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Eating is a protected behavior even in the face of persistent pain in male rats

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Abstract

Feeding is critical for survival. Yet, patients with chronic pain often lose their appetite and eat less. We previously showed that *ad libitum* fed male rats continue to feed rather than withdraw from a brief noxious stimulus. This study examined the effects of a sustained noxious stimulus on feeding by testing *ad libitum* fed male rats for five eating behaviors –latency to eat, time taken to eat each chip, pauses and scanning during eating, and the number of chocolate chips eaten - during the hour following a sham injection or an injection of a low (0.5%) or moderate (1.5%) dose of formalin into the hind paw. Sham-injected rats showed no pain-related behaviors, rats injected with 0.5% formalin showed very few pain-related behaviors, and rats injected with 1.5% formalin showed favoring, lifting and licking of the injured paw with a characteristic biphasic time course. Besides taking less time to commence eating during the first phase of formalin pain, rats injected with either dose of formalin did not differ from sham-injected rats on any of the other eating measures. Rats injected with 0.5% formalin showed no pain behaviors during eating, whereas those given 1.5% formalin typically ate while not exhibiting any pain behaviors but occasionally ate while favoring the paw, rarely while lifting the paw, and never while licking the paw. These results show that eating is a protected activity even in the presence of persistent pain in male rats.

Keywords

eating behavior; formalin; chocolate

A decrease in appetite and eating problems are common complaints associated with acute and chronic pain. In a sample of older adult outpatients with non-malignant chronic pain, approximately 40% reported eating problems [1]. Even among a non-clinical population of children and adolescents surveyed for acute and chronic pain, over 50% attributed appetite problems to their pain experience [2]. Anorexia is extremely prevalent in migraine sufferers, occurring in > 80% of sufferers during attacks [3]. The timing of appetite loss coincides well with the appearance of head pain and precedes the onset of symptoms more directly associated with loss of appetite such as nausea and vomiting [3].

In an animal model of migraine pain, male rats that received noxious chemical stimulation of the dura ate less than those given vehicle stimulation in the first four hours following stimulation [3]. However, dural stimulation produces cutaneous facial allodynia [4,5] and

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possible hypersensitivity in the intraoral and maxillary regions required for feeding, raising the possibility that rats ate less simply because it was painful to move their mouths. Indeed, a painful stimulus distant from the mouth has no effect on food intake in food-restricted male rats [6].

The present study examines the impact of persistent pain elicited by formalin injection into a hind paw on eating behaviors in freely fed male rats. Freely fed rats were used, thereby eliminating any effects of hunger, food deprivation, or glucoprivation upon pain responsiveness [7–9]. Eating behaviors were studied during both the first and second phases of formalin pain. Only the first phase of formalin pain is opioid-sensitive [10], and may therefore be more likely to be associated with changes in feeding, which is also influenced by opioids, than is the second phase [11,12].

Three groups of *ad libitum* fed rats were sham-injected or injected with a low (0.5%) or moderate (1.5%) dose of formalin to the hind paw and were then tested for the latency to eat, the time taken to eat each chip, the number and duration of pauses and scanning during each eating bout, and the number of chocolate chips eaten. Chocolate chips were selected as the test food because they are highly palatable and rats with free access to chow readily eat them [13].

Materials and methods

Subjects

All procedures were reviewed and approved by the University of Chicago Institutional Animal Care and Use Committee. Subjects were 30 male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) weighing 380 – 560 g at the start of the experiment. They were housed in pairs and were kept in a 12:12 h light-dark controlled vivarium maintained at a temperature of $\approx 23^{\circ}\text{C}$. Laboratory chow and water were available *ad libitum* during housing and throughout all phases of the experiment. Apparatus. The apparatus consisted of a Plexiglas box (32 $\times$ 32 cm, 100 cm height) with a wire mesh floor located 40 cm above the base of the box. Three cameras were located in the front, on the right side, and underneath the box to record the rat's behavior. A fourth camera was used to record the display of a timer (1 s resolution). All four images were acquired simultaneously with a Quad processor (Everfocus, Duarte, CA) connected to a video-recorder.

Procedure

All rats were familiarized to handling and to the apparatus and were trained to eat Nestle® Milk Chocolate Morsels (chocolate chips) before the start of the experiment proper. The experiment was conducted across 5 days during the light part of the rat's diurnal cycle in a room maintained at a temperature of $\approx 23^{\circ}\text{C}$. On each of days 1 – 3, rats were assessed for their baseline chocolate chip consumption during a 60 min exposure to the test apparatus. Rats ($n = 2$) that ate < 3 chips per session were excluded from further testing. The remaining 28 rats were assigned to one of three groups in a manner which equated the groups on overall mean baseline chip consumption. On day 5, rats in two of the groups were given a subcutaneous injection of formalin (0.5%, $n = 9$ or 1.5%, $n = 10$, 50 μl) into the ventral surface of the right hind paw. Formalin was diluted to these concentrations from a stock solution of 100% (formaldehyde solution 37% w/w, Fisher Scientific Company, Fairlawn, NJ) and was injected using a single-use 1 ml syringe and a 30-gauge needle. Since we wanted to compare formalin-injected rats with rats without edema and persistent pain and since saline injection into the hind paw produces edema [14], we chose to give control rats a sham injection: a 30-gauge needle was inserted subcutaneously and then withdrawn ($n = 9$). This procedure adequately controlled for the stress due to handling and needle insertion without causing frank edema. Immediately after the formalin or sham

injection, the rat was placed into the test cage for 60 min and given a chocolate chip. On all baseline and test sessions, chocolate chips were given one at a time. All rats were sacrificed immediately after testing.

Behavioral scoring and analyses

Pain and eating behaviors were scored from analyses of video-recordings. The time and duration that the injected paw was normal, favored, lifted, or licked/bitten were scored [15]. The eating behaviors scored were: latency to eat, time taken to eat each chip, eating pauses and scanning during each eating bout, and the number of chips eaten. Latency to eat was defined as the time between the ultimate retrieval of a chip till the commencement of eating. The number and time taken to eat the chips were chosen because they have been shown to be affected by a number of experimental manipulations such as exposure to intense noise (80 – 110 dB), a non-noxious stressor [16–18].

Statistical analyses

The rats were allocated to groups to equate for baseline chip intake. Statistical analysis confirmed that there were no significant differences in the mean number of chocolates eaten on baseline days (Means $\pm$ S.E.M.s = 7.6 ± 0.5 , 7.6 ± 0.7 , 7.6 ± 0.7 , $F_{0.5}(2,25) < 1.0$). The proportion of time rats spent favoring, lifting, and licking the injected paw was calculated. The average latency to eat, time taken to eat each chip, number and duration of eating pauses, proportion of time spent scanning during each eating bout, and number of scans made per eating bout, and the number of chips eaten by each of the three groups were also calculated. Mixed design ANOVAs, with significance level set at 0.05, were used to test for significant differences between groups and across time.

Results

All rats had *ad libitum* access to water and chow throughout the experiment including during testing.

Sham injected rats showed no pain behaviors at any stage during testing. Rats injected with 0.5% formalin spent only 1% of the total time displaying pain-related behaviors. They showed few pain behaviors during either the first (0–10 min) or second (10–60 min) phases of formalin pain, and were not statistically different from sham-injected rats in this respect (Fig. 1A). Rats injected with 0.5% formalin never displayed any pain behaviors during eating. They showed a significantly shorter latency to eat than sham-injected rats during the first phase of formalin pain (Fig. 2A). Yet, they did not differ from sham-injected rats on the proportion of eating trials interrupted by pauses, the mean duration of eating pauses, the proportion of time spent scanning during each eating bout, or the number of scans made per eating bout throughout testing (data not shown). Moreover, both groups of rats took the same amount of time to consume each chip and ate the same number of chocolate chips during both phases of formalin pain (Fig. 2B & C).

Rats injected with 1.5% formalin spent significantly more time paw-favoring (2%), paw-lifting (24.5%), and paw-licking (13.5%) than rats injected with the lower dose ($F_{0.5}(1,12) = 40.2$). They displayed the characteristic biphasic pain response to formalin, with an acute phase that peaked within the first 5 min and a second phase that peaked approximately 30 min post-injection (Fig 1B). While eating, rats injected with 1.5% formalin displayed no pain behaviors most of the time (74%), favoring of the injected paw a small proportion of the time (23%), and paw lifting the remainder of the time (3%). Although it is possible to briefly interrupt feeding to paw-lick or to stop eating mid-chip, none of the 1.5% formalin-injected rats behaved in either of these ways.

Like rats injected with the lower formalin dose, rats injected with 1.5% formalin displayed a significantly shorter latency to eat than sham-injected rats during the first phase (Fig 2A) ($F_{0.5(2,25)} = 5.4$). It must be noted that eating latency was defined as the time between the ultimate retrieval to the start of eating. Both sham- and formalin-injected rats occasionally retrieved a chocolate chip, dropped it, and then retrieved the chip again some time later. Fewer sham-injected rats (2 of 8) displayed this behavior than rats injected with 0.5% (5 of 9) or 1.5% (5 of 10) formalin. Retrieval latencies from the time of first retrieval to the start of eating were the same between the groups. Although rats injected with 1.5% formalin displayed some pain behaviors during eating, they took the same time to eat each chip (Fig. 2B), showed similar interruptions and scanning during eating bouts (data not shown), and ate the same number of chips (Fig. 2C) as rats in the other two groups.

Discussion

Our results show that eating persists even in the presence of persistent pain in *ad lib* fed male rats. Rats injected with 1.5% formalin that exhibited pain behaviors during 40% of the testing time ate the same number of chips and took the same time to do so as rats that displayed pain behaviors for only 1% of the test time or those that showed no pain behaviors at all. The similar eating patterns in animals with very different expressions of pain held despite the fact that a quarter of the time that the 1.5% formalin-injected rats were eating, they also behaved as though they were feeling pain. The most prevalent pain behavior occurring concurrent with eating was favoring the injected paw. Rats were less likely to eat when the pain level was presumably higher as indicated by their lifting of the injected paw. Because it is impossible for a rat to paw lick and eat at the same time, it is not surprising that there were no instances of such concurrent behaviors.

Although the present results show that rats continue to eat even in the presence of moderate and persistent pain, rats are likely to stop eating when pain intensity is severe enough. In a pilot study ($n = 2$), we observed that rats injected with 2.5% formalin licked and bit their paws nearly continuously during the first phase and consequently, ate very few chocolate chips during that time. Thus, it is likely that feeding overrides pain below a certain sensory threshold whereas attending to the injured paw must take priority over feeding above that threshold. In light of our previous finding that withdrawals are delayed or suppressed entirely during eating [13], it appears that pain behaviors are modulated during eating by the active suppression of nociceptive input, a suppression that is only discontinued when conditions pose a serious threat or injury to the animal.

We found that rats injected with formalin began eating faster during the first phase of formalin than did sham-injected rats. Since the first phase of formalin pain is sensitive to opioid receptor antagonists [11,12], the presence of opioids which are well known to increase palatability [19–21] may function to expedite eating during the initial phase of formalin pain.

Unlike previous research [22], 0.5% formalin did not evoke a biphasic pain response. It is unlikely that ingestion of sucrose in the chocolate during formalin testing produced an analgesic effect because sucrose-induced analgesia is present only in infant rats [23–25]. However, it is possible that daily consumption of chocolate prior to testing may have altered responses to formalin since chronic consumption of sucrose alters morphine-induced analgesia [26–34]. Further, it is also possible that either the availability of food or eating itself produces analgesia. The latter possibility is supported by the analgesic effects of eating observed in food-deprived [35,36] and freely fed [13] animals tested for pain responses to acute noxious heat as well as by the analgesic effects of non-nutritive suckling in human babies [37,38]. Thus, both dietary changes and feeding-associated analgesia may account for the greatly reduced effect of 0.5% formalin in the present study compared to previous reports [22].

The present results stand out against reports of decreased eating in chronic pain patients and in freely-fed rats with a model of migraine pain [1–3]. Our findings may differ from widely reported hypophagia in chronic pain patients since we tested rats only once. It is possible, even likely, that chronic pain of longer duration (days, weeks, months) may affect feeding as it does among humans with chronic pain [1,2]. Differences between our findings and those reported in a rat model of migraine pain are likely due to a heightened oral sensitivity produced by dural stimulation.

The present study is part of an on-going project that examines the interaction between eating and pain and the role of raphe magnus cells in modulating ingestion-induced analgesia [13]. Since raphe magnus cells have only been physiologically characterized in male rats [39], we focused on studying feeding behavior during persistent formalin pain in male rats. Because there are sexual differences in pain responses to formalin and in feeding behaviors [40,41], it should be noted that the present findings do not necessarily generalize to female rats.

In conclusion, the present results show that eating is a protected behavior in the presence of persistent pain to the hind limb in *ad lib* fed male rats. The persistence of feeding even in the face of moderate pain underscores the immense need, and thus value of, ingesting food. Under natural conditions, a scarce and/or unpredictable food supply may have driven natural selection to favor mechanisms that ensure that animals feed whenever possible. Feeding analgesia is likely one mechanism that aids in guaranteeing that animals will eat when food is available.

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Figure 1.

Proportion of time that rats favored, lifted, and licked their injected paw during the hour following an injection of formalin. **(A)**. Rats injected with 0.5% formalin showed very few pain-related behaviors. **(B)**. Rats given 1.5% formalin displayed a biphasic pain response, with the first phase occurring within 5 minutes and the second phase peaking 30 minutes after injection. Rats injected with 1.5% formalin spent significantly more time favoring, lifting, and licking their paw than rats injected with 0.5% formalin.

Figure 2.

Eating behaviors in sham- and formalin-injected rats during the first 10 minutes (0 – 10) and the next 50 minutes (10 – 60) after a sham or formalin injection to the hind paw. (A). Mean latency to eat. The time taken from the ultimate retrieval of a chocolate chip to the start of eating was significantly faster in formalin-injected rats than in sham-injected rats during the first phase of formalin pain (*). (B). Average time taken to eat each chip. Formalin-injected rats took the same amount of time to eat each chip as sham-injected rats during either phase of formalin pain. (C). Mean number of chocolate chips eaten. Rats injected with formalin ate the same number of chips as sham-injected rats during the first and second phases of formalin pain.