during the testing phase (drug exposure) of this experiment (Fig. 2, Table 2). Weight also significantly declined initially but showed a rebound toward the end of the testing phase (Fig. 3, Table 3). This weight increase was concomitant with increases in fluid consumption, suggesting that the caloric content of the analgesic solution (commercial brands contain corn syrup) was sufficient to restore body weight if a significant volume of the solution was consumed.

Our results are in partial contrast to those found in the study by Speth et al. (2001), in which adult female Sprague-Dawley rats showed decreased water intake during the 24 h after administration of the novel cherry-flavored acetaminophen solution. One possibility for this divergence of results may lie in the species examined, as our study employed Long Evans hooded rats. Any species differences remain to be tested. Alternatively, the discrepancy may arise from the brand of liquid acetaminophen that was used. Speth and colleagues used Rite-Aid brand, whereas we used Tylenol brand. Possibly the formulations differ in palatability, as the ingredients are similar, but not exactly identical. Qualification of such a difference should be moot, however, as both solutions are similarly novel from water.

Our study addresses the practicality of oral administration of acetaminophen as an analgesic. It does not address the efficacy of such an approach to pain management. A review of the literature indicates, however, that although its mechanism of action remains largely unknown, acetaminophen is an effective analgesic (1, 2, 15). It is also worth mentioning that oral administration of acetaminophen is not subject to some of the drawbacks associated with more potent analgesics. For example, although it is clear that injection of opioids produces potent nociceptive antagonism, peak analgesia is only reached after 30 to 60 min and is followed by a gradual decline in efficacy until the next injection (approximately 1 to 2 h for butorphanol, 2 to 3 h for morphine, and 6 to 8 h for buprenorphine; 16). Our protocol permits the animal to obtain analgesic "around the clock" and may provide more stable, although certainly not as potent, pain relief. In addition, the frequent injections required to maintain opiate analgesia may disrupt diurnal rhythms and prove distressful to the animal merely by the increased handling. In an attempt to overcome these drawbacks, Deeb et al. (17) have adminis-

tered the synthetic opioid buprenorphine via water bottles and reported effective levels of analgesia. These investigators did not, however, report the presence or absence of neophobia.

A more compelling reason to seek alternatives to opiate-induced analgesia is that opiates significantly alter body function during drug exposure. They suppress the respiratory (18), immune (19), and cardiac (20) systems. Further, their effects may persist long after the drug has been metabolized. For example, a study by Eisch et al. (21) examined the proliferation and survival rates of mitotic neurons in adult rats after administration of morphine. They found that chronic administration (5 days of 75-mg pellet administration) was associated with neural proliferation rates in the hippocampus that were significantly lower (-28%) than those of sham-control rats. Furthermore, survival rates of new neurons were significantly lower (-47%) in morphine-exposed animals 4 weeks after the initial administration compared to those animals that had not received the drug. Although the mechanisms of acetaminophen analgesia remain largely unknown, recent studies have implicated interactions with the monoamine neurotransmitters (particularly serotonin) in the antinociceptive process (22-26).

Acetaminophen, of course, is not a magic bullet. Its side effects include nephrotoxicity and hepatotoxicity (for a detailed overview, see [27]). Kocaulu et al. (28) administered a one-time acetaminophen dose of 900 mg/kg via intraperitoneal injection to 12 male Swiss Albino rats. The rats were not allowed to eat for 24 h after administration, during which time their urine was collected and after which their kidneys were removed. The rats displayed elevated levels of urinary gamma-glutamyl transferase (GGT) compared to saline-injected controls. GGT is an enzyme indicative of acute nephrotoxicity (see [29]). Kocaulu et al.'s (28) histological assay, however, revealed "minimal damage" in the kidneys of the acetaminophen-injected rats. They speculated that a possible reason for the lack of histological damage may have been due to the rat strain used in the study, as acetaminophen-induced nephrotoxicity may be strain-dependent (30). Newton et al. (30) discovered that male Sprague-Dawley rats showed no evidence of renal cortical necrosis 24 h after single administrations of acetaminophen of up to 900 mg/kg, whereas male Fischer 344 rats showed significant damage (20% to 30% of the renal cortex was necrotic at the 900 mg/kg dose). Another possible reason for lack of histological damage in the previous report may be that female rats are more susceptible to acetaminophen-induced nephrotoxicity than male rats, at least in the Sprague-Dawley strain (31). A study of chronic acetaminophen exposure in female Fischer 344 rats, however, showed that 40 to 83 weeks at a dosage of 140 to 210 mg/kg body wt daily administration yielded no significant renal damage (32). Another study of chronic acetaminophen exposure (900 mg/kg body wt daily for 2 months) with female Sprague-Dawley rats showed that renal damage had nearly disappeared by 4 months after administration (33).

A study by Colin et al. (34) related the manner of administration route to toxicity potential. Using adult male Sprague-Dawley rats, they administered acetaminophen doses of 100 or 750 mg/kg either intraperitoneally or orally. Hepatorenal toxicity was assessed via urinary creatinine and serum glutamic-pyruvic transaminase activity. Their results indicated the potential for nephrotoxicity and hepatotoxicity at the 750 mg/kg dose, but only when the acetaminophen was administered intraperitoneally. A recent study indicates that the hepatic antioxidant system actually adapts to repeated acetaminophen administration, such that it becomes less sensitive to its hepatotoxic effects (35). In light of the results from our experiment, we suggest that acetaminophen as an adjunct to animal water bottles may serve as a reliable analgesic administration route provided that the drug exposure begins 1 to 2 days before surgery.

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