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Acetaminophen Self-administered in the Drinking Water Increases the Pain Threshold of Rats (*Rattus norvegicus*)

G Andrew Mickley,* Zana Hoxha, Jaclyn M Biada, Cynthia L Kenmuir, and Stephanie E Bacik

Previous studies have suggested that the addition of flavored acetaminophen suspension (for example, Children's Tylenol) in the drinking water of rats may not be effective in producing postoperative analgesia because of low levels of consumption. However, these investigations neither measured analgesia nor compared the consumption by rats that had undergone surgery with that by unmanipulated rats. The present study reports that although unmanipulated rats naive to the taste of flavored acetaminophen do indeed drink significantly less of this liquid than tap water, they drank sufficient amounts of the acetominophen-containing solution to significantly raise pain thresholds, as measured by the hot-plate test. Moreover, rats that had undergone surgery drank significantly more acetaminophen solution than did those that had no surgery. These data suggest that oral self-administration of flavored acetaminophen by rats may be an appropriate means to reduce pain.

Abbreviations: ACET, acetominophen; ANOVA, analysis of variance; HP, hot-plate test

Placing an analgesic drug in the drinking water of rats has been proposed as an appropriate way to provide analgesia after surgery or other pain-inducing procedures.[1,6] Allowing animals to self-administer an analgesic by drinking has distinct advantages. It reduces the stress on animals, because they do not need to be handled or restrained during an analgesic injection. It also reduces the need for staff to attend to the animal overnight or at frequent intervals during the day. Further, there is no disruption of the diurnal rhythm of animals receiving treatment or of those in the same room.[6,21]

In particular, over-the-counter analgesics (for example, acetaminophen) have received particular attention because they are readily available, effective in producing analgesia in rats, and exhibit low toxicity (relative to opiates).[1] However, these drugs may not be as effective as opiates in providing postsurgical analgesia;[25] therefore delivery of an appropriate dose becomes particularly important.

If oral analgesics are to be effective, they must be consumed in sufficient quantity to produce relief from pain. This issue was raised by Speth and colleagues,[21] who reported that rats exhibited neophobia ('fear of the new') for the novel taste of a cherry-flavored children's acetaminophen solution and suggested that self-administration of this analgesic is a counterproductive means of providing pain relief. However, those investigations did not measure the pain responsivity of rats or assess animals that were drinking analgesics postsurgically.

In the present study, we first measured the relative consumption of tap water versus water containing cherry-flavored acetaminophen. We then compared the pain threshold of naive rats drinking acetaminophen–water with that of untreated rats. Finally, we retrospectively compared the acetaminophen–water consumption of unmanipulated rats with that of a group of surgically manipulated rats.

Our study confirmed that rats show initial neophobia to acetaminophen–water and do not drink as much acetaminophen solution as they do tap water (a liquid very familiar to the animals). However, we found that rats that had undergone surgery drink more acetaminophen solution than do unmanipulated animals. Further, if the acetaminophen solution is the only liquid available, unmanipulated rats consume enough to produce an analgesic response.

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Neuroscience Program, Baldwin-Wallace College, Berea, Ohio.
**Corresponding author. Email:amickley@bw.edu*

Materials and Methods

Subjects. We used 15 female and 15 male Harlan-derived Sprague-Dawley rats (Hsd:Sprague-Dawley SD) from the Baldwin–Wallace College breeding colony in the analgesia study (Table 1); these animals weighed 535.0 ± 27.0 g (mean ± standard error). An additional 105 male and female Sprague-Dawley rats were evaluated as part of a retrospective historical analysis of postsurgical use of acetaminophen in drinking water; these rats weighed slightly less (488.01 ± 6.01 g) than the animals used in the analgesia study.

When received, the animals for the colony were negative on measures of serology, polymerase chain reaction analysis, bacteriology, parasitology, and exhibited no significant lesions.[8] No animals from other sources were introduced in the colony. However, no routine sentinel monitoring was performed on the colony's animals.

Animals were individually housed in large (44.45 cm long × 21.59 cm wide × 20.32 cm high) wire-topped plastic cages with corncob bedding (Bed o' cobs, The Andersons Industrial Products, Maumee, OH). The animals were maintained on a 12:12-h light:dark cycle (lights on, 0600; lights off, 1800) in a temperature-controlled room (23 to 26 °C). Rats had constant access to rat chow (no. 5001, PMI Nutrition International, Brentwood, MO) for the entire study. In place of ad libitum tap water, some rats were given acetaminophen–water (see next page).

Animals were cared for according to the recommendations in the *Guide for the Care and Use of Laboratory Animals*,[16] the Animal Welfare Act, and subsequent amendments. The procedures described here were approved by the Baldwin–Wallace College

Table 1. Summary of group nomenclature and treatments

	No. of rats (male/female)	Liquid available prior to testing	Platform temperature (°C) at time of day 1 test	Platform temperature (°C) at time of day 2 test
Acetaminophen–hot plate (ACET-HP)	8 (4/4)	Acetaminophen–water	55.0 ± 0.5 °C	55.0 ± 0.5 °C
Acetaminophen–no hot plate (ACET-No HP)	7 (4/3)	Acetaminophen–water	22.1 ± 0.08 °C	55.0 ± 0.5 °C
H_2O–hot plate (H_2O-HP)	8 (4/4)	Tap water	55.0 ± 0.5 °C	55.0 ± 0.5 °C
H_2O–no hot plate (H_2O-No HP)	7 (3/4)	Tap water	22.1 ± 0.08 °C	55.0 ± 0.5 °C

Rats consumed the same liquid before test days 1 and 2.

Institutional Animal Care and Use Committee.

Hot-plate apparatus. Animals were placed on top of a hot plate (31.5 cm × 31.5 cm) maintained at a constant temperature of either 22.1 ± 0.08 °C (that is, ambient temperature; unheated platform) or 55 ± 0.5 °C (heated), depending on the experimental condition. To prevent animals from escaping from the hot plate, a custom clear acrylic observation enclosure (height, 40.64 cm) was placed on top of the hot plate. The top of the enclosure remained open to ensure proper ventilation as well as to allow placement and removal of the subjects.

Acetaminophen solution and bottles. To prepare the acetaminophen–water, 7 ml of cherry-flavored acetaminophen elixir (Children's Tylenol, McNeil Consumer and Specialty Pharmaceuticals, Fort Washington, PA) was mixed with 43 ml of tap water. The elixir contained 32 mg acetaminophen per ml; therefore, our acetaminophen drinking solution contained 4.48 mg acetaminophen per ml. Both tap water and acetaminophen–water were made available to the rats in 50-ml plastic water bottles with sipper tubes (to prevent dripping).

Experimental groups involved in day-1 analgesia testing. Rats were assigned randomly to the experimental and control groups that were employed in the analgesia study (Table 1). Rats in each of the groups were given ad libitum access to tap water or acetaminophen–water before the start of the analgesia test procedure. The acetaminophen–hot plate group (ACET-HP; n = 8) was given access to acetaminophen water before being tested on the heated platform. The tap water–hot plate group (H_2O-HP; n = 8) had access to tap water before being tested on the heated platform. Comparison of the behavioral responses of the rats in these 2 groups allowed us to evaluate acetaminophen-induced analgesia. The acetaminophen–no hot-plate group (ACET-No HP; n = 7) was given access to acetaminophen before being tested on the unheated platform; observation of this group allowed us to record any motor effects of drinking acetaminophen water alone so that acetaminophen–water consumption itself might be excluded as the cause for any behaviors observed when the platform was heated. The tap water–no hot-plate group (H_2O-No HP; n = 7) was given access to tap water before being tested on the unheated platform; this group allowed us to detect how unmedicated animals behaved with repeated exposure to the unheated plate.

Experimental groups involved in day-2 analgesia testing. The 4 groups described in the preceding paragraph were combined into 2 groups for the second day of analgesia testing. Rats received the same liquid to consume as they did before day 1 testing, but all animals were tested on the hot plate (Table 1).

Procedures. ***Baseline water consumption.*** Baseline water consumption values were obtained for all animals for 3 consecutive days. Animals were given 24-h access to tap water. The amount consumed (measured to 0.1 g) was determined by weighing the bottles before and after the 24-h period. This practice enabled researchers to quantify the amount of liquid the animals regularly consumed each day while allowing the animals to acclimate to the plastic water bottles that were used throughout the experiment.

Testing of day-1 liquid consumption. The day after this period of acclimation, the experiment began with a day of acetaminophen–water or tap water consumption. By means of the previously described procedures, ACET-HP and ACET-No HP animals were given access to acetaminophen water for 23 h. During the same period, H_2O-HP and H_2O-No HP animals were given access to tap water in lieu of acetaminophen–water. At 0730, bottles were removed, and the weights were recorded.

Testing of day-1 analgesia. At approximately 0740, animals were taken individually from the vivarium to the experiment room. To facilitate observation of baseline behaviors, each animal first was placed on an unheated (22.1 ± 0.08 °C) plate. This step familiarized the animals with the equipment being used and allowed recording of each rat's natural behavior in this environment. The animals were observed for 20 s, and any instances of paw licking (the dependent variable) were noted. Each animal was removed from the plate by the base of its tail, replaced into its home cage, and moved back into its original location in the vivarium. All animals tested on the unheated plate failed to lick their paws for the maximum 20 s allowed.

Once this baseline evaluation was completed (by approximately 0820), animals from the 2 control groups (ACET-No HP and H_2O-No HP) were tested individually on the unheated platform by use of the same procedure. After testing of these 2 control groups, ACET-HP and H_2O-HP animals were removed individually from the vivarium in their home cages and placed on a table in the experiment room. Each animal was removed from its cage and placed on the now-heated (55 ± 0.5 °C) hot plate for a maximum of 20 s. This duration was chosen because it was sufficient for the researchers to observe a paw-lick reaction (the dependent variable), which was the first indicator of pain sensation in other studies.[12] In addition, the 20-s exposure time has been reported to be insufficient to cause any persistent pain or tissue injury in the rat.[5,9] Note, however, that we observed slight redness or swelling of the foot tissues 1 h after the day-1 hot-plate test in fewer than 1/3 of the animals in both the acetaminophen–water and tap water groups. Because (a) this redness disappeared before the next test day, (b) an equal number of rats in both experimental and control groups exhibited this response, and (c) these rats did not display aberrant behavioral responses on day-2 testing, we did not exclude them from the data analysis.

One of the authors (GAM), blind to the experimental treatment of the rats, observed each animal for an early indication of pain sensation, that is, the paw-lick reaction. This reaction

consists of the rat licking or placing one of its hindpaws in its oral cavity. At the first sign of this behavior, the rat was removed from the plate. Those animals that did not exhibit such behaviors within 20 s were removed from the plate at that time. Each animal was placed back in its individual home cage and moved back into its original location in the vivarium.

Testing of day-2 liquid consumption. After completion of the first testing period, animals were given 21-h access to the same liquid they had had a day before. At 0730 the next day, bottles were removed from the cages, and the amount of liquid consumed was recorded.

Testing of day-2 analgesia. There was a change in the analgesia testing procedure for the second day of behavioral testing. Starting at approximately 0810, we tested all rats on the heated hot plate after an initial (baseline) observation of the animals on the unheated plate. Therefore, for some animals (ACET-HP and H_2O-HP groups), this analgesia test was the second time they had experienced time on a hot surface, but it was the first time for the ACET-No HP and H_2O- No HP groups. This 2-d procedure was adopted to permit testing of analgesia in rats that may have overcome some of their initial neophobia and therefore would likely drink more of the acetaminophen water than they had on day 1. After testing, all animals were given free access to tap water.

Retrospective data analysis. To place the data from the analgesia study in a clinical context, we analyzed the postsurgical history of rats from our laboratory for which acetaminophen was used as part of a postsurgical analgesic regimen. We analyzed the liquid consumption of 105 male and female rats during the 24-h immediately after surgery; a subset of these animals (n = 78) were followed for 3 d after surgery. Acetaminophen–water was mixed as described and made available to rats in their home cages immediately after surgery. Drinking patterns were analyzed after 2 different surgical procedures: (a) fetal injection and (b) stereotaxic brain surgery.

Pregnant dams undergoing the fetal injection procedure were anesthetized with isoflurane, and a small skin incision was made on their backs before they received a transient spinal block (via injection between the first and second lumbar vertebrae of 0.1 ml of a solution containing 2% lidocaine and 0.001% epinephrine). This procedure was effective in producing: (a) a complete abdominal and hindlimb paralysis; (b) approximately 45 min of spinal anesthesia; and (c) complete recovery after anesthesia.[23] The uterine horns were exposed through a midline laparotomy, and each fetus received oral injection (10 µl) of saccharin-flavored water (0.3% saccharin) or tap water and an IP injection (3 µl) of lithium chloride (81 mg/kg) or physiological saline before the uterus was replaced and the abdominal incisions were sutured and stapled.[14] Local anesthetic (0.5 ml of 2.5 mg/ml bupivacaine, Abbott Laboratories, North Chicago, IL) was infused into the abdominal incision and the incision on the back (from the spinal block). The dam then was placed into a warm cage environment until she regained movement of her legs, after which she was returned to her home cage and given access to acetaminophen–water.

Animals undergoing the stereotaxic procedure were deeply anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg). According to standard procedures for stereotaxic surgery,[22] a midline incision was made on the scalp and 2 or more holes were drilled in the skull. After placement of an electrode, the scalp was sutured, and local anesthetic (0.5 ml of 2.5 mg/ml, bupivacaine, Abbott Laboratories, North Chicago, IL) was infused into the incision. The animal was placed back in its home cage and given access to acetaminophen–water.

The amount of acetaminophen consumed over three, 24-h periods after surgery was recorded for animals from each surgical group. By all measures reported here, the animals undergoing the 2 different surgical procedures were statistically indistinguishable, therefore the animals were combined into a single surgery group.

Statistical analyses. *Sex-based differences.* We used both male and females rats in this study. To determine whether there were sex-based differences in acetaminophen drinking, water drinking, or latencies in the hot-plate test, we used Mann–Whitney U tests (appropriate for our small numbers of subjects).[20] These analyses revealed no differences between sexes in any of these measures during either day 1 or day 2 of behavioral testing. Therefore sex was not considered as a factor in the subsequently described analyses.

Analgesia study—consumption data. We used repeated-measures analysis of variance (ANOVA; drug treatment [acetaminophen–water or tap water] × test condition [hot plate or unheated plate) × test day (repeated factor; day 1 or day 2]) and subsequent Spjøtvoll–Stoline post-hoc tests[10] ($\alpha = 0.05$) to compare the amount of acetaminophen–water or tap water consumed by rats on day 1 in order to determine whether the animals exhibited neophobia for acetaminophen.

Moreover, we were able to determine whether previous experience with acetaminophen on day 1 reduced neophobia and increased consumption of the drug on day 2. Comparison of the dose of acetaminophen (mg acetaminophen/kg body weight) self-administered across the 2 d of the study allowed us to determine the extent to which being on a heated plate versus an unheated plate on day 1 altered consumption of acetaminophen during the subsequent hours (repeated-measures ANOVA; test condition [hot plate or unheated plate] × test day [repeated factor; day 1 or day 2]).

Analgesia study—hot-plate test data. To establish the effectiveness of our hot-plate testing, we used a preliminary *t* test to compare the paw-lick latencies of rats standing on heated versus unheated plate conditions on day 1 of the study (H_2O-HP versus H_2O-No HP).

Rats received different exposures to the hot plate on day 1 and day 2; thus not all animals could be included in a simple repeated-measures ANOVA. However, we analyzed the paw-lick latencies of the subset of rats exposed to the same hot-plate conditions on days 1 and 2 by using repeated-measures ANOVA (drug treatment [acetaminophen–water or tap water] × test day [repeated factor; day 1 or day 2]) and subsequent Spjøtvoll–Stoline post-hoc tests[10] ($\alpha = 0.05$).

Finally, we compared the paw-lick latencies from day 2 of rats that had a history of being tested on the hot plate on day 1 with those that had been on the unheated plate on day 1 (ANOVA; drug treatment [acetaminophen–water or tap water] × test condition [hot plate or unheated plate]). This comparison revealed that exposure to the hot plate on day 1 did not influence paw-lick latencies (nonsignificant effect of test condition). Therefore, we combined all the rats into their respective drug-treatment groups (acetaminophen–water or tap water) and used a *t* test to compare the effects of the analgesic treatment on paw-lick latencies on day 2.

Relationship between consumption of acetaminophen–water and pain responsivity. We calculated the correlation between the dose of acetaminophen consumed (mg/kg) and the latency (s) to lick a paw for each of the 2 test days.

Postoperative consumption of acetaminophen–water: retrospective data analysis. We calculated the mean volume of acetaminophen–water consumed during the 24 h after a surgical

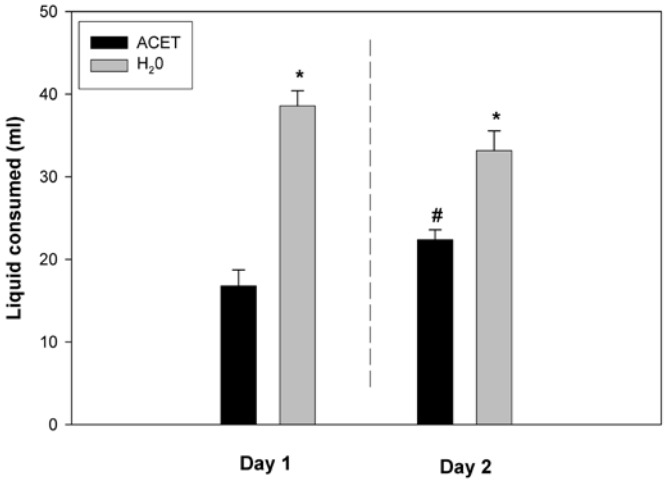

Figure 1. Consumption (mean ± standard error of the mean) of acetaminophen–water (ACET) or tap water (H_2O) by rats prior to analgesia testing. ACET, combination of ACET-HP and ACET-No HP groups; H_2O, combination of H_20-HP and H_20-No HP groups. See Table 1 for group treatments. Consumption of tap water was significantly higher than that of acetaminophen–water on both days of the study. However, as neophobia diminished on day 2, consumption of acetaminophen–water increased significantly. *, Significantly ($P < 0.05$) different from rats drinking acetaminophen–water on the same day; #, significantly ($P < 0.05$) different from rats in the same treatment groups drinking acetaminophen–water on day 1.

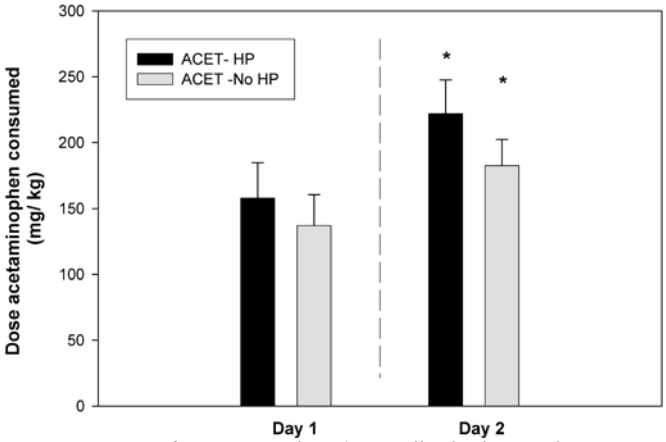

Figure 2. Dose of acetaminophen (in mg/kg body weight; mean ± standard error of the mean) consumed by rats in the period before the analgesia tests conducted on 2 successive days (see Table 1 for descriptions of groups). The dose of acetaminophen voluntarily consumed increased significantly from day 1 to day 2. *, Significant ($P < 0.05$) increase in consumption compared with day-1 dose. The experience with the hot plate on day 1 did not significantly alter subsequent drinking of acetaminophen–water prior to the day-2 hot-plate test.

procedure and, using *t* tests, compared these drinking levels with those recorded from rats that had not undergone a surgical procedure. We also used a repeated-measures ANOVA to compare the volume of acetaminophen–water consumed during 3 d postsurgery.

All analyses were conducted by use of SPSS software (Chicago, IL).

Results

Analgesia study. During the 3 d of baseline water consumption before the start of the study, rats drank 40.74 ± 1.14 ml (mean ± standard error) daily; this value was roughly comparable to the volume of tap water consumed by rats on day 1 of the study (38.58 ± 1.83 ml; Figure 1). Rats with access to only the acetaminophen–water mixture drank significantly less than did their tap-water-drinking counterparts on both days 1 and 2 before analgesia testing (drug treatment effect: $F[1,26] = 51.46$, $P < 0.001$]. However, from day 1 to day 2 there was a significant increase in acetaminophen–water consumption whereas that of tap water declined (drug treatment × test day interaction: $F[1,26] = 13.39$, $P = 0.001$), indicating a reduction in the neophobia effect noted on the first day of the study. The body weights of the rats in the various treatment groups were similar (535.71 ± 28.43 g). Therefore, the dose (mg/kg) of acetaminophen consumed exhibited the same trends as described earlier, with the self-administered acetaminophen dose increasing significantly from day 1 to day 2 (Figure 2; $F[1,12] = 6.01$, $P = 0.03$). This increase on day 2 evidenced itself independent of the animal's experience with the hot plate (or unheated plate) on day 1.

We first established that the hot plate test is an effective way to discern heat sensitivity. Rats that are placed on a 55 °C heated plate exhibited significantly shorter latencies to lick their paw than did rats placed on the unheated plate ($t[13] = 15.0$, $P < 0.001$).

Rats that consumed acetaminophen before hot-plate testing on day 1 exhibited significantly longer latencies to lick their paws than did control rats that consumed water (Figure 3). Repeated-measures ANOVA and post-hoc tests revealed a significant drug treatment × test day interaction ($F[1,13] = 79.18$, $P = 0.02$). The rats that drank acetaminophen–water before the test took significantly longer to sense and respond to the heat than did the tap-water-drinking control rats on the first test day.

A comparison of the latency to paw lick on day 1 versus day 2 revealed that, on day 2 of analgesia testing, the increased consumption of acetaminophen was accompanied by a corresponding significant ($P < 0.05$) increase in time spent on the hot plate (17.59 ± 0.91 s). However, the paw-lick latencies of the acetaminophen-drinking rats and water-drinking rats did not differ significantly on day 2, because tap-water-drinking rats also increased their time on the hot plate. Still, there was significant correlation between the dose of acetaminophen consumed and paw-lick latencies recorded on day 2 of behavioral testing ($r[14] = 0.611$, $P = 0.020$).

The day after surgery, the rats that had had surgery drank significantly more acetaminophen–water than did the no-surgery controls that were part of the analgesia study (ACET-HP and ACET-No HP; $t[118] = 1.78$, $P = 0.039$, 1-tail test; Figure 4). This consumption did not change significantly during the 3 d after surgery. The weights of the rats in our historical surgical analysis were not significantly different from those in our analgesia study that did not undergo surgical treatment.

Discussion

Consistent with findings reported by Speth and colleagues[21] and Bauer and colleagues,[1] our data suggest that rats exhibit a neophobic response upon their first exposure to cherry-flavored acetaminophen–water. These rats drank significantly less of the flavored acetaminophen solution when compared with the tap-water drinking of weight-matched controls. However, we also report that the amount of acetaminophen that is consumed is sufficient to produce an analgesic response in an initial hot-plate test. Moreover, we found that, after surgery, other rats exhibited significantly higher acetaminophen consumption as compared with that of unmanipulated rats. Together these findings suggest that the administration of acetaminophen in the drinking water of rats can be an effective means of raising the pain threshold

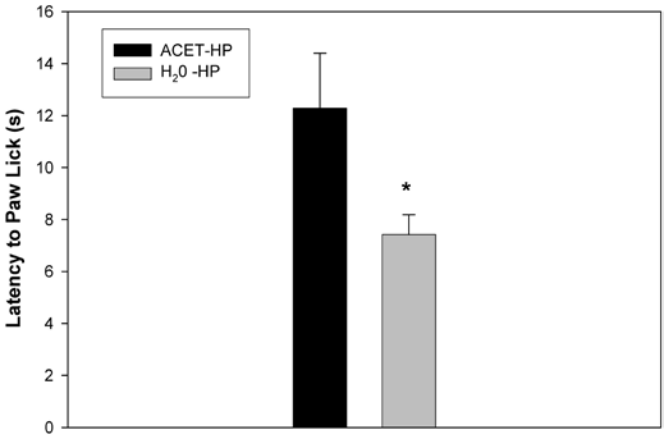

Figure 3. Latency (mean ± standard error of the mean) to lick a paw after placement on a hot plate (HP) (see Table 1 for descriptions of groups) on the day of the first analgesia test. Rats that had been drinking acetaminophen–water before the analgesia test exhibited significantly longer latencies to paw lick than did rats that drank tap water. *, Significantly ($P < 0.05$) different from the acetaminophen–water-drinking rats tested on the hot plate. All rats placed on an unheated plate (ACET-No HP and H_2O-No HP groups, see Table 1; data not shown here) failed to lick a paw for the entire 20-s test period.

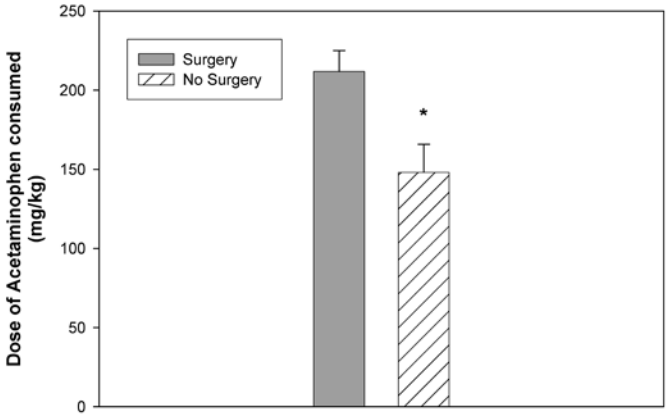

Figure 4. Daily dose (mean ± standard error of the mean) of acetaminophen consumed by naive rats that had recently undergone surgery compared with rats that had no such procedure. The postsurgical consumption of acetaminophen of the surgically manipulated rats was significantly higher than that of rats having no surgery (see also Figure 2). *, Significantly ($P < 0.05$) lower dose consumed than surgery rats.

despite the fact that the volume of the acetaminophen solution consumed may be less than that of tap water.

The dose of acetaminophen that our rats consumed over a day was approximately 200 to 250 mg/kg. This dose, when delivered via intraperitoneal injection, has been shown to be insufficient to produce a significant change in pain sensitivity.[18] But it should be noted that the pharmacokinetics and some of the behavioral measurement parameters of that previous study were very different from the ones we used. Bianchi and Panerai[3] reported that, when received orally, a much lower dose of acetaminophen (25 mg/kg, PO) reduced centrally mediated hyperalgesia (as measured by a tail-flick test). Somewhat higher doses (50 and 100 mg/kg orally) also reduced peripheral analgesia (measured by paw-withdrawal) and enhanced nociceptive thresholds to a mechanical stimulus in an uninflamed paw. It should be noted that Bianchi and Panerai[3] performed oral gavage (in contrast to our animals' self-administration over a longer time period), and therefore our data are not directly comparable. Oral ingestion may well produce different analgesic outcomes and durations than do more direct routes of drug administration.

Our data indicate that self-administration of acetaminophen via the drinking water can produce a measurable analgesia as measured with the hot-plate test. However, our data do not reveal the extent to which this analgesia is complete or sufficient to be clinically important. Other investigators have treated rats with several doses of morphine (intraperitoneally) and then tested them on a hot plate heated to temperatures that fell within 0.5 °C above[13] and below[26] the range we used. Untreated control animals placed on a hot plate exhibited paw-lick latencies comparable to ours (approximately 7 s).[13] Our rats drinking acetaminophen–water stayed on the hot plate for 12.29 ± 4.34 s, which is comparable to the latencies produced by 7.5 to 10.0 mg/kg of morphine intraperitoneally. A dose of 12.5 mg/kg morphine produced a latency to paw lick of approximately 20 s,[13] that is, the maximum latency allowed by our procedures. Therefore, we can say that rats drinking acetaminophen–water obtain moderate, but incomplete, analgesia comparable to that of low doses of morphine used in other studies.

We observed different paw-lick responsivity on test days 1 and 2. As noted earlier, on day 1 rats drinking acetaminophen–water exhibited significantly longer paw-lick latencies than did tap-water-drinking rats. However, on day 2, this difference disappeared. The paw-lick latencies were very high (16.94 ± 1.00 s) for all of our subjects on day 2, and any drug effect may have been obscured by a ceiling effect. Note that, by the time of the second behavioral test, all animals had been in the apparatus 3 times. Rats were placed in the apparatus twice as we established baseline paw-licking rates on the unheated platform and another time for the day 1 test itself (during which half the animals were on the heated plate, whereas the other half were on the unheated platform). Perhaps the general familiarity that the animals had with the apparatus reduced their responsivity during this second analgesia test. However, despite this general increase in paw-lick latency, we did note a significant positive correlation between acetaminophen dose and pain responsivity on day 2.

We chose to limit the observation time on the hot plate to 20 s, because this exposure time has been reported to be safe for animals and insufficient to cause any persistent pain or tissue injury in the rat.[5,9] However, this limitation may have inhibited our ability to detect group differences during day 2 of the analgesia test when latencies to paw lick became longer. In fewer than 1/3 of our rats, we observed a slight redness or swelling of the foot tissues an hour after the day 1 hot-plate test. This redness disappeared before the next test day, and these rats did not display aberrant behavioral responses on the hot plate. Still, for future studies, we do not recommend extending this observation period beyond 20 s for fear of producing a protracted change in the foot pads.

We did not measure responsivity to painful stimuli in rats after surgery. However, the analysis of the acetaminophen consumption of rats during a postsurgical period has relevance to our rats tested for pain sensitivity. Both groups of rats weighed about the same. But after surgery, rats drank more than the animals that had not undergone surgical manipulation. This difference is especially interesting because the natural prediction would be that after surgery and treatment with a local analgesic, rats would be less inclined to self-administer a novel-tasting acetaminophen solution. That is to say, we would expect that the pain alleviation produced by the other analgesics and the neophobia evoked by the novel taste would reduce the volume

of acetaminophen solution consumed. The opposite seems to be the case.

Because we did not measure pain sensitivity in postsurgical rats by using hot-plate tests, we cannot directly know the extent to which the self-administration of acetaminophen attenuates pain postsurgically. However, the combined facts that rats after surgery drink more acetaminophen solution than do unmanipulated rats and that surgically unmanipulated rats self-administer enough acetaminophen–water to produce a moderate analgesic response on the hot-plate test suggest that acetaminophen consumption after surgery may be sufficient to produce moderate analgesia in these animals as well.

Our data do not allow us to determine why rats drink more acetaminophen–water after a surgical procedure. Although blood loss is minimal in our surgical procedures, it may be that after surgery, our animals are drinking to combat some level of dehydration rather than because the acetaminophen–water produces analgesia. Still, the volumes of acetaminophen solution our surgery subjects drink surpass those consumed by surgically unmanipulated rats. Perhaps incidentally, our surgery subjects derive some analgesic benefit from this drinking behavior, although we did not directly confirm this theory in the current study.

Our data are, in some ways, similar to those reported by Persinger,[17] who showed that rats with acute injuries increased their consumption of acetaminophen–water relative to that when injuries were not present. Further, the amount of acetaminophen consumed was positively correlated with the severity of the injury. Persinger[17] suggests that the consumption of acetaminophen is a behavior that results in the removal of an aversive stimulus (negative reinforcer) and, as such, this behavior will be repeated. Our data are consistent with this perspective but do not exclude a role for dehydration or other postsurgical factors in producing the results we report here.

It should be noted that our analgesia testing was performed in the morning within 3 to 4 h of the end of the rat's dark cycle (lights on at 0600) and within 2 h of the removal of the water bottles. Rats do most of their eating and drinking during the dark period, [6,24] and acetaminophen in plasma reaches a peak in 30 to 60 min. The drug's half-life in plasma is about 2 h.[7] The fact that we measured a reliable analgesia so long after the time when the animals consumed the acetaminophen suggests that considerable amounts of drinking occurred in the early morning hours. Analgesia measures taken closer to, or during, the dark phase of the cycle may produce different results.

The study of pain is especially challenging because of the wide diversity of painful stimuli, with their differing characters, time courses, and intensities.[4,25] Evaluation of acute peripheral pain such as that described here may produce different results than the measurement of visceral or incisional pain which have longer time courses and different subjective characteristics and neural centers of control. However, Millecamps and colleagues[15] reported that oral acetaminophen is also effective in reducing cutaneous allodynia in monoarthritic rats treated over a 7-d period, findings that suggest that this drug may be used to address more than acute pain such as that administered in the present study. The paw-licking response after placement on a hot plate is considered to be an integrated reaction to pain involving supraspinal control,[11,19] and acetaminophen apparently works on brain to produce its analgesic effects. Other analgesia tests do not engage supraspinal neurons.[11] Therefore the data presented here should be interpreted in the limited context of the specific behavioral testing paradigm we used.

Here we demonstrate an increase in pain threshold despite the neophobia that reduced drinking of a cherry-flavored acetaminophen solution. Presumably, the analgesic reaction could have been heightened further if rats drank more of the acetaminophen water. For this reason, others[1,21] have suggested starting rats on an acetaminophen-containing solution several days before surgery in order to familiarize them with the taste and reduce the neophobic response. Consistent with the findings of Bauer and colleagues,[1] our data from surgically unmanipulated animals indicate that drinking of acetaminophen–water increases significantly from the first to the second day of consumption. The time spent on the hot plate also increased on this second day. Therefore, if repeated administration of acetaminophen does not interfere with the experimental manipulation at hand, pre-exposure to the novel taste may improve the efficacy of this treatment. It is unknown the extent to which this approach might produce adverse effects (for example, hepatotoxicity and nephrotoxicity).[1,2]

Using methods of oral self-administration of analgesic agents after surgery has several advantages over drug injections. There is less stress associated with handling and constraint, no disruption of diurnal rhythms, and less disturbance of other animals in the vivarium. But these advantages are diminished if the rats do not drink enough of the analgesic to produce pain relief. Our findings indicate that unmanipulated rats drink sufficient amounts of flavored acetaminophen solution to produce an increase in pain threshold. Moreover, our historical data from rats that had recently undergone surgical procedures show that these animals drank significantly more acetaminophen water than did the unmanipulated animals. These data lead us to conclude that the oral, self-administration of flavored acetaminophen solution after surgery may be helpful in reducing moderate pain.

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References

1. **Bauer DJ, Christenson TJ, Clark KR, Powell SK, Swain RA.** 2003. Acetaminophen as a postsurgical analgesic in rats: a practical solution to neophobia. Contemp Top Lab Anim Sci **42(2):**20–25.
2. **Bessems JG, Vermeulen NP.** 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues, and protective approaches. Crit Rev Toxicol **31:**55–138.
3. **Bianchi M, Panerai AE.** 1996. The dose-related effects of paracetamol on hyperanalgesia and nociception in the rat. Br J Pharmacol **117:**130–132.
4. **Buffington CA.** 2001. Visceral pain in humans: lessons from animals. Curr Pain Headache Rep **5:**44–51.
5. **Dubuc I, Remande S, Costentin J.** 1999. The partial agonist properties of Levocabastine in neurotensin-induced analgesia. Eur J Pharmacol **381:**9–12.
6. **Flecknell PA.** 1996. Post-operative care. In: Flecknell PA, editor. Laboratory animal anesthesia. London: Academic Press. p 127–157.
7. **Flower RJ, Moncada S, Vane JR.** 1985. Analgesic–antipyretics and anti-inflammatory agents: drugs employed in the treatment of gout. In: The pharmacological basis of therapeutics. 7th ed. Gilman AG, Goodman LS, Rall TW, Murad F, editors. New York: Macmillan Publishing. p 674–715.
8. **Harlan Laboratories** [Internet]**.** U.S. health reporting [cited on 6 Feb 06]. Available at http://www.harlan.com/healthreports.

9. **Harris JA, Westbrook RF.** 1995. Midazolam impairs the acquisition of conditioned analgesia if rats are tested with an acute but not a chronic noxious stimulus. Brain Res Bull **39:**227–233.
10. **Kirk RE.** 1982. Experimental design: procedures for the behavioral sciences. 2nd ed. Monterey (CA): Brooks–Cole Publishing.
11. **Le Bars D, Gozariu M, Cadden SW.** 2001. Animals models of nociception. Pharmacol Rev **53:**597–652.
12. **McKim WA.** 2000. Drugs and behavior: an introduction to behavioral pharmacology. 4th ed. Upper Saddle River (NJ): Prentice–Hall.
13. **Michaluk J, Karolewicz B, Antkiewicz-Michaluk L, Vetulani J.** 1998. Effects of various Ca2+ antagonists on morphine analgesis, tolerance, and dependence and on blood pressure in the rat. Eur J Pharmacol **352:**189–197.
14. **Mickley GA, Remmers-Roeber DR, Dengler CM, Kenmuir CL, Crouse C.** 2001. Paradoxical effects of ketamine on the memory of fetuses of different ages. Dev Brain Res **127:**71–76.
15. **Millecamps M, Jourdan D, Leger S, Etienne M, Eschqlier A, Ardid D.** 2005. Circadian pattern of spontaneous behavior in monoarthritic rats. Arthritis Rheum **52:**3470–3478.
16. **National Research Council**. 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press.
17. **Persinger MA.** 2003. Rat's preferences for an analgesic compared to water: an alternative to "killing the rat so it does not suffer." Percept Motor Skills **96:**674–680.
18. **Pini LA, Sandrini M, Vitale G.** 1996. The antinociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain. Eur J Pharmacol **308:**31–40.
19. **Sandrini M, Vitale G, Pini LA, Lopetuso G, Romualdi P, Candeletti S.** 2005. Nociceptin/orphanin FQ prevents the antinociceptive action of paracetamol on the rat hot plate test. Eur J Pharmacol **507:**43–48.
20. **Siegel S.** 1956. Nonparametric statistics of the behavioral sciences. New York: McGraw-Hill.
21. **Speth RC, Smith MS, Brogan RS.** 2001. Regarding the inadvisability of administering postoperative analgesics in the drinking water of rats (*Rattus norvegicus*). Contemp Top Lab Anim Sci **40(6):**15–17.
22. **Skinner JE.** 1971. Neuroscience: a laboratory manual. Philadelphia: WB Saunders.
23. **Smotherman WP, Robinson SR, Miller BJ.** 1986. A reversible preparation for observing the behavior of fetal rats in utero: spinal anesthesia with lidocaine. Physiol Behav **37:**57–60.
24. **Stephan FK, Zucker I.** 1972. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci U S A **69:**1583–1586.
25. **St A Stewart L, Martin WJ.** 2003. Evaluation of postoperative analgesia in a rat model of incisional pain. Contemp Top Lab Anim Sci **42(1):**28–34.
26. **Valone JM, Randall CK, Kraemer PJ, Bardo MT.** 1998. Olfactory cues and morphine-induced conditioned analgesia in rats. Pharmacol Biochem Behav **60:**115–118.