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Emotional fever after habituation to the temperature-recording procedure

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Abstract

To examine whether habituation to having temperatures taken might reduce the emotional fever induced by other stressors, 20 rats were habituated by having three colonic temperatures taken within 6 min twice a week for 8 weeks. Two novel stressors were added during Week 9; rats were given an intraperitoneal saline injection before taking their temperatures on Day 1, and on a second day, they were housed with a group of unfamiliar animals for 5 min before taking their temperatures. Temperatures during Weeks 10–11 were taken as during Weeks 1–8. Results showed that, overall, the third daily temperature averaged 1 °C higher than the first, indicating that taking temperatures was stressful. Between weeks, temperatures rose between Weeks 1 and 2, suggesting a conditioned fever. Temperatures fell 1.5 °C from Weeks 2–8, indicating habituation. During Week 9, temperatures after the intraperitoneal injection were no higher than during Week 8; however, 5 min of group housing raised temperatures 1.5 °C for males and 2.5 °C for females. Temperatures during Weeks 10 and 11 were below those of Week 8, suggesting that the fever induced by group housing in Week 9 was situation specific. These results indicate that rats habituated to having their temperatures taken exhibit emotional fever only to selected new stimuli. Body temperatures do not rise after an intraperitoneal injection given by a familiar handler, but briefly placing rats with unfamiliar animals induces a substantial febrile response.

Keywords: Body temperature; Conditioned fever; Emotional fever; Group housing; Habituation; Intraperitoneal injection; Sex differences; Stress

1. Introduction

Gently handling or otherwise disturbing rats and other laboratory animals causes them to rapidly develop an emotional fever. For example, picking an animal up, measuring its colonic temperature, administering an intraperitoneal injection, immobilizing it, or moving it to a new environment typically increases central temperature by 1–2 °C within 10 min [1–10]. Similarly, when the rectal temperatures of several animals in the same room are taken sequentially, the temperatures of the animals taken last are significantly higher than those of the animals taken first [11,12].

Stress-induced increases in body temperature appear to reflect a fever or rise in thermoregulatory “set point” rather than a rise above the set point. Rats housed in a cold environment exhibit the same stress-induced increase in body temperature as rats kept at temperatures within their thermoneutral zone [1,3], and administering the antipyretic drug sodium salicylate blocks a stress-induced rise in temperature in species as diverse as rats [10,13], ducks [8], and lizards [9].

Previous research indicates that stress-induced fever can be classically conditioned, such that temperatures after the second exposure to a stressor are higher than they were following initial exposure [14]. On the other hand, animals that are sufficiently habituated to a procedure that initially is stressful may eventually exhibit a reduced hyperthermic response to that stressor [15].

The phenomena of conditioned fever and habituation typically have been demonstrated by repeatedly exposing animals to the same situation. The degree to which habituation to the temperature-recording procedure affects emotional fever to new stimuli is not well understood. In one previous study, rats habituated to having their temperatures taken nevertheless exhibited a dramatic increase in temperature on a day that a cat was held nearby during the recording procedure, prompting the investigators to conclude that novel stimuli cause the febrile response to reappear in habituated animals [7]. In the present study, we extended this procedure by investigating whether rats that have been habituated to having their temperatures taken

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will subsequently exhibit emotional fever in response to an intraperitoneal saline injection or to being housed with unfamiliar rats of the same gender for 5 min. If habituated rats do not exhibit an emotional fever to either or both of these stimuli, this would suggest that the effects of habituation to the temperature-taking procedure may generalize to situations containing a substantial novel component.

2. Method

2.1. Subjects

Twenty Sprague–Dawley albino rats (10 male and 10 female) were acquired from Harlan (Indianapolis, IN). Animals were 58 days old at delivery and were individually housed in standard wire cages. Water and food (Harlan Teklad Rodent Diet #8604) were provided ad libitum. The laboratory was kept at 22 °C with a 12:12-h light–dark cycle (lights on at 0700 h).

2.2. Apparatus

Rats were weighed using a triple beam balance. Colonic temperatures were determined to an accuracy of 0.1 °C using a Bailey Instruments (Saddlebrook, NJ) model BAT-12 digital thermometer, with the thermistor probe inserted 50 mm into the rectum.

2.3. Procedure

On the Monday, 6 days after delivery to our laboratory, the 64-day-old rats were weighed and a single temperature was taken. On Tuesday and Thursday of that week, one group of 10 animals (5 males and 5 females) had their temperatures taken three times during a 6-min period, at 2-min intervals. The second group of 5 males and 5 females had their temperatures taken in like manner during the 6 min immediately following. Temperatures were taken at approximately 1300 h.

The weekly procedure described above was repeated for seven additional weeks (Weeks 2–8), with the exception that no Monday temperatures were taken after the first week. In addition, beginning with Week 3, the two groups of 10 animals were alternated so that during each week, both groups of rats had their temperatures recorded first on one test day and second on the other. This allowed an assessment of whether any elevation in body temperature occurred for rats that were tested immediately after taking the temperatures of 10 other animals.

The ninth week was designated as "stress" week during which rats were exposed to two novel procedures. Animals were weighed as usual on Monday. The first novel procedure occurred for half the rats on Tuesday and for the remaining half on Thursday. On each of these days, five males and five females had a single temperature taken immediately after which they received an intraperitoneal injection of isotonic saline in a volume of 0.25 ml/kg body weight. Two additional temperatures then were taken: the first, 2 min after the intraperitoneal injection and the second, 2 min later. On Saturday, all 20 rats were exposed to a second novel procedure. Same-sex groups of five rats were placed together in a group cage and taken to an adjacent room. Beginning 5 min after entry into the group cage, the rats had three temperatures taken at 2-min intervals.

During Weeks 10 and 11, rats were weighed as usual on Mondays. On Tuesdays and Thursdays, all rats had three temperatures taken at 2-min intervals, as described above for Weeks 3–8.

2.4. Statistical analyses

Data were analyzed using analysis of variance (ANOVA) procedures conducted with the BMDP 2V statistical program (Release 7.0). This program allows factors involving repeated measures (e.g., three daily temperatures, 11 test weeks) to be divided into orthogonal components, permitting examinations of linear, quadratic, cubic, and higher order trends in the data. When significant higher order trend components (i.e., beyond cubic) involving test weeks indicated that a comparison of temperatures during adjacent weeks would be useful, subsequent ANOVAs were used to compare adjacent pairs of weeks. All data are reported as means ± S.E. $P \leq .05$ were considered statistically significant.

3. Results

3.1. Body weight

Body weight increments across test weeks and sex differences in weight were evaluated using a two-factor ANOVA, with the 11 test weeks as a within-group factor and the two sexes as a between-group factor. The Sex × Weeks interaction was statistically significant, $F(10,180) = 2{,}781.63$, $P < .0001$, as was the linear component of this interaction, $F(1,18) = 134.05$, $P < .0001$. At 64 days of age, male rats weighed an average of 279.3 ± 1.9 g and they weighed 418.2 ± 6.7 g 10 weeks later, at 134 days. Females weighed an average of 203.8 ± 2.9 g at 64 days and 268.4 ± 2.9 g at 134 days. As expected, males weighed more than females during all test weeks ($P < .0001$) and they gained weight more rapidly than females during the 11-week period.

3.2. Temperature: differences within days, between sexes, and across weeks

The overall analysis of body temperature (excluding differences between the first and second 10 animals on any given day) utilized a S(Sex) × Order × Weeks ANOVA.

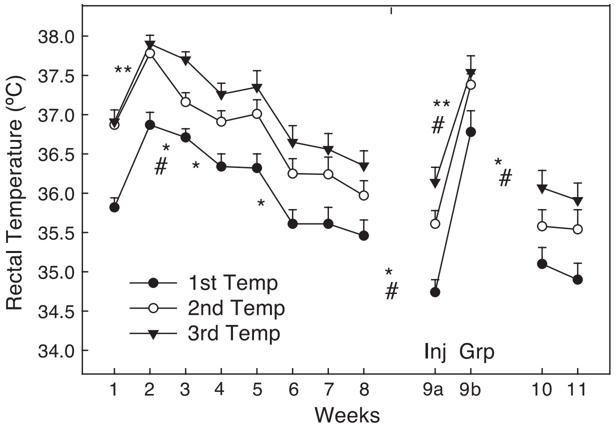

Fig. 1. A significant Order × Weeks interaction ($P < .01$) between the mean temperatures (± S.E.) taken first, second, and third (order) during a 6-min period across 11 test weeks. * * Combined temperatures rose significantly between these two adjacent weeks. * Combined temperatures dropped significantly between these 2 weeks. #A significant Order × Weeks interaction also occurred between these 2 weeks.

Sex (male, female) was a between-group variable, whereas order (first, second, and third daily temperatures) and weeks (average temperatures across Weeks 1–11) were within-group variables.

The order main effect was significant, $F(2,36) = 383.13$, $P < .0001$, and the linear and quadratic components of the effect were significant, $F(1,18) = 425.10$, $P < .0001$ and $F(1,18) = 102.11$, $P < .0001$. The first daily temperature averaged 35.8 ± 0.1 °C, the second averaged 36.5 ± 0.1 °C, and the third averaged 36.8 ± 0.1 °C. Thus, body temperature increased approximately 1 °C during the 6 min that the three daily temperatures were taken, with the greatest increase (0.7 °C) occurring between the first and second temperatures.

3.2.1. Order × Weeks interaction

The Order × Weeks interaction was significant, $F(22,396 = 2.64$, $P < .01$), and this interaction is shown in Fig. 1. Numerous higher level components involving weeks were significant, and so individual ANOVAs were used to assess the effects of order and sex between successive weeks. In Fig. 1, adjacent weeks during which overall temperatures rose significantly are marked with "* * " and it can be seen that this occurred twice. The first such increase occurred between Weeks 1 and 2; this likely reflects a conditioned emotional response during Week 2 that was formed on the basis of the Week 1 experience. The second temperature increase occurred between Weeks 9A (injection) and 9B (group housing), indicating that a greater fever was generated by group housing than by an intraperitoneal injection. Adjacent weeks during which overall temperatures fell significantly are marked with " * " in Fig. 1. These decreases occurred between Weeks 2 and 3, 3 and 4, 5 and 6, 8 and 9A, and 9B and 10. It is apparent that decreases in body temperature occurred at a fairly steady pace starting with Week 3, with the only reversal of this downward trend occurring during Week 9B, when the temperatures of animals housed in groups increased to a level as high as they had been in Week 2. It is noteworthy that body temperatures did not increase in response to the intraperitoneal injection in Week 9A. It was not expected that the first of the three daily temperatures in 9A would increase since this measurement was taken prior to the injection, but it is evident from Fig. 1 that the second and third temperatures, obtained after the injection, also were no higher than they had been during Week 8.

Adjacent pairs of weeks in which the Order × Weeks interaction was significant are marked with "#" in Fig. 1. The figure shows that when emotional fever was greatest—during Weeks 2 (conditioned emotional response) and 9B (group housing)—the second daily temperature more closely approximated the third temperature than it did on days when the febrile response was less pronounced. During Week 10, the second daily temperature dropped to almost the midpoint between the first and third temperatures, accounting for the interaction between order and Weeks 9B and 10. Finally, although the three combined temperatures fell significantly between Weeks 8 and 9A, the intraperitoneal injection in Week 9A nevertheless raised the second daily temperature above the first to a greater degree than had occurred during Week 8.

3.2.2. Sex × Week interaction

The sex main effect was significant, $F(1,18) = 156.02$, $P < .0001$, with females exhibiting temperatures that averaged 1.3 °C higher than those of males (37.0 ± 0.1 and 35.7 ± 0.1 °C) over the 11-week period. The sex factor also interacted significantly with weeks, $F(11,198) = 9.03$, $P < .0001$, and this interaction is shown in Fig. 2.

The Sex × Weeks interaction contained a significant linear component, $F(1,18) = 41.40$, $P < .0001$, and inspec-

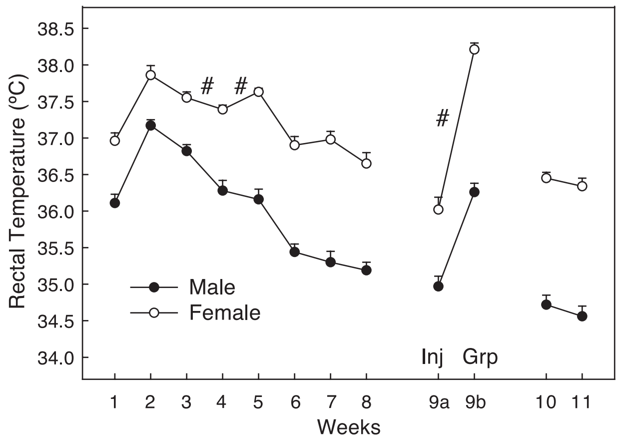

Fig. 2. A significant Sex × Weeks interaction ($P < .0001$) in the mean body temperatures (± S.E.) of male and female rats tested across 11 weeks. #A significant Sex × Weeks interaction also occurred between these adjacent weeks.

tion of Fig. 2 indicates that although both males and females exhibited a reduction in core temperature as habituation continued, the reduction was greater for males than for females. The interaction also contained several nonlinear components, and so individual ANOVAs were used to assess sex differences between successive weeks. Adjacent weeks during which a significant Sex × Week interaction occurred are marked with "#" in Fig. 2. The interaction between Weeks 3 and 4 indicates that habituation caused a greater temperature decrease in males than in females. Between Weeks 4 and 5, male temperatures continued to decrease, whereas female temperatures actually increased somewhat. The interaction between Weeks 9A and 9B indicates that the stress of group housing raised female temperatures to a greater degree than in males.

3.3. Temperature differences between first and second 10 rats

"Session-half" effects assessed the effects of having temperatures taken with either the first or second group of 10 animals (sexes balanced) each day. These effects were assessed beginning with Week 3, from which time the temperatures of each group of 10 rats were taken first once, and second once, each week. These temperature data were evaluated using an S(Sex) × Half session × Order × Weeks ANOVA. Sex (male, female) was a between-group factor and session half (first or second group tested), order (first, second, and third daily temperature), and weeks (3–11, excluding Week 9) were within-group factors. Data collected after the intraperitoneal injection during Week 9 could not be assessed for session-half effects because only 10 animals received the intraperitoneal injection on each of the two test days so that all rats essentially were tested "first" on both days. Data following 5 min of group housing during Week 9B also were not included in this analysis because the procedure differed dramatically from that used during "nonstress" tests, and it seemed desirable to assess session-half effects under test conditions that remained relatively constant.

The session-half main effect was significant, $F(1,18) = 43.98$, $P < .0001$. Fig. 3 shows that rectal temperatures averaged 0.7 °C lower when the rats were among the first 10 to have their temperatures taken on a given day compared to when they were among the second 10.

The sex main effect was significant, $F(1,18) = 132.30$, $P < .0001$. Female body temperatures during Weeks 3–8 and 10–11 averaged 1.4 °C, higher than those of their male counterparts (36.9 ± 0.1 °C vs. 35.5 ± 0.1 °C).

The order main effect was significant, $F(2,36) = 651.16$, $P < .0001$, with significant linear and quadratic components, $F(1,18) = 761.37$, $P < .001$ and $F(1,18) = 60.52$, $P < .0001$. The first temperature recorded on any given day averaged 35.7 ± 0.1 °C, the second temperature averaged 36.3 ± 0.1 °C, and the third averaged 36.7 ± 0.1 °C. Thus, the rats exhibited a steady increase in average temperature across the 6 min of each daily test period, although the 0.6 °C increase between the first and second temperatures was somewhat greater than the 0.4 °C increase between the second and third temperatures.

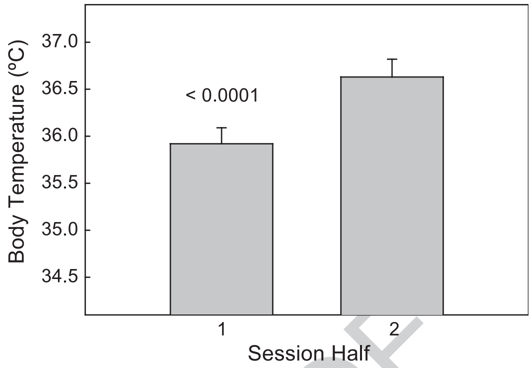

Fig. 3. Differences in mean colonic temperatures (± S.E.) for rats that were among the first or second 10 animals to be assessed on a given day (session half 1 and 2). Session-half effects were measured during Weeks 3–8 and 10–11 (Week 9 was excluded). Three temperatures were taken in 6 min during each session half.

The weeks main effect was significant, $F(7,126) = 104.80$, $P < .0001$, with significant linear and quadratic components, $F(1,18) = 486.32$, $P < .0001$ and $F(1,18) = 9.69$, $P < .01$. Overall means (°C ± S.E.) were as follows: Week 3 = 37.1 ± 0.1, Week 4 = 36.8 ± 0.1, Week 5 = 36.9 ± 0.1, Week 6 = 36.1 ± 0.1, Week 7 = 36.1 ± 0.2, Week 8 = 35.9 ± 0.1, Week 10 = 35.6 ± 0.2, and Week 11 = 35.4 ± 0.2. It is apparent that average body temperatures decreased steadily beginning with Week 3 (ignoring Week 9), except for a small increase of 0.1 °C between Weeks 4 and 5.

4. Discussion

The present results replicate and extend several earlier findings. We clearly replicated the finding that emotional fever occurs in rats [1–3,6,7,10,13,16,17], a phenomenon that also has been observed in a variety of other species including mice [5,11,12,15,18,19], rabbits [20], lizards [9], ducks [8], and humans [21–23]. Perhaps, the most direct evidence for emotional fever was the observation that rectal temperatures increased approximately 1 °C during the 6-min period that the three daily recordings were made. This degree of fever is well within the 1–2 °C increase that has been reported to occur within 10 min following the onset of an assortment of "stressful" handling procedures in the studies listed above.

The present findings also extend an observation previously reported in mice [11,12] that increased body temperature can be induced in animals, which are not handled directly but that are merely in the same room where other animals are having their temperatures taken. Daily temperatures averaged 0.7 °C higher when rats had their temperatures recorded with the second group of 10 animals rather than with the first group.

Previous investigators have reported that rats develop conditioned increases in body temperature in response to stimuli that are predictive of handling [14] or of being moved from their home cage shelves to a laboratory bench [17]. In the present study, it seems likely that a conditioned febrile response accounted for the increased body temperatures that were evident during the second week of testing, relative to those obtained during the first week. In fact, average body temperatures were somewhat above Week 1 levels until the sixth week of testing.

Starting with the third week of testing, however, any conditioned fever was progressively diminished by habituation to the temperature-recording procedure, and from Week 6 onward, body temperatures fell increasingly below Week 1 levels. By Week 11, average temperatures were 1.1 °C lower than they had been during Week 1. This reduction in body temperature is in accord with previous reports that habituation to stressful procedures reduces the emotional febrile response of rats [7] and mice [5].

In a previous study, investigators reported that rats that had been habituated for several weeks to having their temperatures taken nevertheless exhibited an abrupt rise in body temperature on a day when a cat was held nearby while temperatures were taken [7]. These investigators concluded that novel stimuli cause emotional fever to reappear in rats that have been habituated to the temperature-taking procedure. The results of the present study indicate that not all novel stimuli cause fever to reappear in habituated animals. Certainly, body temperatures increased dramatically within minutes when rats were housed for the first time with four other same-sex animals, even though all rats were habituated to having their temperatures taken. On the other hand, giving the rats an intraperitoneal saline injection after 8 weeks of habituation did not cause their temperatures to rise above the level recorded during the week prior to the injection. In the present study, we did not demonstrate that intraperitoneal injections increase body temperatures in nonhabituated animals. However, the injection procedure certainly was a "new" stimulus, and others have reported that intraperitoneal injections raise the body temperatures of nonhabituated rats [6] and that oral injections raise the temperatures of nonhabituated mice [5]. Therefore, we conclude that when rats are habituated to having their rectal temperatures taken, they do not exhibit a temperature increase to all new stimuli and particularly not to an intraperitoneal injection that is administered by the same handler who previously has taken their temperatures.

It is noteworthy that the increased temperature in response to group housing during Week 9 did not interrupt the progress of habituation to the conditions preceding and following that week. Temperatures during Weeks 10 and 11 were lower than during Week 8, indicating that the emotional fever induced by group housing in Week 9 was temporary and that introduction of a stressful stimulus during Week 9 did not disrupt habituation to the testing that characterized Weeks 1–8 and 10–11.

In all phases of the study, body temperatures of female rats averaged more than 1 °C higher than those of males. Female rats are more active and have higher metabolic rates than males, and it is possible that this accounts for their higher body temperatures. It seems unlikely that the higher temperatures of the 10 females were associated with a particular phase of the estrus cycle since temperatures were taken at 2- and 5-day intervals (Tuesdays and Thursdays) for 11 weeks, and the length of the rat estrus cycle is 4–5 days.

Compared to males, female rats may exhibit a greater degree of emotional fever that habituates relatively slowly. In this study, the rate that body temperatures declined during habituation was slower for females than for males and group housing raised body temperatures an average of 2.5 °C for females, which was a full degree more than occurred for males. It is possible that sex differences in activity and metabolic rate interact with emotional fever and that male–female differences in temperature would disappear if such factors were controlled. Whatever the explanation, however, the stress of group housing clearly elevates body temperatures to a greater degree in females than in males, and the emotional fever associated with taking colonic temperatures habituates more slowly in females than in males.

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