Title
Paradoxical surrogate markers of dental injury-induced pain in the mouse

Journal
Pain, 154(8)

ISSN
0304-3959

Authors
Gibbs, Jennifer L
Urban, Rochelle
Basbaum, Allan I

Publication Date
2013-08-01

DOI
10.1016/j.pain.2013.04.018

Peer reviewed

Published in final edited form as:
Pain. 2013 August ; 154(8): 1358–1367. doi:10.1016/j.pain.2013.04.018.

Paradoxical surrogate markers of dental injury-induced pain in the mouse

Jennifer L. Gibbs[2,3,*], **Rochelle Urban**[1,*], and **Allan I. Basbaum**[1]

[1]Department of Anatomy, University of California San Francisco, San Francisco, CA, USA

[2]Department of Preventive and Restorative Dental Sciences, Division of Endodontics, UCSF School of Dentistry, San Francisco, CA, USA

[3]Current Address: Department of Endodontics, New York University College of Dentistry, New York, NY, USA

1. Introduction

Odontalgia, or toothache, is one of the most common types of pain experienced by both adults and children [39]. The pain can be severe, leading to disruption of daily activities, including missed work, sleep disruption, difficulty eating, weight loss, and mood alterations [1; 28; 40; 55]. Although toothache is usually readily treated by clinical procedures, the economic, cultural, and psychological barriers to accessing such treatments results in a high prevalence of toothache, especially in persons of lower socioeconomic status (SES) [58; 66]. For example, a recent study in low SES Americans found that 44% of the population experienced more than 5 separate toothache experiences in a 10-year period [13] and many subjects reported the pain intensity to be “the highest level possible”, suggesting that management of odontogenic pain is a significant public health concern.

The neurobiology of odontalgia focuses on involvement of the dental pulp, as this connective tissue contained within the tooth is densely innervated by a unique class of trigeminal neurons. Activation of pulpal fibers by thermal, mechanical or electrical stimuli almost always results in a sensation of pain [42; 49]. Interestingly, the majority of pulpal neurons are, in fact, low threshold mechanosensitive fibers rather than classical nociceptors [24; 29]. Outside of the tooth the axons of these neurons are myelinated, but they become unmyelinated upon entering the tooth, where the fibers branch extensively [9; 23; 26; 63]. Under normal conditions pulpal afferents are quiescent. However, when the enamel is compromised, innocuous stimuli such as air or modest temperature changes evoke pain. When bacteria approach the pulp, the afferents are sensitized by inflammatory mediators exacerbating the thermal and mechanical hypersensitivity and producing spontaneous pain. Ultimately, the pulpal tissues succumb to the bacterial infection and degenerate, producing severe paroxysmal pain in some patients.

Correspondence: Dr. JL Gibbs, Department of Endodontics, Endodontics/Clinic 7W, New York University College of Dentistry, 345 East 24th St. New York, NY, 10010-4020, jlg15@nyu.edu, Phone: 212-998-9438, Fax Number: 212-995-4834.
*Equal contributors.

It is not known which receptors and signaling mechanisms confer the dental pulp with its unique pain transducing abilities. A better understanding of the neurobiology of dental pain requires the availability of clinically relevant animal models and validated behavioral outcomes. Previous studies in ferrets and rats demonstrate that administering inflammatory agents adjacent to the dental pulp increases short term spontaneous behaviors, decreases exploratory behavior, and lowers evoked mechanical withdrawal thresholds, which are interpreted as signs of pain [10-12; 64]. Despite the advantages provided by the genetic tractability of mice, to our knowledge there are no reported behavioral outcomes of dental injury models in mice (although behavioral changes subsequent to trigeminal nerve injury have been described [46; 69]). In the present report, we induced dental pulp injury in mice and examined a panel of behavioral and physiological indices of pain that, based on known clinical signs and symptoms in humans experiencing odontogenic pain, might manifest in the mouse, including changes in body weight, feeding and drinking, spontaneous locomotor activity, and cold aversion. Although our findings did not parallel the human experience of odontogenic pain, they illustrate the complexity of non-exogenous stimulus evoked rodent pain behaviors and identify novel outcomes useful to the study of orofacial pain.

2. Methods

2.1 Animals

Adult male and female C57Bl/6 mice were purchased from Charles River Laboratory, and arrived at least 2 weeks before testing began. The animals had freely available food (Purina 5058 chow, pellets) and water under a standard 12-hour light/dark cycle with a regulated ambient temperature of 20-22°C and were housed in groups of 3-5, except for the home cage monitoring experiments (see below). Experimental manipulations were performed at 10-12 weeks of age. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California San Francisco and followed the guidelines of the Committee for Research and Ethical Issues of International Association for the Study of Pain.

2.2 Experimental dental pulp injury (DPI)

Under ketamine-xylazine anesthesia (100-10mg/kg, respectively), we created a dental pulp injury (DPI) in mice by mechanically exposing the dental pulp. This procedure produces pulpal inflammation (pulpitis) followed by necrosis of the pulpal tissues (Fig 1A). For the DPI, the animal's mouth was held open by placing the curved tip of a partially opened forcep on the opposite side of the mouth while retracting the tongue. Next the left, or left and right maxillary first molar was drilled with a ¼ round bur at low speed until two pulp horns were exposed. The pulp exposures were visually confirmed using an illuminated operating microscope. The use of the microscope also allowed for clear observation of the depth of the drilling, and assured that the drilling did not extend deeper than the pulp chamber. In rare cases when accidental tissue damage occurred beyond the intended pulp exposure, the mice were excluded from the study. All procedures were performed by the same experienced operator (JG) who is also clinically trained as an endodontist, which allowed us to minimize the variability in technical performance of the DPI procedure. Control animals received the same anesthesia and had their mouths held open with forceps for the amount of time it takes to complete the DPI procedure, approximately 10 minutes. After recovery from anesthesia, animals were returned to their cages, where they had access to water-softened food pellets.

2.3 Home cage monitoring (HCM)

To monitor behavior of the mice in their home cage, we used the automated monitoring system developed by Goulding, et al [27; 65]. Briefly, this system consists of 32 cages each

placed on a pivoting platform with two load beams calibrated to detect the position of the mouse. Photobeams at the feeder (containing Purina 5058, powdered food) and a lickometer at the water bottle detected bouts of feeding and drinking, respectively. Daily monitoring proceeded continuously, except for 1.5-2 hours daily maintenance of the system, when the previous day's food and water were removed and weighed before replacing with new food and water.

Mice were assigned to either DPI or control group based on randomly selecting mice from their original cages where they were group housed, and both groups were dispersed amongst the 32 cages so experimenters were not aware of the treatment allocation. The mice were evenly split between control and DPI and singly housed among the 32 monitoring cages. All animals were initially placed in the system for 9 days to measure baseline activity (Fig. 1A). On the day of DPI or control manipulation, animals were removed, weighed and immediately anesthetized and underwent the DPI or control procedure. As soon as the mice recovered from anesthesia, they were returned to their home cage in the HCM system. Monitoring was reinitiated when all animals were returned (the system was off for a total of 5 hours).

The HCM classified states as inactive or active. In the active state, the behavior was further classified based on location, movement, and feeder/lick spout data. These data points respectively represent feeding, drinking, time in motion, or other (which includes small movements that can be separated by location). As noted in the results, because of missing data due photobeam blockage by powdered chow at the feeder, we excluded some animals in each group from the detailed analysis of bouts and time budgeting. All other data was included in the analysis. The automated nature of the data collection limits the possibility for experimenter bias to influence results.

2.4 Sucrose consumption assay

All sucrose consumption assays were initiated 40 minutes before the start of the dark cycle (19:00), so that the animals were active and more likely to consume sucrose. Food and water were removed 3.5 hours before the start of the assay. At the time of food removal, the mice were weighed, and the cages were brought into the testing room. Sucrose solutions were made fresh each day and put in bottles filled with reusable ice cubes (Icy Cools®) that were either frozen, when testing cold sucrose consumption, or thawed, when testing room temperature consumption. The bottles were weighed just prior to the start of the assay. The test began when each animal was individually placed in the test cage containing one sucrose bottle. The animals could drink freely for 2 hours, after which they were removed from the test chambers and returned to their home cages where they were group housed. Sucrose bottles were weighed and in the case of cold sucrose tests, the temperature of the solution was measured. If the temperature was above 6°C then that animal's data were excluded, although this was rare. At least 48 hours, but not more than 4 days, separated test days.

To train animals in the room temperature (RT) sucrose consumption assay, we first exposed them to 2 days of 5% room temperature sucrose. Animals were then trained at 2.5% for 4 additional days, at which point all animals reached a stable baseline. We then performed 3 tests to assess baseline sucrose consumption. For the cold assay, animals received RT sucrose on the first two training sessions (once at 5%, once at 2.5%). Then animals received the same training and baseline schedule as the RT animals, except with cold sucrose. Any animal that failed to have an average baseline consumption of 0.45g was excluded from the rest of the study. This rarely occurred in animals consuming RT, but was observed in 10-15% of those consuming cold sucrose.

The morning after the 3rd baseline, animals were assigned to control or DPI groups, in a manner allowing for a stratified distribution of baseline levels of sucrose consumed between the two treatment groups. Animals underwent either DPI or control intervention between 9:00 AM and 1:00 PM and were subsequently tested on days 2, 5, 7, 9 (or 10), 12, 14, 17 and 19 (or 20) (See Figure 1B for timeline of room temp tests.) In these experiments the outcomes measured included total sucrose consumption (in grams of liquid consumed) and percent change from the average baseline consumption. Investigators were unaware of group allocation when weighing water bottles.

2.5 Drug studies

To test the sensitivity of the DPI-induced sucrose behavior to a non-steroidal anti-inflammatory drug (NSAID), we administered indomethacin (Sigma-Aldrich, St. Louis, MO; 5.0 mg/kg in 1.7% ethanol and 0.9% saline) once a day for the first 4 days after injury. Two 3.0 mg/kg subcutaneous were made on the day of DPI, one immediately after exposure of the dental pulp and the second at the start of the dark cycle. Each subsequent injection (also subcutaneous) was given at the start of the dark cycle, except on day 2 after DPI when the injection was given immediately following sucrose consumption testing (1.5 hours after lights out). Thus, at the time of sucrose testing on days 2 and 5, drug was last administered about 24 hours earlier. To habituate animals to any effects of the injections themselves, animals were given an injection of saline 24 hours prior to each sucrose test during the training and baseline periods. One animal was excluded because of a failed injection. Finally, to test the involvement of endogenous opioids, we administered 1.0 mg/kg naloxone (Sigma-Aldrich, St. Louis, MO) (i.p.) 30 minutes prior to the sucrose assay. We tested once immediately after training (before injury) and on day 2 after DPI or sham manipulation. To control for possible effects of the injection, during all other training and baseline test days, animals received a saline injection prior to the test (Fig. 1B).

2.6 Histological evaluation of maxillae

Mice were injected with 2.5% Avertin and perfused transcardially with 10 ml of 0.1 M phosphate-buffered saline (PBS), followed by 20 ml of 10% formalin in 0.1 M sodium phosphate buffer. The maxillary jaws were collected, post-fixed for 4 hrs, and then cryoprotected overnight in 30% sucrose in 0.1 M PB. Maxillae were decalcified in 10% EDTA (pH 7.6) for 2 to 3 wks. Tissues were sectioned in a cryostat at 40 μm and thaw-mounted onto Superfrost Plus Microscope Slides. Slides underwent a standard H&E staining protocol for visualization of gross histological structures.

2.7 Statistical Analysis

Results are expressed as mean ± S.E.M. and p values less than 0.05 were considered significant (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Comparisons were analyzed with one-way or repeated measure analysis of variance (ANOVA). In experiments with only one control group, we used Student's t-test, except in cases where data were non-parametric, in which case we used the Mann-Whitney U-test. For ANOVAs, we used post-hoc analysis with Bonferroni tests. Data were analyzed and visualized using GraphPad Prism 5 for Mac.

3. Results

Histological changes after dental pulp injury

We evaluated gross histological changes with H&E staining on slide-mounted cryo-sections of decalcified maxillae. Although we observed degradation of the coronal pulpal tissues seven days after DPI, degradation of the radicular pulp did not occur until 14- 21 days after pulp exposure. The latter can be appreciated in Figure 2A, which illustrates degradation of

coronal and some radicular pulp, and inflammation of the remaining pulpal tissues, 17 days after DPI. Although there is inherent variability in the progression of inflammation and necrosis subsequent to DPI, we consistently observed that the majority of pulpal tissue remains vital for the first 3-5 days. After a week, the dental pulp proper, located within the pulp of the tooth, becomes necrotic and the inflammation begins to progress into the pulp that is located within the roots of the tooth. Generally, at the end point of the behavioral experiments presented here, the pulp is largely necrotic, with some viable tissue remaining in the deepest, most apical part of the root. What is significant is that in the time frame of the behavioral experiments described here (often 15-20 days), the dental pulp progresses from fully vital to partially vital/partially necrotic, with at least some vital pulpal tissue remaining in the deeper root canal at the end of the experiment.

Weight change after DPI and in other models of chronic pain

Short term decreases in total weight and prolonged attenuation of weight gain rates are commonly observed after manipulation of rodents in experimental models of neuropathic and inflammatory pain [5; 8; 65; 68]. Consistent with those findings, we observed that bilateral DPI led to greater deficits in body weight relative to control animals. Two days after the procedure, control animals lost about 2% of their body weight, while animals receiving DPIs lost twice as much weight (Fig. 2B). An evaluation of the time course of weight change showed that the largest difference between the two groups occurred 2 days after injury (Fig. 2C). The control animals resumed gaining weight on the second day after surgery, but the DPI mice did not. Compensation for the weight loss in the injured mice did not occur until day 5, at which point the mice actually gained slightly more weight that the control mice. To determine whether the weight loss was related to inflammatory pain, animals were given daily injections of indomethacin (5.0 mg/kg) or vehicle after DPI or control manipulation. Figure 2D shows that indomethacin indeed prevented the weight loss in mice with DPI during the first 2 days after injury, but had no effect in control animals (one-way ANOVA $p<0.05$; Fig. 2D). This differential effect suggests that the weight loss is, in fact, secondary to inflammation-associated mechanical allodynia and/or spontaneous pain in the DPI animals.

This pattern of weight loss in animals with dental pulp injury was observed in multiple experiments, in both male and female mice with bilateral DPI, and in male mice with a unilateral DPI (Supplemental Figure 1). As this finding suggests that short-term weight loss is a consistent outcome measure in the DPI model, we hypothesized that the weight loss could be used to develop a reliable behavioral endpoint for the assessment of ongoing pain. Thus, in the following experiments, we asked whether the bilateral DPI procedure impacted food consumption or other activities of daily living that might influence body weight.

Daily home cage behavior in animals with DPI

To observe a panel of behaviors that might contribute to the diminished weight gain in DPI animals, we compared the home cage behavior of animals before and after the bilateral DPI or control procedures. The home cage monitoring (HCM) system houses one mouse per cage and allows for continuous monitoring of the animal's behavior, including overall locomotor activity, as well as bouts of eating and drinking [27]. All animals were initially monitored for 9 days to habituate them to the HCM and to collect baseline measures of eating, drinking and movement.

The DPI and control groups had similar total daily life measures, including distance moved, food and water consumed, and percent increase in body weight during the 9 day pre-manipulation monitoring period (Fig. 3 A-D). In the 12 observation days after DPI or control manipulation, the total movement, total food, and total water intake remained similar

in the two groups (Fig 3 A-C). On the other hand, despite comparable food intake in the control and DPI groups, DPI animals again failed to gain weight normally. Thus, in the 12 day monitoring period after manipulation, the two groups differed significantly in the amount of weight gained. Control animals gained a similar percent weight during this period as they did in the pre-manipulation period (post-manipulation: 6.0±1.1%); DPI animals only gained 1.8±0.9% (One-way ANOVA, $p<0.01$; post-tests $p<0.01$ Pre DPI vs. Post DPI and Post DPI vs. Post control, Fig. 3D). To summarize, the simultaneous monitoring of weight, activity, and food and water consumption established that despite minimal or no changes in consumption and activity, there is a significant change in weight gain between the control and DPI groups.

Although total pre-manipulation and post-manipulation feeding, drinking and movement totals for the two groups did not differ, it is possible that time dependent changes in intake or movement occurred in the initial days after injury. We, therefore, analyzed the data by day. Compared to average daily baseline intake/movement before DPI or control manipulation, we did record a large decrease in feeding, drinking and movement in DPI and control animals, but only in the days immediately following anesthesia and manipulation (Fig. 3E-G). We observed a modest effect of the DPI injury on movement in the first 48 hours after the DPI or sham procedures; movement was 44±5% of baseline in control animals and only 28±3% in DPI animals (One Way ANOVA, $p<0.01$, Fig. 3E). In both groups, full recovery of to pre-manipulation levels occurred a week after the DPI or control manipulation, demonstrating the long-lasting effects of anesthesia on total distance moved. On the other hand, although feeding initially decreased in both DPI and control groups to 80% of baseline amounts and recovered to baseline levels within a week, we found no between group differences on any post-injury day (Fig. 3F). Similarly, water intake decreased by 15% in the first 24 hours after manipulation and returned to normal by 48 hours after anesthesia, with no differences between DPI and controls (Fig 3G). This analysis further supports our finding that DPI did not affect food or water consumption relative to controls and only modestly inhibited overall movement in the immediate post-manipulation period.

A great advantage of the HCM system is that it is possible to monitor specific features of the animal's daily life patterns, notably the proportion of time spent performing different tasks, which is collectively referred to as time budgeting. Previous studies that evaluated the effects of dental injury in the rat found that the pattern of feeding altered, but there was minimal change in total food intake [36] . For this reason, we analyzed the eating and movement patterns of animals in the days after injury. Not surprisingly, we found that on the first day after anesthesia/manipulation there were changes in intake and movement bout properties compared to the baseline period. Surprisingly, however, there were no differences between controls and DPI group in any of the measures (Table 1). Thus, in both groups of mice, for the first 24 hours, feeding bout number decreased, while the size and duration of each bout increased, but there was no difference between the groups.

As sleep can be significantly disrupted by persistent pain, including toothache, we were especially interested in assaying the effects of dental injury on the duration and pattern of sleep. The home cage monitoring system does not directly measure sleep, however, we can obtain an approximate measure using the time spent in the inactive state (for detail methods see Goulding, et al, 24). Once again, we did not replicate the expected results. In fact, we found that the average time spent inactive in the 5 days before DPI or sham manipulation and in the first 5 days after did not significantly differ between the two groups (supplemental Fig 2). In summary, using the HCM system, we did not detect behavioral changes in mice with DPI that would have been consistent with what is known about the human experience of dental pain, including sleep disruption, changes in eating and drinking, and long lasting

changes in activity level. Weight loss, however, was also observed in the HCM system, as well as a short-term change in activity.

Sucrose consumption in naive mice

To identify other behavioral endpoints that correlate with a pain outcome that we hypothesize should associate with DPI, we turned our attention to another hallmark of dental inflammation in humans, namely cold hypersensitivity. In the course of these studies, we developed a cold-water consumption assay, in which sucrose solution was used to increase the motivation of the animals to drink readily measurable quantities. We first evaluated how much cold (2-5°C) and room temperature solutions mice would consume at various concentrations of sucrose, during a 2 hour test period. As expected, the mice consumed more (RT) 5% sucrose than RT 2.5% sucrose (T-test, 2.30±0.08g vs. 1.14±0.08g, $p<0.001$), and more RT 2.5% than cold 2.5% (T-test, 1.14±0.08g vs. 0.72±0.08g, $p<0.001$; Fig. 4A). Interestingly, most animals drank more sucrose at either temperature or concentration than RT water. This indicates that while mice preferred RT to cold liquids, they were clearly motivated to drink sucrose regardless of temperature. Based on these preliminary observations, we performed all subsequent experiments with the 2.5 % concentration of sucrose.

Influence of DPI on sucrose consumption

We trained animals in the sucrose consumption test until their baseline consumption was stable, usually 3 sessions, after which they underwent bilateral DPI or sham manipulation (Fig IB). As there is significant cold hypersensitivity after dental injury in patients, we hypothesized that DPI animals would reduce their consumption of cold sucrose. Surprisingly, male mice with bilateral DPI increased the amount of cold-sucrose consumed, to over 150% of baseline; controls remained at baseline consumption levels (Fig 4D). Perhaps even more surprising is that the temperature did not affect the sucrose solution consumption in DPI animals, (RT DPI: 176±14% vs. cold DPI: 207±31%, $p=0.64$; Fig 4B&C). In fact, at both temperatures, sucrose consumption remained elevated for more than 2 weeks after injury, with a significant effect of DPI manipulation in each case (2-way ANOVA, effect of treatment with cold sucrose p=005; effect of treatment with RT sucrose $p=0.005$; Fig. 4B&C). Furthermore, the average sucrose consumed during the first week after manipulation was significantly increased in DPI compared to control, for both cold and RT sucrose solutions (Cold: cntl vs DPI $p<0.01$; RT: cntl vs DPI, $p<0.05$; Fig. 4D). Interestingly, in contrast to the results after the bilateral injury, we found that male mice with unilateral DPI did not have a significant increase in cold sucrose consumption compared to controls (Supplemental Fig 3), suggesting that the magnitude of alteration in sucrose consumption is, in fact, directly related to the magnitude of the injury.

Finally, as a number of studies in mice demonstrated that pain behaviors are gender dependent [17; 48; 50], we repeated the sucrose consumption test in female mice. In these studies, both male and female mice demonstrated significant increases in sucrose consumption after bilateral DPI (Supplemental Figure 3). Based on this finding of no effects of gender or temperature on the outcome of the assay, for the remaining experiments we used female mice, because of the strong association between female gender and orofacial pain, and room temperature sucrose.

Relationship of sucrose intake to weight change

To address the factors that might contribute to DPI animals' increased sucrose intake, we first examined the relationship of weight change to sucrose intake. During the baseline test period (i.e. in unoperated mice), we did not find a correlation between daily average weight change and baseline sucrose consumption (Pearson $r = -0.06$, $p=0.27$, Supplemental figure

4A). On the other hand, when data from all animals (DPI and Control) on day 2 after manipulation were analyzed, we observed a significant inverse relationship between sucrose intake (normalized to baseline) and percent weight change (n=94, Pearson r= −0.478, p<0.0001, Supplemental figure 4B). However, when only DPI animals were included in the analysis (r=−0.17, p=0.25, n=48, Supplemental figure 4C), the correlation of weight loss to percent of baseline intake disappeared. This finding suggests that the amount of weight loss does not correlate with the amount of sucrose solution consumed immediately after the injury. At the minimum, we can conclude that weight loss and increased consumption of sucrose solution are not linearly related in mice with DPI. Furthermore, because the increased sucrose consumption was maintained for an extended period (at least 2 weeks) after the weights normalized (2-3 days), we do not believe that weight loss is the primary factor contributing to the sucrose consumption behavior.

Indomethacin normalizes sucrose consumption after DPI

Because sucrose consumption appears not to be related directly to the early weight loss observed after DPI, we next tested the hypothesis that consumption served as a palliative, providing some relief of the pain associated with the dental injury. In fact, there is considerable evidence that sucrose or other hedonic substances can provide analgesia, including inhibition of spontaneous behaviors in the formalin test in rodents and reduction of the response to acute painful stimuli in psychophysical studies in humans [4; 16; 21; 33]. Therefore, in the next experiment we asked whether the enhanced sucrose consumption that we observed was, in fact, driven by DPI-induced pain. In these studies, animals were given daily injections of 5.0 mg/kg indomethacin after bilateral DPI or control procedures, at the start of the dark cycle. The NSAID had no effect on sucrose consumption in control animals. In contrast, although vehicle-treated female mice with DPI again significantly increased their consumption of the sucrose solution, those that received indomethacin did not (Figure 5). Taken together with the fact that indomethacin also prevented the increase of weight loss in mice with DPI during the first 2 days after injury, we suggest that both sucrose intake and weight change are reliable surrogate outcome measures of inflammatory pain associated with dental tissue injury in mice.

Naloxone does not alter baseline sucrose intake or DPI-induced increased consumption

As the effect of consuming palliative substances on pain is thought to involve the endogenous opioid system, we next tested whether the opiate antagonist naloxone influences DPI-enhanced sucrose consumption [16; 53]. In these studies we first administered naloxone (1.0 mg/kg) or saline to naïve animals, 30 min prior to the sucrose consumption test. Although this dose of naloxone has been shown to decrease motivation to drink sucrose [2], we observed no effect of naloxone on the volume of 2.5% sucrose consumed (Supp Fig. 5A). However, it is also known that naloxone is more effective the more palatable/rewarding the substance. Since the motivation to drink sucrose after DPI was increased, which suggests that the reward value of the sucrose had increased, we also tested the 1.0 mg/kg dose of naloxone two days after DPI, when the maximal increase in sucrose consumption was observed. Here too, we found no effect of naloxone in control (109±8% baseline) or DPI animals (One-way ANOVA, F=4.4, p=0.03; Supplemental Figure 5B). Taken together, these data suggest that the endogenous opioid system does not contribute to the observed DPI-induced increase in sucrose consumption.

4. Discussion

While there is a clear benefit to developing and using pre-clinical models to study dental pain, there are limited reliable behavioral measures in rodents, especially mice. Here, we uncovered at least two measures that are associated with dental pulp injury, namely sucrose

consumption and weight loss. Surprisingly, the rodent equivalent of daily activities known to be impacted in humans with toothache, largely did not change as a result of DPI. Specifically, in the home cage monitoring experiments, mice with DPI ate and drank equivalent amounts as controls, and were inactive for the same amount of time as the control mice, suggesting no change in sleeping patterns. Intriguingly, although food consumption did not change, DPI animals nevertheless gained significantly less weight than did controls. Furthermore, in the first two weeks after DPI, injured animals, but not controls, increased their intake of a sucrose solution in a 2-hour test, and this increase was eliminated by indomethacin. These data indicate that both weight loss and sucrose consumption might be useful surrogate markers of dental pain. Our findings raise a number of questions regarding the root causes of the change in behavior. Why are there changes in weight gain without a parallel change in feeding? What mechanisms drive the increased sucrose consumption?

4.1 Weight loss after dental injury

There is considerable evidence in humans that weight loss occurs in association with dental injury [45; 57]. Importantly, untreated dental decay in children is associated with a failure to thrive, and when dental interventions are performed that alleviate pain and infection, children quickly recover their weight and achieve normal growth [1; 19]. Several hypotheses have been proposed to explain the relationship between dental decay and weight deficiency in children [60]. One hypothesis is that it simply hurts to eat and therefore persons with dental pain eat less. Indeed in clinical studies, nearly 60% of patients with toothache responded “a lot” to the question whether pain kept them from eating [13]. Our experimental design provides insight into this question.

We observed that although all animals, whether injured or not, decreased food intake in the first 3 days after anesthesia, there was no additional decrease of feeding due to DPI. Intriguingly, only in the DPI group was this manifest as a lasting change in weight gain. Furthermore, there was no evidence that complex aspects of feeding behavior (e.g. feeding bouts), differed in DPI and control animals. This suggests that pain from the dental injury did not affect the feeding behaviors in mice. These observations are consistent with the hypothesis that feeding is a strongly protected behavior in rodents, despite the presence of ongoing pain, and this hypothesis is supported in the literature. For example, *ad libitum* consumption of chocolate chips, a highly palliative food for rats, is unaffected by a hindlimb injection of formalin. Also, spontaneous nocifensive behaviors evoked by formalin, such as paw lifting and shaking, are inhibited in rats eating chocolate [21]. Taken together, these findings indicate that it is the ongoing pain or inflammatory processes initiated at the site of tissue injury that drives the change in weight gain. However, the paradox of weight loss without a change in food consumption remains.

One possible explanation for this paradox is that chronic inflammation affects metabolic pathways via pro-inflammatory cytokines. Though chronic inflammatory diseases, such as rheumatoid arthritis and cancer, are associated with cachexia-anorexia syndrome [15; 38; 43; 52], it is unclear that chronic pain and/or peripheral tissue inflammation are able to initiate a cytokine-mediated decrease in weight. However, if after dental pulp injury there were a systemic increase in cytokines from the IL-1 and IL-6 families, both of which have been implicated in cancer-related cachexia, then mice would likely also display weight loss. There is certainly evidence for increased expression of IL-1 and IL-6 in inflamed human dental pulp relative to healthy dental pulp [61; 70]. However, it is unknown whether the local upregulation of cytokines in the pulp has systemic consequences. Given that pulpal inflammation is concomitant with dental caries, and dental caries causes weight deficiencies in children, it is quite feasible that the inflammatory processes that occur in pulpitis contribute to weight changes. It is worth noting that there is growing evidence for the contribution of oral infections, especially periodontitis, to systemic health problems such as

atherosclerosis [14; 30; 54]. Rodent models of pulpitis, such as DPI, should prove useful to better understand the role of pulpal inflammation on systemic health.

4.2 Dental pulp injury leads to increased sucrose consumption

Mice with pulpal exposure increased intake of a sweet solution regardless of its temperature. Although we demonstrated that this behavior is reproducible in male and female mice, and reversed with an NSAID, the mechanisms driving this change in behavior are not immediately obvious. One hypothesis is that mice with DPI are more motivated to drink sucrose solutions because this activity provides analgesia when there is ongoing pain. There is, in fact, substantial evidence that consumption of highly palliative substances, such as sucrose, is associated with analgesia. For example, sucrose consumption produces analgesia in human infants during painful procedures, including heel lance and needle prick [6; 62]. In adults, pain tolerance to pressure is increased following ingestion of highly palliative foods [44]. And in both rodent pups and adult animals, the analgesic effect of sucrose and other highly palatable foods has been linked to endogenous opioids and is reversible with naloxone [4; 22; 59]. Based on these previous reports, we hypothesize that in the DPI model, the observed behavior of increased sucrose consumption serves to counter the unpleasantness of ongoing dental pain. As we found that a moderate dose of naloxone (1mg/kg) did not alter the increased motivation to drink sucrose after DPI, it appears that endogenous opioids might not influence this behavior. Clearly, more work is needed to rule out definitively the contribution of endogenous opiates to the observed increase in sucrose consumption. Further studies should assess higher doses of naloxone as well as different routes of administration.

Whether the sucrose consumption produces analgesia or not, the observed behavior likely involves a convergence of neuronal pathways that underlie pain and reward, which could occur in the mesostriatal dopamine circuit. This circuit is involved in both food and drug reward, and the nucleus accumbens (NAc) is a critical component of the circuit, implicated in reward seeking behaviors, pain avoidance behaviors, and analgesia [7; 20; 31]. As such, microinjection of opiate antagonists into the NAc of rats decreases sucrose consumption, while microinjection of opiate agonists produces analgesia and increased consumption of palliative substances [18; 25; 34]. Although the consequence of the intersection of pain behaviors and reward behaviors in the NAc are not known, it is conceivable that an injury producing an ongoing pain state produces plasticity in neurons of the NAc, and that this, in turn, could affect reward motivated behaviors, such as the consumption of palliative substances. This plasticity could be dependent or independent of the sucrose consumption producing analgesia. Clearly, further work is needed to evaluate the role of the NAc in injury-evoked changes in sucrose consumption.

Also of interest is whether increased sucrose consumption after dental injury is unique to this model, or whether similar behaviors occur in other injury models. A recent publication suggests that the increased consumption of palliative substances after acute tissue injury may indeed be a more general behavioral phenomenon in rodents [41]. In the latter study, rats were introduced into a novel round-shaped testing arena that presented a bowl of palliative food treats. However, the bowl was positioned in the center of a brightly lit, fully exposed arena, a condition to which the rat is normally averse as they avoid bright light and prefer to stay in the periphery. Of interest was the observation that rats that had undergone a planter incision injury spent more time in the center of the test environment, i.e., near the treats, and consumed more treats than did the uninjured rats. These findings suggest not only that acute pain evoked by tissue injury can indeed enhance consumption of palliative substances, but that the finding is not limited to the DPI model or even to the trigeminal system.

4.3 Increased sucrose consumption as a measure of ongoing nociception

Recently an important discussion involving the validity of outcome measures used in animal models of pain has taken place among researchers using pre-clinical pain models. Specifically, there is concern that the assays of mechanical and thermal hypersensitivity involving reflex withdrawal evoked by experimenter applied stimuli do not adequately capture the pain experience [47; 56]. With this new concern in mind, several research groups have independently identified novel behavioral outcomes that could relate to the underlying pain or unpleasantness associated with tissue or nerve injury, including delayed burrowing behavior, interpreting facial cues suggestive of ongoing pain, and invoking conditioned place preference in response to analgesia administration to a rodent experiencing ongoing pain, amongst others [3; 32; 35; 37; 51; 67]. Similarly, we hypothesize that increased sucrose consumption relates to ongoing pain such that the motivation to consume a highly palliative substance is enhanced in rodents with persistent tissue injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants 1K23DE019461 and NS14627 and DA29204. None of the authors have any conflict of interest to report.

References

[1]. Acs G, Shulman R, Ng MW, Chussid S. The effect of dental rehabilitation on the body weight of children with early childhood caries. Pediatr Dent. 1999; 21:109–113. [PubMed: 10197335]

[2]. Agustin-Pavon C, Martinez-Ricos J, Martinez-Garcia F, Lanuza E. Sex versus sweet: opposite effects of opioid drugs on the reward of sucrose and sexual pheromones. Behav Neurosci. 2008; 122:416–425. [PubMed: 18410180]

[3]. Andrews N, Legg E, Lisak D, Issop Y, Richardson D, Harper S, Pheby T, Huang W, Burgess G, Machin I, Rice AS. Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. Eur J Pain. 16:485–495. [PubMed: 22396078]

[4]. Anseloni VCZ, Weng H-R, Terayama R, Letizia D, Davis BJ, Ren K, Dubner R, Ennis M. Age-dependency of analgesia elicited by intraoral sucrose in acute and persistent pain models. Pain. 2002; 97:93–103. [PubMed: 12031783]

[5]. Arras M, Rettich A, Cinelli P, Kasermann HP, Burki K. Assessment of post-laparotomy pain in laboratory mice by telemetric recording of heart rate and heart rate variability. BMC Vet Res. 2007; 3:16. [PubMed: 17683523]

[6]. Blass EM, Watt LB. Suckling- and sucrose-induced analgesia in human newborns. Pain. 1999; 83:611–623. [PubMed: 10568870]

[7]. Borsook D, Becerra L, Carlezon WA Jr. Shaw M, Renshaw P, Elman I, Levine J. Reward-aversion circuitry in analgesia and pain: implications for psychiatric disorders. Eur J Pain. 2007; 11:7–20. [PubMed: 16495096]

[8]. Brennan MP, Sinusas AJ, Horvath TL, Collins JG, Harding MJ. Correlation between body weight changes and postoperative pain in rats treated with meloxicam or buprenorphine. Lab Anim (NY). 2009; 38:87–93. [PubMed: 19229225]

[9]. Cammer W, Tansey FA. Immunocytochemical localization of carbonic anhydrase in myelinated fibers in peripheral nerves of rat and mouse. J Histochem Cytochem. 1987; 35:865–870. [PubMed: 3110266]

[10]. Chattipakorn SC, Sigurdsson A, Light AR, Narhi M, Maixner W. Trigeminal c-Fos expression and behavioral responses to pulpal inflammation in ferrets. Pain. 2002; 99:61–69. [PubMed: 12237184]

[11]. Chidiac JJ, Rifai K, Hawwa NN, Massaad CA, Jurjus AR, Jabbur SJ, Saade NE. Nociceptive behaviour induced by dental application of irritants to rat incisors: a new model for tooth inflammatory pain. Eur J Pain. 2002; 6:55–67. [PubMed: 11888229]

[12]. Chudler EH, Byers MR. Behavioural responses following tooth injury in rats. Arch Oral Biol. 2005; 50:333–340. [PubMed: 15740712]

[13]. Cohen LA, Bonito AJ, Akin DR, Manski RJ, Macek MD, Edwards RR, Cornelius LJ. Toothache pain: behavioral impact and self-care strategies. Spec Care Dentist. 2009; 29:85–95. [PubMed: 19284508]

[14]. D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, Tonetti MS. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. J Dent Res. 2004; 83:156–160. [PubMed: 14742655]

[15]. Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Annals of the New York Academy of Sciences. 2001; 933:222–234. [PubMed: 12000023]

[16]. de Freitas RL, Kubler JM, Elias-Filho DH, Coimbra NC. Antinociception induced by acute oral administration of sweet substance in young and adult rodents: the role of endogenous opioid peptides chemical mediators and mu(1)-opioid receptors. Pharmacol Biochem Behav. 101:265–270. [PubMed: 22197708]

[17]. DeLeo JA, Rutkowski MD. Gender differences in rat neuropathic pain sensitivity is dependent on strain. Neurosci Lett. 2000; 282:197–199. [PubMed: 10717425]

[18]. Dill RE, Costa E. Behavioural dissociation of the enkephalinergic systems of nucleus accumbens and nucleus caudatus. Neuropharmacology. 1977; 16:323–326. [PubMed: 194170]

[19]. Elice CE, Fields HW. Failure to thrive: review of the literature, case reports, and implications for dental treatment. Pediatr Dent. 1990; 12(3):185–189. [PubMed: 2150222]

[20]. Fields HL. Understanding how opioids contribute to reward and analgesia. Reg Anesth Pain Med. 2007; 32:242–246. [PubMed: 17543821]

[21]. Foo H, Crabtree K, Thrasher A, Mason P. Eating is a protected behavior even in the face of persistent pain in male rats. Physiology and Behavior. 2009; 97:426–429. [PubMed: 19321150]

[22]. Foo H, Mason P. Sensory suppression during feeding. Proc Natl Acad Sci USA. 2005; 102:16865–16869. [PubMed: 16275919]

[23]. Fried K, Arvidsson J, Robertson B, Brodin E, Theodorsson E. Combined retrograde tracing and enzyme/immunohistochemistry of trigeminal ganglion cell bodies innervating tooth pulps in the rat. Neuroscience. 1989; 33:101–109. [PubMed: 2481244]

[24]. Fried K, Sessle BJ, Devor M. The paradox of pain from tooth pulp: low-threshold "algoneurons"? Pain. 152:2685–2689. [PubMed: 21889261]

[25]. Gear RW, Aley KO, Levine JD. Pain-induced analgesia mediated by mesolimbic reward circuits. J Neurosci. 1999; 19:7175–7181. [PubMed: 10436070]

[26]. Gibbs JL, Melnyk JL, Basbaum AI. Differential TRPV1 and TRPV2 channel expression in dental pulp. J Dent Res. 90:765–770. [PubMed: 21406609]

[27]. Goulding EH, Schenk AK, Juneja P, MacKay AW, Wade JM, Tecott LH. A robust automated system elucidates mouse home cage behavioral structure. Proc Natl Acad Sci USA. 2008; 105:20575–20582. [PubMed: 19106295]

[28]. Heaivilin N, Gerbert B, Page JE, Gibbs JL. Public health surveillance of dental pain via Twitter. J Dent Res. 90:1047–1051. [PubMed: 21768306]

[29]. Henry MA, Luo S, Levinson SR. Unmyelinated nerve fibers in the human dental pulp express markers for myelinated fibers and show sodium channel accumulations. BMC Neurosci. 2012; 13:29. [PubMed: 22429267]

[30]. Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. J Gen Intern Med. 2008; 23:2079–2086. [PubMed: 18807098]

[31]. Ikemoto S, Panksepp J. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev. 1999; 31:6–41. [PubMed: 10611493]

[32]. Jirkof P, Cesarovic N, Rettich A, Nicholls F, Seifert B, Arras M. Burrowing behavior as an indicator of post-laparotomy pain in mice. Front Behav Neurosci. 4:165. [PubMed: 21031028]

[33]. Kakeda T, Ogino Y, Moriya F, Saito S. Sweet taste-induced analgesia: an fMRI study. Neuroreport. 21:427–431. [PubMed: 20220542]

[34]. Kelley AE, Bless EP, Swanson CJ. Investigation of the effects of opiate antagonists infused into the nucleus accumbens on feeding and sucrose drinking in rats. J Pharmacol Exp Ther. 1996; 278:1499–1507. [PubMed: 8819538]

[35]. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. Nat Neurosci. 2009; 12:1364–1366. [PubMed: 19783992]

[36]. Kramer PR, He J, Puri J, Bellinger LL. A Non-invasive Model for Measuring Nociception after Tooth Pulp Exposure. J Dent Res. 2012; 91:883–887. [PubMed: 22797321]

[37]. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS. Coding of facial expressions of pain in the laboratory mouse. Nat Methods. 2010; 7:447–449. [PubMed: 20453868]

[38]. Langhans W. Anorexia of infection: current prospects. Nutrition. 2000; 16:996–1005. [PubMed: 11054606]

[39]. Lipton JA, Ship JA, Larach-Robinson D. Estimated prevalence and distribution of reported orofacial pain in the United States. J Am Dent Assoc. 1993; 124:115–121. [PubMed: 8409001]

[40]. Locker D, Grushka M. Prevalence of oral and facial pain and discomfort: preliminary results of a mail survey. Community Dent Oral Epidemiol. 1987; 15:169–172. [PubMed: 3474103]

[41]. Low LA, Fitzgerald M. Acute pain and a motivational pathway in adult rats: influence of early life pain experience. PLoS One. 2012; 7:e34316. [PubMed: 22470556]

[42]. McGrath PA, Gracely RH, Dubner R, Heft MW. Non-pain and pain sensations evoked by tooth pulp stimulation. Pain. 1983; 15:377–388. [PubMed: 6866536]

[43]. Means RT, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. Blood. 1992; 80:1639–1647. [PubMed: 1391934]

[44]. Mercer ME, Holder MD. Antinociceptive effects of palatable sweet ingesta on human responsivity to pressure pain. Physiol Behav. 1997; 61:311–318. [PubMed: 9035263]

[45]. Miller J, Vaughan-Williams E, Furlong R, Harrison L. Dental caries and children's weights. Journal of Epidemiology and Community Health. 1982; 36:49–52. [PubMed: 7069355]

[46]. Miyamoto M, Tsuboi Y, Takamiya K, Huganir RL, Kondo M, Shinoda M, Oi Y, Iwata K. Involvement of GluR2 and GluR3 subunit C-termini in the trigeminal spinal subnucleus caudalis and C1-C2 neurons in trigeminal neuropathic pain. Neurosci Lett. 491:8–12. [PubMed: 21215292]

[47]. Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009; 10:283–294. [PubMed: 19259101]

[48]. Mogil JS, Sternberg WF, Kest B, Marek P, Liebeskind JC. Sex differences in the antagonism of swim stress-induced analgesia: effects of gonadectomy and estrogen replacement. Pain. 1993; 53:17–25. [PubMed: 8316385]

[49]. Mumford JM, Bowsher D. Pain and protopathic sensibility. A reveiw with particular reference to the teeth. Pain. 1976; 2:223–243. [PubMed: 800250]

[50]. Painsipp E, Wultsch T, Shahbazian A, Edelsbrunner M, Kreissl MC, Schirbel A, Bock E, Pabst MA, Thoeringer CK, Huber HP, Holzer P. Experimental gastritis in mice enhances anxiety in a gender-related manner. Neuroscience. 2007; 150:522–536. [PubMed: 17945426]

[51]. Pereira Do Carmo G, Stevenson GW, Carlezon WA, Negus SS. Effects of pain- and analgesia-related manipulations on intracranial self-stimulation in rats: further studies on pain-depressed behavior. Pain. 2009; 144:170–177. [PubMed: 19435650]

[52]. Plata-Salamán CR. Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. Nutrition. 2000; 16:1009–1012. [PubMed: 11054608]

[53]. Pomonis JD, Jewett DC, Kotz CM, Briggs JE, Billington CJ, Levine AS. Sucrose consumption increases naloxone-induced c-Fos immunoreactivity in limbic forebrain. Am J Physiol Regul Integr Comp Physiol. 2000; 278:R712–719. [PubMed: 10712293]

[54]. Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V. Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident

cardiovascular disease events. Arterioscler Thromb Vasc Biol. 2007; 27:1433–1439. [PubMed: 17363692]

[55]. Reisine ST. The impact of dental conditions on social functioning and the quality of life. Annu Rev Public Health. 1988; 9:1–19. [PubMed: 3288228]

[56]. Rice AS, Cimino-Brown D, Eisenach JC, Kontinen VK, Lacroix-Fralish ML, Machin I, Mogil JS, Stohr T. Animal models and the prediction of efficacy in clinical trials of analgesic drugs: a critical appraisal and call for uniform reporting standards. Pain. 2008; 139:243–247. [PubMed: 18814968]

[57]. Ritchie CS, Joshipura K, Silliman RA, Miller B, Douglas CW. Oral health problems and significant weight loss among community-dwelling older adults. Journals of Gerontology Series A, Biological Sciences and Medical Sciences. 2000; 55:M366–371.

[58]. Sanders AE, Slade GD, Lim S, Reisine ST. Impact of oral disease on quality of life in the US and Australian populations. Community Dent Oral Epidemiol. 2009; 37:171–181. [PubMed: 19175659]

[59]. Segato FN, Castro-Souza C, Segato EN, Morato S, Coimbra NC. Sucrose ingestion causes opioid analgesia. Brazilian Journal of Medical and Biological Research. 1997; 30:981–984. [PubMed: 9361728]

[60]. Sheiham A. Dental caries affects body weight, growth and quality of life in pre-school children. British Dental Journal. 2006; 201:625–626. [PubMed: 17128231]

[61]. Silva AC, Faria MR, Fontes A, Campos MS, Cavalcanti BN. Interleukin-1 beta and interleukin-8 in healthy and inflamed dental pulps. J Appl Oral Sci. 2009; 17:527–532. [PubMed: 19936537]

[62]. Slater R, Cornelissen L, Fabrizi L, Patten D, Yoxen J, Worley A, Boyd S, Meek J, Fitzgerald M. Oral sucrose as an analgesic drug for procedural pain in newborn infants: a randomised controlled trial. Lancet. 2010; 376:1225–1232. [PubMed: 20817247]

[63]. Sugimoto T, Takemura M. Tooth pulp primary neurons: cell size analysis, central connection, and carbonic anhydrase activity. Brain Res Bull. 1993; 30:221–226. [PubMed: 8457869]

[64]. Tsuboi Y, Iwata K, Dostrovsky JO, Chiang CY, Sessle BJ, Hu JW. Modulation of astroglial glutamine synthetase activity affects nociceptive behaviour and central sensitization of medullary dorsal horn nociceptive neurons in a rat model of chronic pulpitis. Eur J Neurosci. 2011; 34:292–302. [PubMed: 21707791]

[65]. Urban R, Scherrer G, Goulding EH, Tecott LH, Basbaum AI. Behavioral indices of ongoing pain are largely unchanged in male mice with tissue or nerve injury-induced mechanical hypersensitivity. Pain. 2011; 152:990–1000. [PubMed: 21256675]

[66]. Vargas CM, Macek MD, Marcus SE. Sociodemographic correlates of tooth pain among adults: United states, 1989. Pain. 2000; 85:87–92. [PubMed: 10692606]

[67]. Vierck CJ, Acosta-Rua AJ, Johnson RD. Bilateral chronic constriction of the sciatic nerve: a model of long-term cold hyperalgesia. J Pain. 2005; 6:507–517. [PubMed: 16084465]

[68]. Vos BP, Hans G, Adriaensen H. Behavioral assessment of facial pain in rats: face grooming patterns after painful and non-painful sensory disturbances in the territory of the rat's infraorbital nerve. Pain. 1998; 76:173–178. [PubMed: 9696471]

[69]. Xu M, Aita M, Chavkin C. Partial infraorbital nerve ligation as a model of trigeminal nerve injury in the mouse: behavioral, neural, and glial reactions. J Pain. 2008; 9:1036–1048. [PubMed: 18708302]

[70]. Zehnder M, Delaleu N, Du Y, Bickel M. Cytokine gene expression--part of host defence in pulpitis. Cytokine. 2003; 22:84–88. [PubMed: 12849707]

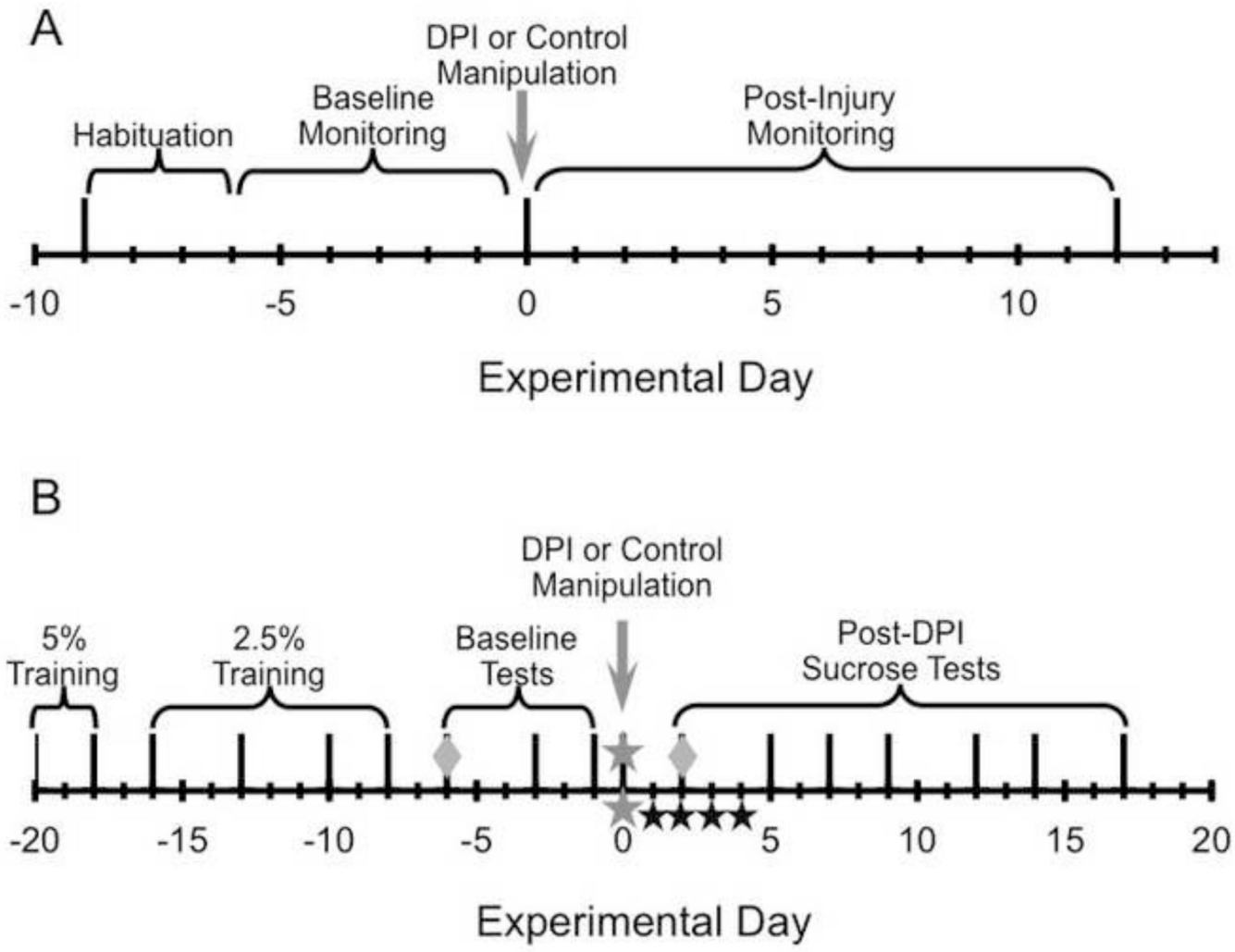

Figure 1.

Timeline of experiments. (A) Home cage monitoring experiment proceeded with 3 days of habituation, 6 days of baseline monitoring and 12 days of post-manipulation monitoring. (B) Timing of sucrose consumption test for room temperature tests (cold tests included an additional two training sessions before this timeline began). Each bar denotes a sucrose consumption assay. The arrow indicates the day of DPI/sham manipulation. The diamonds show the timing of naloxone injection and the stars show the timing of indomethacin injection, with grey stars indicating injection of 3.0 mg/kg and black stars indicating injection of 5.0 mg/kg.

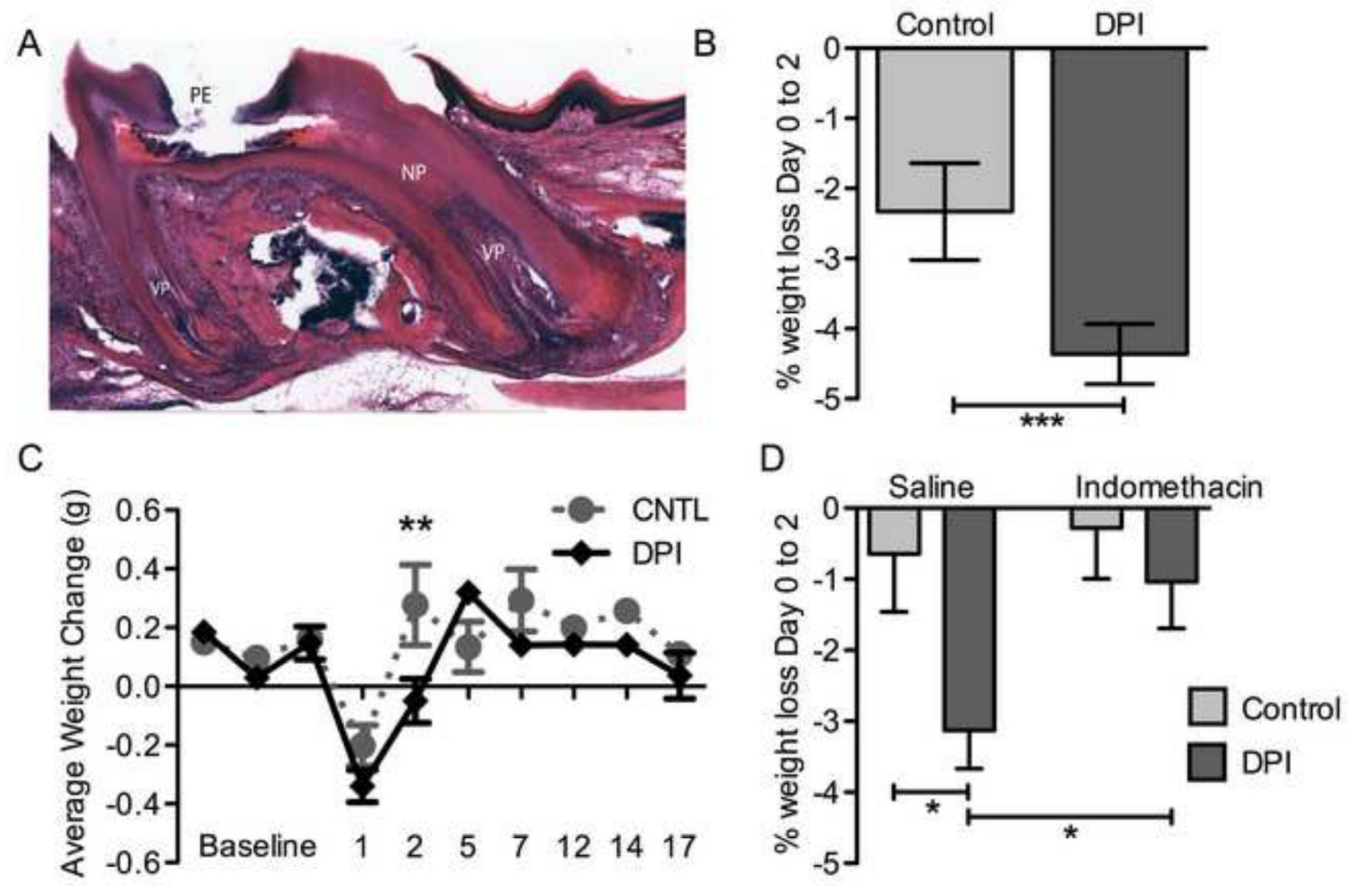

Figure 2.

Effect of dental pulp injury on weight. (A) Hematoxylin and eosin stain of an injured tooth 17 days after initial pulp exposure, illustrates the extent of the initial mechanical pulp exposure (**PE**). There is evidence of necrotic pulp tissue **(NP**) in throughout the coronal pulp, extending into the right root canal space. Vital pulp tissue remains in both the leftmost and rightmost roots. (B) Weight loss over the first two days after bilateral DPI, measured by percent change in body weight immediately preceding injury or control manipulations. (DPI: n=28-31 Mann-Whitney p=0.0005) (C) Average daily weight change on the three baseline days and each day after DPI (Two-way ANOVA, effect of treatment, p=0.001) CNTL: Control. (D) Daily injections of indomethacin in the first 2 days after DPI or control manipulation protected DPI animals from weight loss relative to controls (one-way ANOVA: F=3.5, p=0.02 n=11-12).

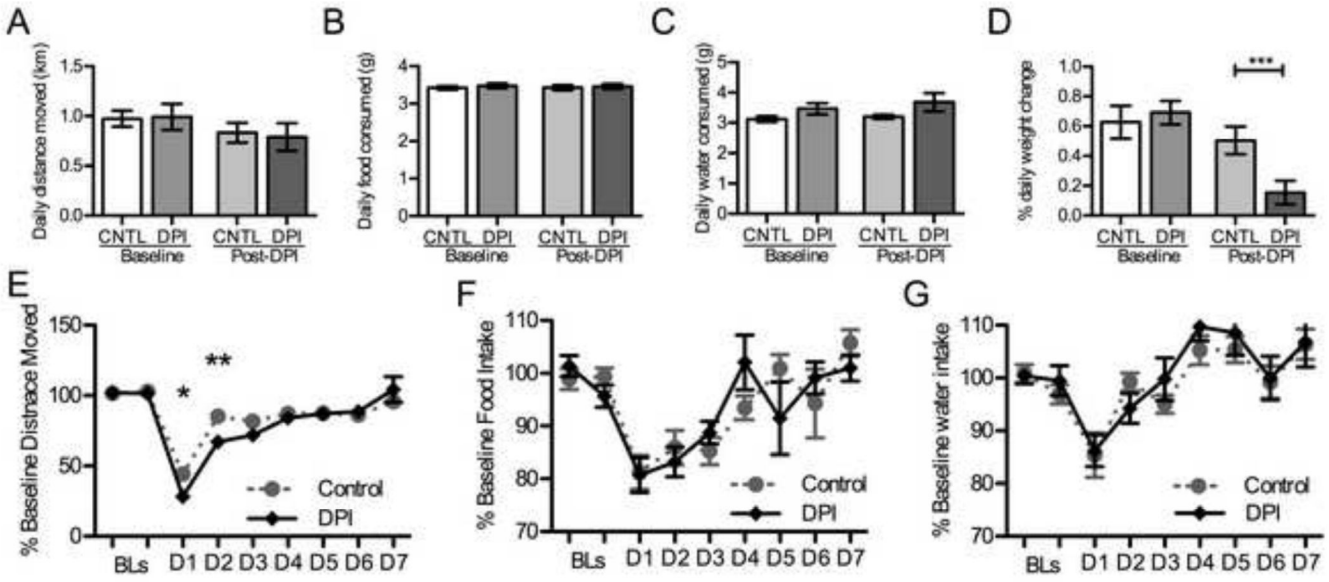

Figure 3.

The effect of bilateral DPI and sham manipulation on home cage behaviors. Average daily movement (A), food (B) and water (C) in the 9 days prior to manipulation and 12 days after in animals that underwent control or DPI manipulations. There were no significant differences between the two groups, either before or after manipulation. (D) In the 12 days after DPI, mice gained less weight per day compared to controls and compared to the mice 9 days prior to manipulation (n= 15-16, One-way ANOVA, $F=4.55$, $p=0.0062$; post-tests $p<0.01$ Pre DPI vs. Post DPI and Post DPI vs. Post Control) (E-G) Average daily movement (E), food (F) and water (G) decreased as a result of anesthesia. There was an additional significant decrease in locomotion on days 1 and 2 after DPI (Repeated measures baselines through day 4 only, effect of treatment p-0.018). CNTL: control

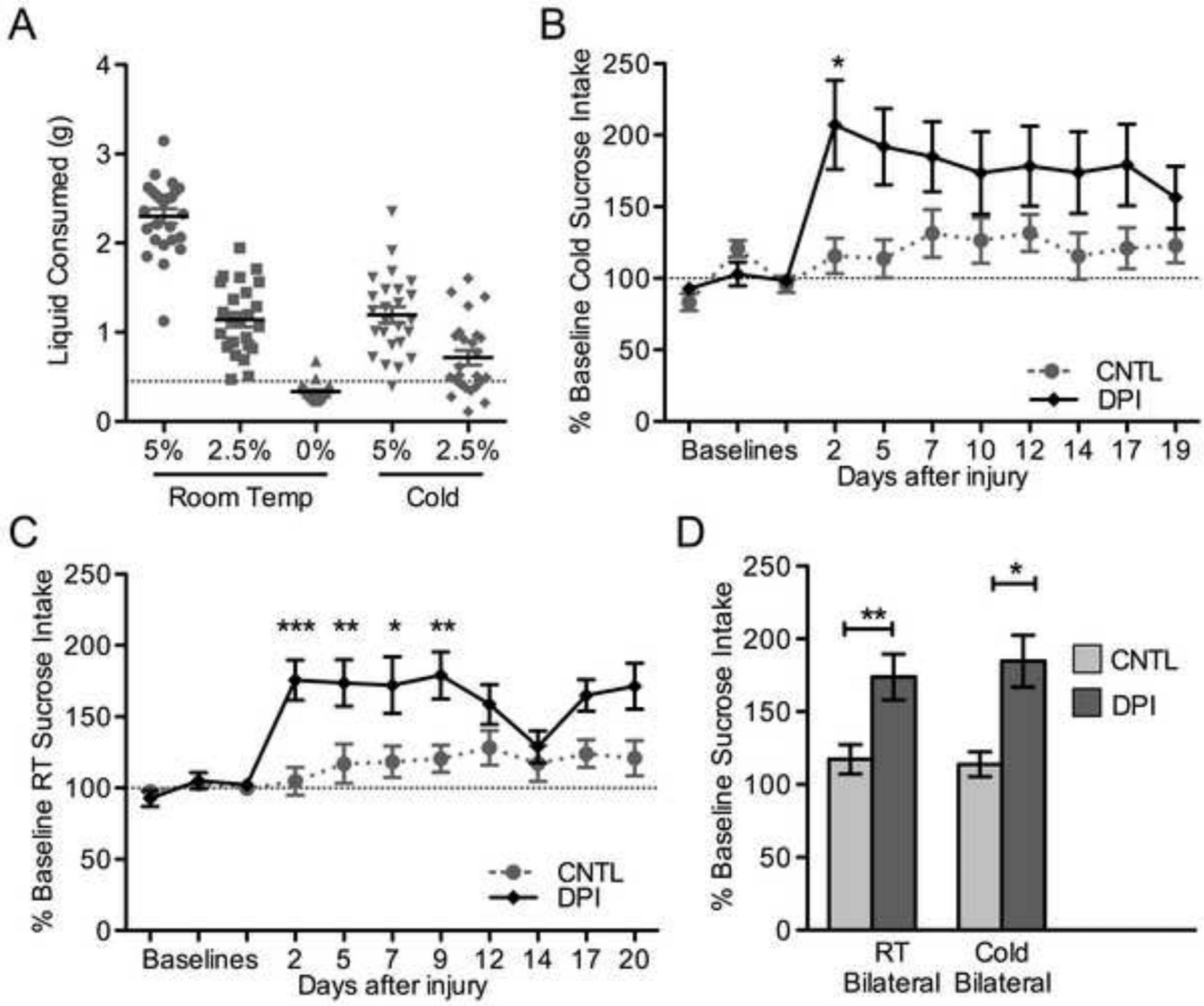

Figure 4.

Sucrose consumption in mice with bilateral DPI. (A) Sucrose consumption in naïve male mice reveals a concentration and temperature dependence on motivation to drink during the two-hour test. Dotted line indicates the level below which animals were excluded from the DPI studies. (B,C) Mice with DPI drank more cold (B) or RT (C) sucrose after injury than control mice. Data are normalized to each mouse's baseline average and individual baseline days are also shown compared to the average of all baselines ((B)2-way ANOVA, effect of treatment $p=0.05$, post-hoc t-test at day 2 $p<0.05$, n=14-17; (C) 2-way ANOVA, effect of treatment $p=0.005$, post-hoc t-test on days 2-9 $p<0.05$, n=13). (D) The average sucrose consumption for the first week after injury (day 2-7) normalized to baseline consumption is increased in mice with bilateral DPI in either cold or room temperature sucrose tests (Cold vs control 117±10%; cold, DPI 174±16%, t-test $p<0.01$ compared to control; RT vs control 120±12; RT, DPI 195±27, t-test $p<0.05$ compared to control).

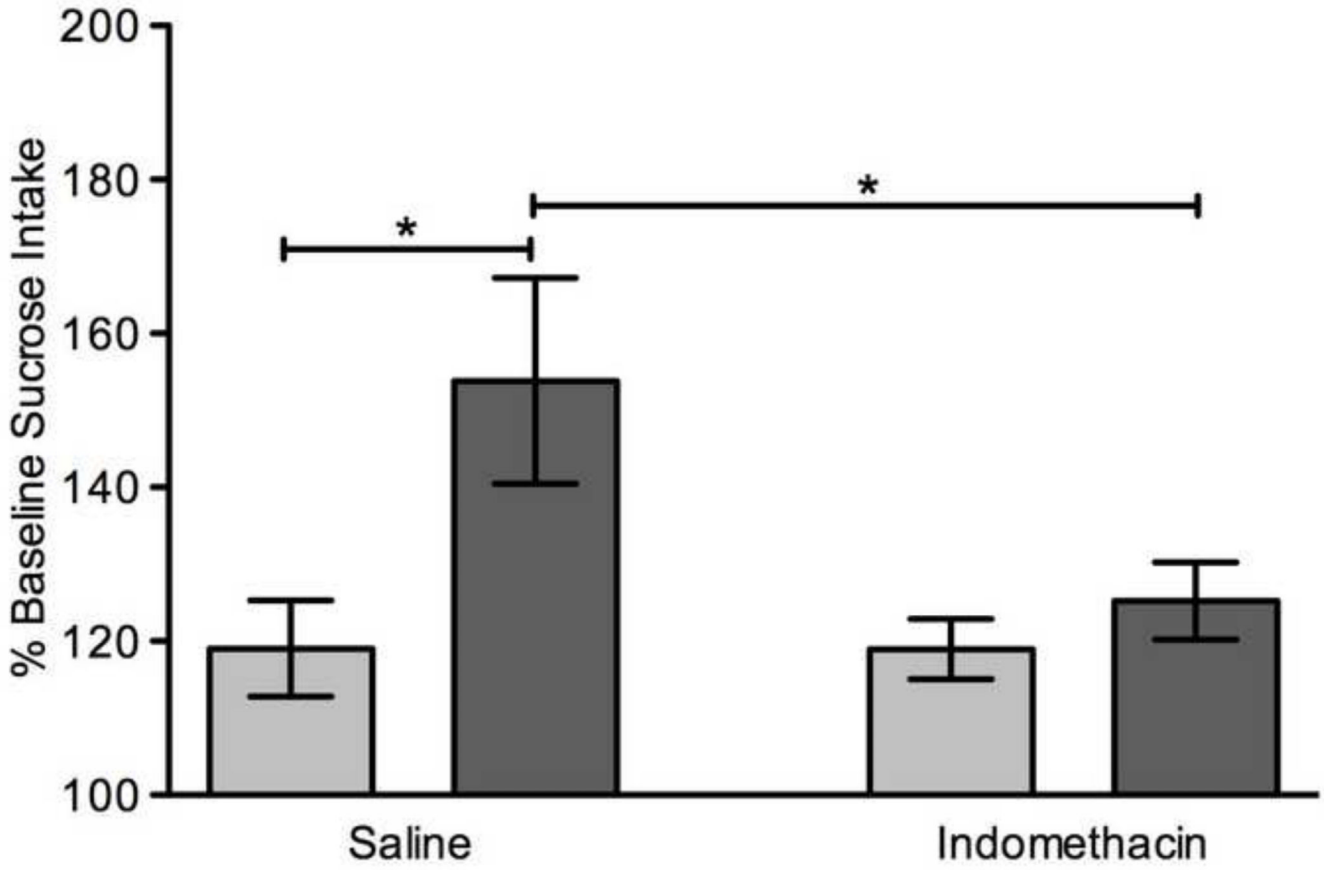

Figure 5.

Daily injections of indomethacin in the first 2 days after bilateral DPI blocked the injured animals increase in sucrose intake as measured on day 2 after manipulation (one-way ANOVA: F=4.3, p=0.01, n=11-12 per group).

Dental pain, including toothache, is one of the most prevalent types of orofacial pain, causing severe, persistent pain that has a significant negative effect on quality of life, including eating disturbances, mood changes, and sleep disruption. As the primary cause of toothache pain is injury to the uniquely innervated dental pulp, rodent models of this injury provide the opportunity to study neurobiological mechanisms of tissue injury induced persistent pain. Here we evaluated behavioral changes in mice with a dental pulp injury (DPI) produced by mechanically exposing the pulp to the oral environment. We monitored the daily life behaviors of mice with DPI, including measures of eating, drinking, and movement. During the first 48 hours the only parameter affected by DPI was locomotion, which was reduced. There was also a significant short-term decrease in the amount of weight gained by DPI animals that was not related to food consumption. As cold allodynia is frequently observed in individuals experiencing toothache pain, we tested whether mice with DPI demonstrate an aversion to drinking cold liquids using a cold-sucrose consumption test. Surprisingly, mice with DPI increased their consumption of sucrose solution, to over 150% of baseline, regardless of temperature. Both the weight loss and increased sucrose intake in the first 2 days of injury were reversed by administration of indomethacin. These findings indicate that enhanced sucrose consumption may be a reliable measure of orofacial pain in rodents, and suggest that alterations in energy expenditure and motivational behaviors are under recognized outcomes of tooth injury.

Table 1

Bout averages ± SEM in DPI and control animals (n=6) in the first 48 hours after manipulation. The low number of mice in this analysis is due primarily to loss of data from blocked photobeams.

		Control			DPI	
Feeding Bouts Averages		**Baseline average**	**Day 1**	**Day 2**	**Baseline average**	**Day 1**
	Bout Number	90.8±9.2	60±8.3	74.5±7.7	106.2±28	54±10.9
	Bout Duration (s)	55±3.8	78.3±7.1	64.3±7.6	47.8±3	79.2±13.4
	Bout Size (mg)	39.3±4.4	50.3±5.3	38.8±4.4	40.3±7.9	62.7±8.1
	Intensity (mg/s)	0.71±0.06	0.65±0.05	0.61±0.04	0.84±0.14	0.81±0.05
Movement Bout Averages						
	Bout Number	4628±1071	1962±289	3848±667	4269±463	1385±281
	Bout Duration (s)	1.3±0.1	1.47±0.12	1.34±0.11	1.48±0.1	1.53±0.06
	Bout Size (cm)	17±1.1	15.4±0.9	17.1±1.4	26.4±7.5	14±0.9
	Speed (cm/s)	13±1.3	10.5±0.4	12.5±0.9	13.1±1.4	8.9±0.4