There's a Rat in My Room! Now What? Mice Show No Chronic Physiological Response to the Presence of Rats

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There’s a Rat in My Room! Now What?
Mice Show No Chronic Physiological Response to the Presence of Rats

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In general, guidelines on housing and care of animals in the laboratory state that rats and mice should not be housed in the same room. Mice may perceive rats as predators. Although one theory says this can cause stress, there is little scientific evidence to support this theory. In the wild, rats and mice usually do not share the same microhabitat, but this appears to be true for most small rodent species. Furthermore, reports of predatory behavior of rats toward mice mainly originate from experimental settings using rats with high inbred levels of aggression. This experiment measured heart rate (HR), body temperature (BT), activity (AC), and urinary corticosterone in female C57BL/6 mice before, during, and after introducing Wistar rats into their room. The study found no chronic effects of rat introduction on any parameters. The study concluded that housing rats and mice in the same room is at least less disturbing than cage cleaning, which caused a temporary increase of HR, BT, and AC. Current results do not support legislation based on compromised welfare.

Guidelines on the housing and care of animals in the laboratory state that rats and mice should not be housed in one and the same nonhuman animal room (Dutch Inspectorate for Health Protection and Veterinary Public Health, 1998).

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The basis of this statement, however, is not clear. Article 1.2.4 of the European guidelines of 1986 (Council of Europe, 1986) indicate that “care should be taken not to house together incompatible species”; it has been argued that this should be the case for rats and mice, as gastric ulcers had been occasionally reported in rats housed in the same room with mice (Dutch Inspectorate for Health Protection and Veterinary Public Health & Dutch Association for Laboratory Animal Science, 1989). However, no reference has been made to any supporting scientific evidence of this gastric ulceration in rats.

Today, still no evidence has been found for these possible gastric ulcers in rats. It is often assumed that the guidelines were implemented because of welfare issues concerning mice. Mouse may be frightened by the presence of rats, as it is sometimes said that rats are predators of mice (Calvo-Torrent, Brain, & Martinez, 1999). However, this argument is somewhat controversial. Although there are studies in which rats (the so-called killer rats) were observed killing mice (Hsuchou et al., 2002), these studies were performed in laboratory settings using aggressive (inbred) rat strains and often involved experimentally induced muricidal behavior (Bac et al., 2002; Ho, Chen, Tai, & Tsai, 2004; Hsuchou et al., 2002).

To our knowledge, there has been no evidence of mice being a regular prey for rats in the wild, and Sage (1981) did not refer to it in his extensive review on wild mice. In his review, Sage paid attention to interspecific interactions between wild house mice and other small rodents, including rats. He concluded that there was a substantial body of mainly anecdotal information saying that house mice frequently had negative interactions with other rodent species and that the nature of these negative interactions most frequently involved not only nest sites but also possible competition for food resources. Rats and mice did not appear to share the same microhabitat in the wild; however, this was also true for house mice in combination with other rodents such as voles and other mouse species (Sage, 1981). Hence the question remains: Does avoiding each other in the wild mean that sharing a room in confinement is stressful and a definite challenge to welfare? Therefore, research is required to provide evidence-based legislation with respect to the communal housing of rats and mice.

Some experiments were already performed in this field. Mice who lived for 7–14 days in a room that contained rats showed an increase in sympathetic neurotransmitter release, an effect that was even bigger when the mice were individually housed (D’Arbe, Einstein, & Lavidis, 2002). Activation of the sympathetic nervous system can be considered a sign of stress. In addition, circadian rhythms of heart rate and activity were found to be disturbed in a subsequent experiment (I. Chin, personal communication, February 13, 2006). Furthermore, chronic exposure (21 days) to rats was found to reduce reward sensitivity and (in male mice) prevent habituation to the plus-maze (Calvo-Torrent et al., 1999), which can also be considered signs of stress.
Other studies demonstrating stress in mice when confronted with the presence of rats were usually performed not to establish the effect of communal housing but merely to investigate the effect of rats as an acute stressor. As a result, these studies involved only short-term exposures to rats and, more important, rats being placed in very close proximity to the mice, literally on top of their cages (Grootendorst, De Kloet, Dalm, & Oitzl, 2001; Grootendorst, De Kloet, Vossen, Dalm, & Oitzl, 2001) or even within their cages behind a Perspex wall (Linthorst, Flachskamm, Barden, Holsboer, & Reul, 2000). The latter, however, has also been demonstrated to be stressful in mice during postoperative recovery when (familiar) mice were placed behind a grid partition in the cage (Van Loo et al., 2007).

To gain more insight into the effects rats may have on mice when housed in the same animal room, we performed a pilot study in which we recorded heart rate (HR), body temperature (BT), and general activity (AC) patterns by means of radiotelemetry and measured urinary corticosterone (CORT) in female C57BL/6 mice (surplus from a previous experiment) before and during a 2-week exposure to rats in their room.

MATERIALS AND METHODS

All procedures were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of Utrecht University.

Nine female mice of the C57BL/6Jico inbred strain (Charles River, Maastricht, The Netherlands) had been housed at the facility from the age of 3 weeks. Mice and rats were not routinely housed together at the vendor. The mice were housed in a conventional animal room (temperature 18–24°C) with a 12/12 h light/dark cycle (lights on: 0700h, light intensity at shelf level about 100 lux, during lights off: 2 lux). A radio was on during the light phase. The mice were housed in elongated Makrolon® II cages (floor area 530 cm²) provided with sawdust bedding (Lignocel® 4, Rettenmaier&Sohne, Ellwangen-Holzmühle, Germany), two Kleenex® tissues (Kimberly Clark, Ede, The Netherlands), food pellets (CRM-E, SDS, Witham Essex, United Kingdom), and tap water ad libitum. Each experimental mouse was socially housed together with two nonexperimental female cage mates. All mice were surplus from a previous experiment.

At the age of 12 weeks (body weight 19.8 ± 0.2 g), the mice had been implanted with radiotelemetry transmitters (TA10ETA-F20, DataSciences International, St. Paul, MN). Meijer, Spruijt, Van Zutphen, and Baumanns (2006) provide details on the surgical procedure and postoperative care. After transmitter implantation, the mice had been used in an experiment in which they were subjected to repeated trials of restraint by hand for approximately 10 s. During
this period, the mice had been individually housed. At the time of testing of the current experiment, there were no indications that the physiological parameters as used in the study, based on their baseline values, were still affected by the previous experiences of the mice.

Nine weeks after the end of the first experiment, when the mice were about 6 months old, they were confronted with three cages of adult male and female Wistar rats (Charles River, Maastricht, The Netherlands) who had been living at the facility from early age onward and were normally used for educational purposes. According to the routinely monitored health status of both rats and mice, following Federation of European Laboratory Animal Science Associations (FELASA) guidelines (Rehbinder et al., 1996), there was no risk of disease transmission between the two species. The day the rats were introduced into the mouse room is referred to as Day 0. There were two cages housing a group of 4 female rats each and a third cage housing a group of 4 males. The cages were introduced at 11.45h and were positioned on a table about 1.5 m away from the shelves where the mouse cages were standing; thus, the mice could receive olfactory, auditory, and (possibly) visual stimuli from the rats. The rats remained in the room for a period of 2 weeks during which all usual procedures of an animal laboratory such as daily animal checking and weekly cage cleanings were performed (cage cleaning on Days 8 and 14 by transferring the animals from their soiled cage to a clean one). On Day 15 at 13.30h, the rats were removed from the room.

By means of the radiotelemetry system, HR, BT, and AC data were collected 24 hr a day, starting 4 days prior to the arrival of the rats. Transmitter signals were sent to a PC and saved to disk; data acquisition and analysis were performed using Dataquest A.R.T. Version 2.3 (DSI, St. Paul, MN). In addition, urine was collected from the mice three times on Monday mornings at 9.00h to measure urinary CORT levels. The first collection was on the morning just prior to the arrival of the rats (Day 0) in order to assess a baseline value. Urine collections were repeated twice on the following Mondays, 1 and 2 weeks after the rats had been housed in the animal room (Days 7 and 14). To collect their urine, the mice were placed individually in plastic buckets (1.1 litre volume; Emergo, Landsmeer, The Netherlands) provided with a plastic salad dish (250 cc, Depa®, Veriplast BV, Apeldoorn, The Netherlands). The mice who did not urinate spontaneously were picked up and restrained by the scruff and the base of the tail; if necessary, the bladder was gently massaged until the mouse urinated. It was possible to collect urine of all mice in 15 min using this modified method of Dahlborn, Van Gils, Van de Weerd, Van Dijk, and Baumans (1996) and Van Loo, Mol, Koolhaas, Van Zutphen, and Baumans (2001). Urine was collected with a 1 ml syringe and stored in polypropylene tubes at −20°C. CORT levels were measured using a solid-phase $^{125}$I radio immunoassay (CAC® Rat Corticosterone TKRC1, Diagnostic Products
Corporation, Los Angeles, CA). Creatinine concentrations, indicative for the dilution of urine, were determined with the use of a commercial test combination (ABX Diagnostics, Montpellier, France) on a COBAS-MIRA-S autoanalyzer (ABX, Montpellier, France).

Statistics

Normal distribution of all data was established using the 1-sample Kolmogorov-Smirnov test.

Hourly averages of HR, BT, and AC of three 4-day periods were calculated (around rat introduction from Day −1 to Day 2, 1 week later from Day 6 to Day 9, and another week later around rat removal from Day 13 to Day 16). Correlations between Day −1 and the other days within these three periods as well as correlations between other days of interest were calculated using a Pearson’s correlation. Bonferroni correction was applied because of multiple tests; correlations were considered statistically significant when \( p < .002 \). Telemetry data are presented as mean values ± standard error of the mean.

CORT/creatinine ratios were analyzed using a Linear Mixed Effect Model with Week as fixed effect and Mouse ID as random effect. Differences were considered statistically significant when \( p < .05 \). CORT/creatinine data are presented as median values with interquartile ranges and highest and lowest nonoutlying values. All statistical analyses were performed in SPSS for Windows, release 12.1.0.

RESULTS

Figures 1–3 represent HR (top), BT (middle), and AC (bottom) of the mice in the three time periods, averaged per hour. Within-day, HR, BT, and AC variation typically showed a circadian rhythm with highest levels during the dark period (represented by the dark horizontal bars). In Tables 1 and 2, Pearson correlation coefficients are listed for HR, BT, and AC. For all correlations, \( \alpha = 0.002 \) was used to assess significance. HR, BT, and AC measurements on all days after rat introduction correlated significantly with measurements on Day −1 \( (p < .001) \) with the exception of Days 0, 8, and 14 (Table 1). On Days 0, 8, and 14, the cages of the mice had been cleaned, resulting in a sharp rise of all three parameters immediately following cage cleaning. HR, BT, and AC on these days significantly correlated with each other \( (p < .001) \) with the exception of AC between Days 8 and 14 (Table 2). Within the second time period (1 week after rat introduction, Figure 2), HR, BT, and AC on Days 6, 7, and 9 correlated with each other \( (p < .001) \). Furthermore, HR and BT on Day 7 (urine collection) correlated with Day 8 (cage cleaning; \( p_{HR} < .004, p_{BT} < .001 \).
FIGURE 1  Day −1 until Day 2. Mean (±SEM) values per hour of heart rate (top panel), body temperature (middle panel) and activity (bottom panel) of 9 female mice from Day −1 until Day 2 of the experiment. The separate days are indicated by vertical lines, black horizontal bars indicate the dark periods, gray shaded area indicates the period before the rats were introduced into the animal room. Correlations are listed in Tables 1 and 2. SEM = Standard Error of the Means.
FIGURE 2. Day 6 until Day 9. Mean (±SEM) values per hour of heart rate (top panel), body temperature (middle panel), and activity (bottom panel) of 9 female mice from Day 6 until Day 9 of the experiment, when rats were present at all times. The separate days are indicated by vertical lines, black horizontal bars indicate the dark periods. Correlations are listed in Tables 1 and 2. SEM = Standard Error of the Means.
FIGURE 3  Day 13 until Day 16. Mean (±SEM) values per hour of heart rate (top panel), body temperature (middle panel), and activity (bottom panel) of 9 female mice from Day 13 until Day 16 of the experiment. The separate days are indicated by vertical lines, black horizontal bars indicate the dark periods, gray shaded area indicates the period after the rats were removed from the animal room. Correlations are listed in Tables 1 and 2. SEM = Standard Error of the Means.
### TABLE 1
Correlations Between HR, BT, or AC Compared With Day −1, as Indicated by Pearson’s $\rho$

<table>
<thead>
<tr>
<th>Day:</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>-.064</td>
<td>.918*</td>
<td>.886*</td>
<td>.891*</td>
<td>.774*</td>
<td>.200</td>
<td>.853*</td>
<td>.886*</td>
<td>.038</td>
<td>.773*</td>
<td>.723*</td>
</tr>
<tr>
<td>BT</td>
<td>.453</td>
<td>.964*</td>
<td>.969*</td>
<td>.964*</td>
<td>.884*</td>
<td>.508</td>
<td>.973*</td>
<td>.969*</td>
<td>.452</td>
<td>.949*</td>
<td>.936*</td>
</tr>
<tr>
<td>AC</td>
<td>.018</td>
<td>.945*</td>
<td>.940*</td>
<td>.876*</td>
<td>.835*</td>
<td>.005</td>
<td>.928*</td>
<td>.888*</td>
<td>.064</td>
<td>.857*</td>
<td>.852*</td>
</tr>
</tbody>
</table>

*Note.* Bonferroni $\alpha = 0.002$. HR = heart rate. BT = body temperature. AC = activity.

* $p < .002$.

### TABLE 2
Correlations Between HR, BT, or AC on Different Days, as Indicated by Pearson’s $\rho$

<table>
<thead>
<tr>
<th>Days:</th>
<th>0–8</th>
<th>0–14</th>
<th>8–14</th>
<th>6–7</th>
<th>6–8</th>
<th>6–9</th>
<th>7–8</th>
<th>7–9</th>
<th>8–9</th>
<th>13–14</th>
<th>13–15</th>
<th>13–16</th>
<th>14–15</th>
<th>14–16</th>
<th>15–16</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>.684*</td>
<td>.649*</td>
<td>.621*</td>
<td>.745*</td>
<td>.191</td>
<td>.822*</td>
<td>.569**</td>
<td>.671*</td>
<td>.224</td>
<td>.183</td>
<td>.815*</td>
<td>.820*</td>
<td>.158</td>
<td>.080</td>
<td>.843*</td>
</tr>
<tr>
<td>BT</td>
<td>.894*</td>
<td>.839*</td>
<td>.736*</td>
<td>.887*</td>
<td>.556</td>
<td>.939*</td>
<td>.726*</td>
<td>.871*</td>
<td>.549</td>
<td>.535</td>
<td>.933*</td>
<td>.953*</td>
<td>.489</td>
<td>.470</td>
<td>.958*</td>
</tr>
<tr>
<td>AC</td>
<td>.891*</td>
<td>.753*</td>
<td>.532</td>
<td>.842*</td>
<td>.048</td>
<td>.892*</td>
<td>.300</td>
<td>.799*</td>
<td>.007</td>
<td>.056</td>
<td>.841*</td>
<td>.864*</td>
<td>.056</td>
<td>.023</td>
<td>.904*</td>
</tr>
</tbody>
</table>

*Note.* Bonferroni $\alpha = 0.002$. HR = heart rate. BT = body temperature. AC = activity.

* $p < .002$, ** $p < .004$ (trend).
In the third period (Figure 3), HR on days 13, 15 (rat removal), and 16 correlated significantly with each other ($p < .001$) but not with Day 14 (urine collection and cage cleaning).

Figure 4 represents CORT levels corrected for creatinine concentration in the urine of the mice prior to rat introduction on Day 0, 1 week after rat introduction (Day 7) and 2 weeks thereafter (Day 14). No statistical differences between the 3 days were found.

**DISCUSSION**

The results show that at the time the rats were introduced in the mouse room, HR, BT, and AC of the mice were still recovering from the urine collection and cage-cleaning procedures a few hours earlier. However, no direct effects of the rat introduction were found as all three parameters continued to decrease until they reached baseline values normal for the time of day. Therefore, the overall
conclusion that can be drawn is that the introduction of rats in the animal room did not result in any chronic effects on the physiological parameters that were measured in the mice.

CORT levels were found to be equal on Days 0, 7, and 14 and were actually lower than the baseline value found in female C57BL/6 mice in a previous study (Meijer, Lemmens, Van Zutphen, & Baumans, 2005). HR, BT, and AC on all days after rat introduction correlated with Day –1 except for those days on which the cages were cleaned (Table 1). The effect of cage cleaning, whether or not combined with urine collection, was also seen in the second and third period (Figures 2 and 3) as Days 8 and 14 did not correlate with the other days in those periods (with the exception of Day 7, discussed later); the other days did show correlations with each other (Table 2). Although visual inspection of HR, BT, and AC data indicates a temporary increase after urine collection on Day 7 (Figure 2), the overall pattern of that day still showed a strong correlation with Day –1 (Table 1) and Days 6 and 9, on which no special treatments took place (Table 2). Only the trend in correlation of HR and the absence of correlation of AC between Days 7 and 8 (Table 2) indicate that the urine collection had some effect on the mice; however, to conclude that urine collection had less impact than cage cleaning seems justified, even though the mice had never been subjected to this procedure before. This can be explained by the fact that mice are known to be active explorers of novel environments, whereas after urine collection the mice soon went back to rest (personal observation).

In contrast to the results of D’Arbe et al. (2002) and Calvo-Torrent et al. (1999), the results of this study suggest that the mice did not experience chronic stress as a result of the presence of rats. As demonstrated for HR in individually housed mice (Einstein, Rowan, Billing, & Lavidis, 2000), a chronic stress state likely would have been expressed, for at least the duration of the study, in increased baseline values.

The current study, however, had two major differences compared with the protocols of the studies by D’Arbe et al. (2002) and Calvo-Torrent et al. (1999). First, our study used female mice; the other three studies used male mice. As gender differences in both physiology and behavior are common in mice, this might have influenced the results. Second, and perhaps even more important, in the current study rats were introduced into the room where the mice already had been housed for months; in the other three studies, the mice were brought into a room other than their own—a room already occupied by rats. As a consequence, the mice had to be transported before they were exposed to the rats. It is known that short, in-house transport by itself causes stress in mice (Drozdowicz, Bowman, Webb, & Lang, 1990; Tabata, Kitamura, & Nagamatsu, 1998; Tuli, Smith, & Morton, 1995); therefore, the preceding transport might have triggered a stress response that was continued by the subsequent novelty
of the exposure to rats. Further research can possibly point out if similar signs of stress are found if mice are transported shortly before the introduction of rats into their own room.

In conclusion, the absence of long-term changes in the physiological parameters used in this study indicates that housing rats and mice in the same animal room does not per se mean that mice experience stress. Therefore, the results do not support legislation based on compromised welfare; however, some precautions are necessary as the stress response to the presence of rats might be context dependent and might be different between genders and/or strains. Furthermore, this experiment does not provide insight into the possible detrimental effects that mice may have on rats; hence, the claim of gastric ulceration in rats still needs to be demonstrated by scientific evidence.

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