

The Physiological and Behavioral Impact of Sensory Contact Among Unfamiliar Adult Mice in the Laboratory

Andreas Rettich, Hans Peter Käsermann, Pawel Pelczar,
Kurt Bürki, and Margarete Arras

*Institute of Laboratory Animal Science
University of Zurich*

Housing mice in the laboratory in groups enables social interaction and is the way a laboratory should house mice. However, adult males show reciprocal aggression and are therefore frequently housed individually. Alternatively, a grid divider, which allows sensory contact by sight and smell but prevents fighting and injuries, can separate mice within 1 cage. This study examined the influence of this housing method on various physiological and behavioral parameters. Adult male mice housed for 10 days with sensory contact to an unfamiliar male displayed significant increases in heart rate (HR), body core temperature (BT), and motor activity (ACT). Furthermore, the mice suffered impaired nest-building behavior and significantly reduced body weight. Conversely, males housed in a similar manner with a female companion showed only a transient elevation of ACT, BT, and HR. Although no clear beneficial effect of housing males with sensory contact to females was evident, this study could not exclude it. On the other hand, housing of mature males in this way leads to sustained detrimental alterations of physiology and behavior, thus implying severe impairment of animal well-being.

It is commonly stipulated that, for their welfare, mice in the laboratory should be housed in stable, compatible groups, enabling normal social behavior (Jennings et al., 1998). However, this group housing approach, which allows social interaction, can lead to serious problems when applied to males. Mature male mice tend to fight with other males when housed in groups, and such reciprocal aggression frequently

results in physical injury (Brain & Hui, 2003; Van Loo, Van Zutphen, & Baumans, 2003) and can even lead to the death of subordinate animals.

Reciprocal male-to-male aggression complicates the housing of male mice, particularly in long-term biomedical studies (aging, diabetes, and metabolism models; arteriosclerosis studies; prion and slow virus infection experiments) and in specific breeding purposes (generation and maintenance of genetically modified lines: mating of stud and donor animals, breeding of founders). In addition, the current trends in animal experiment design stipulate that greater care should be taken to reduce the numbers of animals used in experiments (Kramer & Kinter, 2003). This, among others, can be achieved by reusing mice for several subsequent treatments or experiments to extend their effective experimental life span. The problem of maintaining and housing of aging male populations is certainly one of the reasons that makes it very difficult to effectively apply this practice to male mice.

At the moment, individual housing of adult males is common (Van Loo, Van de Weerd, Van Zutphen, & Baumans, 2004), even if such practice is thought to induce social stress due to isolation, as reflected in an increase of adrenocortical reactivity (Brain, 1975) and altered responses to behavioral tests (Voikar, Polus, Vasar, & Rauvala, 2005) and biomedical experiments (Bartolomucci et al., 2003; Chida, Sudo, & Kubo, 2005; D'Arbe, Einstein, & Lavidis, 2002; Dong et al., 2001; Guidotti et al., 2001; Wu et al., 2001).

The welfare of male animals can be improved by housing them with females. This approach is frequently been adopted when housing valuable mature males (after the mice have been chronically instrumented, after transplantation of liver, heart, and lung). The drawback of this approach is the production of a large number of unwanted offspring.

To avoid symptoms of isolation and avoid production of unwanted progeny, a male can also be housed together with an infertile female, thus constituting group housing of compatible animals while preventing unnecessary reproduction. Research has shown a beneficial effect of this housing model, as demonstrated by the lowered heart rate of males housed in pairs over that of individually housed males (Spani, Arras, Konig, & Rulicke, 2003).

If infertile females are not available, then companion animals can be separated by a perforated grid (commercially available from cage suppliers) that divides the cage into two parts (Van Loo, de Groot, Van Zutphen, & Baumans, 2001). In this housing arrangement animals are prevented from fighting as well as from breeding but can see, hear, and smell each other. This trans-grid sensory contact is regarded as passive social contact (Van Loo et al., 2004), and it may alleviate symptoms of isolation.

We must also mention that in some studies the housing of male animals with partitions coupled to selection of dominant-subordinate counterparts was used to induce depression in mice (Kudryavtseva & Avgustinovich, 1998; Kudryavtseva,

Bakshantovskaya, & Koryakina, 1991) and changes of immunological response (Beitia et al., 2005). These studies, although interesting, were aimed at selecting subordinates by frequent changes of the dominant counterpart and daily physical conflict situations and therefore hardly mirrored the long-term routine housing of male mice.

We therefore wanted to address the question of whether partition housing without physical confrontation would be beneficial or distressing for mature male mice and how the housing conditions could be optimized, to prevent isolation symptoms in the long-term housing.

This study was designed to test whether permanent trans-grid contact can counteract the symptoms of social isolation of mature male mice housed in a common laboratory environment. Bearing in mind the results of Spani et al.'s (2003) study, we were particularly interested in whether housing with sensory contact of a male to a companion would establish heart rate values similar to those detected in group housing, that is, below the values of single housing, thus indicating an overall improvement in the animals' well-being.

MATERIALS AND METHOD

Animals

Sixteen male Hsd:NMRI mice, aged 4 weeks, were obtained from Harlan (Horst, The Netherlands). During the adaptation period of 4 weeks, they were housed as groups of 2 to 4 males. After adaptation, the mice received telemetric transmitter implants at the age of 8 weeks. After surgery, mice were housed individually during a 2-week recovery phase, after which they were used in a 4-month, unrelated study outlined later.

Each transmitter-bearing male was housed in pairs with an ovariectomized female to allow normal social interaction while reproduction was prevented. At the age of 4 to 6 months, each male was vasectomized in two separate sessions, first on the right and then on the left side, during which time they underwent two 2-week periods of being housed singly. After vasectomy was completed on both sides, the adult male was housed with fertile females (for social interaction including mating but not reproduction). After the successful conclusion of the study just described, the 8- to 9-month-old mice were entered in the experiments outlined in this study.

Experimental Setting

The mice were separated from their female cage mates and placed in a fresh Type 3 (T3) cage (425 mm × 266 mm × 150 mm, floor area 820 cm²) with

autoclaved, dust-free, sawdust bedding (80 to 90 g/cage). Autoclaved hay (18 to 20 g/cage) for use as nesting material was randomly distributed over the entire floor area to provide the animal with a free choice of nesting place. The mice were fed a pelleted mouse diet (Kliba No. 3431, Provimi Kliba, Kaiseraugst, Switzerland) ad libitum and had free access to sterilized drinking water. The light:dark cycle in the room consisted of 12:12 hr (07:00 to 19:00) of artificial light (approximately 40 lux in the cage). The temperature was $21 \pm 1^\circ \text{C}$, with a relative humidity of $50 \pm 5\%$ and 15 complete changes of filtered air per hour (HEPA H 14 filter).

After 4 days of single housing, mice were again switched to a fresh T3 cage that was separated into two parts lengthwise with a stainless steel grid divider. Another 4 days later, a fertile adult companion, either a male or a female of the inbred strain A129, aged 4 to 6 months, was placed in the vacant half of the cage. For 10 days, both animals could see, hear and smell each other through the perforated divider. In the case of male:female companionship, the experiment was terminated at 18 days, whereas in the case of the male:male experiment the male companion was removed and the experimental, transmitter-bearing male was observed for another 8 days.

Transmitter-bearing male mice were allocated randomly to the male:male or male:female experiment, resulting in 8 animals per group.

Transmitter Implantation

TA10ETA-F20 transmitters (Data Sciences International, St. Paul, MN), which can measure heart rate (HR), core body temperature (BT), and motor activity (ACT) in freely moving mice, were implanted as previously described (Spani et al., 2003). The mice were anesthetized by inhalation of the volatile anesthetic sevoflurane (SevoraneTM, Abbott, Cham, Switzerland) at a concentration of 4% to 8% in 100% oxygen at a flow rate of 200 ml/min. The anesthetic gas was administered with a nose mask. Ketamine (Ketasol-100TM, Dr. Graub, Bern, Switzerland) was injected subcutaneously as premedication at a dosage of 45 mg/kg body weight for pre-emptive analgesia. The telemetric transmitter body was implanted under aseptic conditions in the abdominal cavity of the mouse. One telemetry lead was tunneled subcutaneously from the thorax to the neck, where the wired loop electrode was fixed between the muscles located to the right of the trachea. The other wired loop lead was sutured to the xiphoid process with a silk thread. The muscle layers and the skin were then closed with resorbable sutures. Postoperative pain was treated with buprenorphine (TemgesicTM, Reckitt and Colman Products Ltd., Hull, England), at a dose of 0.1 mg/kg body weight, injected subcutaneously twice per day for 4 days. Animals were monitored (clin-

ical investigation: appearance, body weight, and food and water consumption) daily for 10 days after surgery and were allowed to recover for 8 weeks.

Data Acquisition

For telemetric data acquisition, the transmitter was switched on by touching the animal with a magnet; signals were detected by a receiver plate placed underneath the animal's cage. Telemetric data were recorded with the program Dataquest LabPRO Version 3.11 (Data Sciences International, St. Paul, MN). HR and BT values were sampled for 30 sec and 10 sec, respectively, every 5 min. ACT was recorded continuously and stored at 5-min intervals. Data were sampled for an 18-day period in male:female experiments and for 26 days in male:male experiments. For each day (24-hr period), average values were calculated for each individual.

Body weight and food and water consumption were determined at the beginning of the experimental phase and after changing the housing conditions (Experimental Days 0, 4, 8, 18, and 26). Each transmitter-bearing mouse, as well as the mouse's water bottle and food pellets, were weighed at the same time each day (18:00 to 19:00 hr at the end of the daylight phase in the animal room) using a precision balance appropriate for weighing moving animals (PR 2003 Delta Range, Mettler-Toledo AG, Greifensee, Switzerland). Daily food and water intake was calculated for each mouse.

Sketches of each cage in the form of a map and a visual appraisal of nest appearance were recorded on Experimental Days 9 (after 4 days in individual housing with divider), 18 (after 10 days with companion behind the divider), and 26 (at the end of the experiment/8 days after removal of the male companion in male:male experiment only).

Data Analysis and Statistics

Sketches and nest records were condensed as categorical variables, that is, the number of animals/cages showing a defined status. Continuous measurements were presented as means \pm standard deviation. Statistical analysis was carried out with SPSS Version 13.0 for Windows.

For each animal, the baseline data were calculated as a daily mean from individual housing in a T3 cage. A novel environment such as a new cage leads to increases in activity and physiological values that persist for a few hours of adaptation (Kramer et al., 1993). To avoid the influence of such novelty, the telemetric baseline data were determined from Days 2 through 4. Student's paired *t* test was used to compare baseline data with data from each experimental day to

test whether the effect of housing model (with divider, with female or male companion) on the parameters measured was significant compared with individual housing in the entire area of a T3 cage. Effects were considered significant at $p < .005$.

RESULTS

Time courses of HR, BT, and ACT over the course of the experiment are shown in Figure 1. As noted earlier, changing of cages at the end of Days 0 and 4 gave rise to greater motor activity and raised HR values on the following day. Because BT remained unchanged, the increase in HR was probably due to higher bodily activity resulting from exploring the new environment and nest-building efforts. However, comparison of the data from single housing in an entire T3 cage area (Days 1 to 4) with that from cages divided by a grid (Days 5 to 8) did not reveal any significant differences.

Larger changes were detected after a companion was accommodated behind the divider grid (end of Day 8). All telemetric measures peaked significantly on the subsequent day, regardless of whether a female or a male companion was presented, but returned to baseline values within 1 day in the case of male:female companionship. In contrast, if a male was present behind the grid, HR and BT remained high during the entire period of residence of the companion animal. Although initially also significantly elevated, ACT decreased after a few days (see Figure 1), indicating that extreme locomotor movements cannot be the cause of the consistent significant elevation of HR and BT. Even after removal of the male companion, HR required a further 2 days to return to baseline levels.

Changes in body weight and in food and water consumption are shown in Figure 2. Body weight was significantly ($p = .001$) reduced after 10 days of sensory contact with a male—but not with a female—companion. Food intake was significantly ($p = .004$) increased after removal of the male conspecific. Water intake increased significantly ($p = .000$) in the male:male group. However, it is possible that the apparent increase in water consumption may have been confounded partially by incidental loss of water from the bottle (touching the bottle while climbing on the grid or disturbance of bedding or nesting material resulting in contact with the nipple of the water bottle).

Records of nest appearance are summarized in Figure 3. The telemetric measurements were consistent with the observations of nest building and the appearance of the cage. In male:female companionship, all mice built proper nests in good condition. In male:male pairing, half the experimental males destroyed their nests. The location of the nest was not correlated with other findings; in female:male companionship, nests were frequently at a distance from each other,

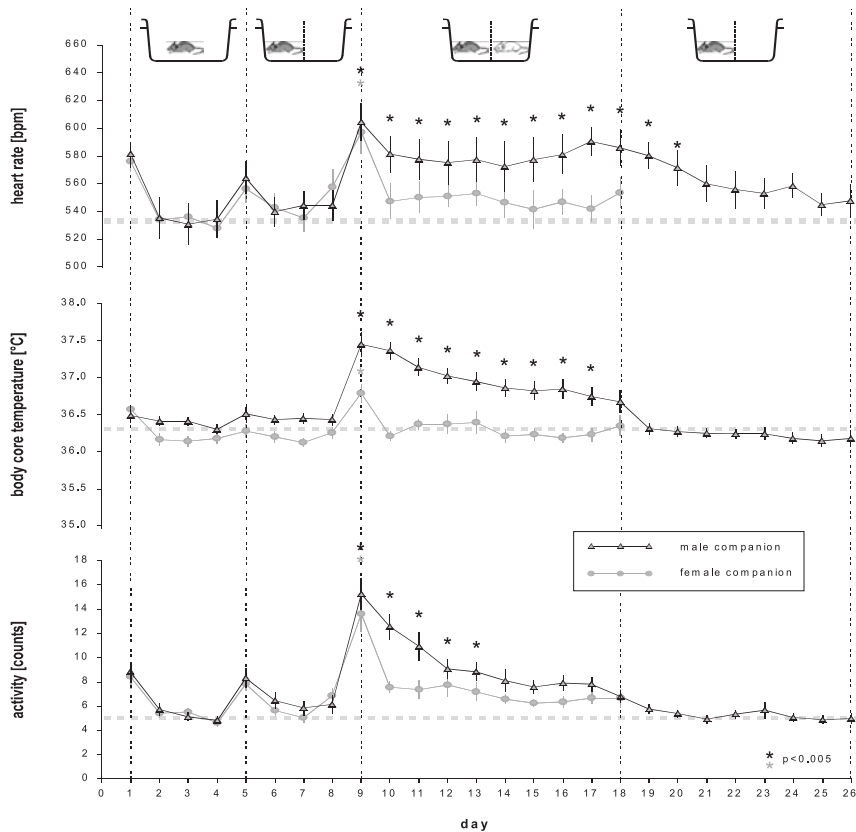


FIGURE 1 Telemetric measurements of heart rate, body core temperature, and motor activity of transmitter-bearing male mice. Data were recorded at 5-min intervals and expressed as means of 24 hr. Bars indicate standard deviations ($n = 8$). Statistical comparison (paired Student's t test) with baseline data from individual housing (dashed line) was considered significant at $p < .005$ (indicated by asterisks above data points).

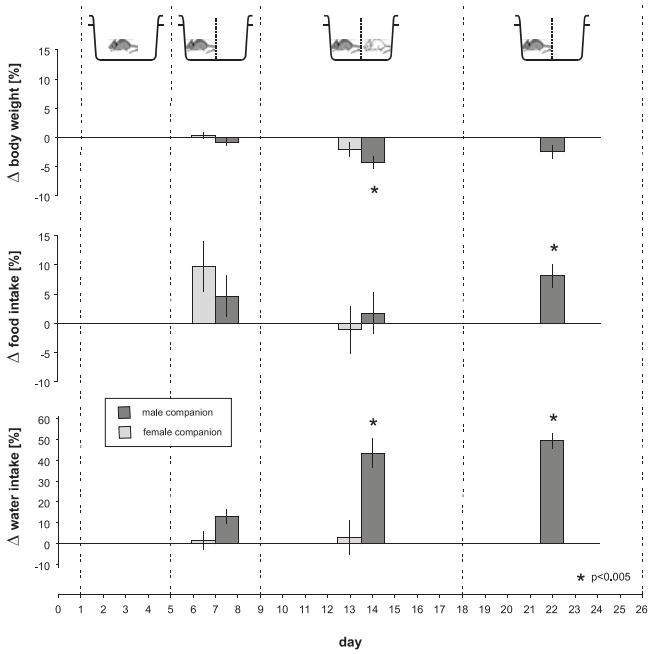


FIGURE 2 Changes in body weight and food and water consumption of transmitter-bearing male mice. Means \pm standard deviations ($n = 8$) related to the baseline value extrapolated from 4 days of individual housing (Days 1 to 4). Asterisks indicate statistical significance (paired Student's t test) at $p < .005$.

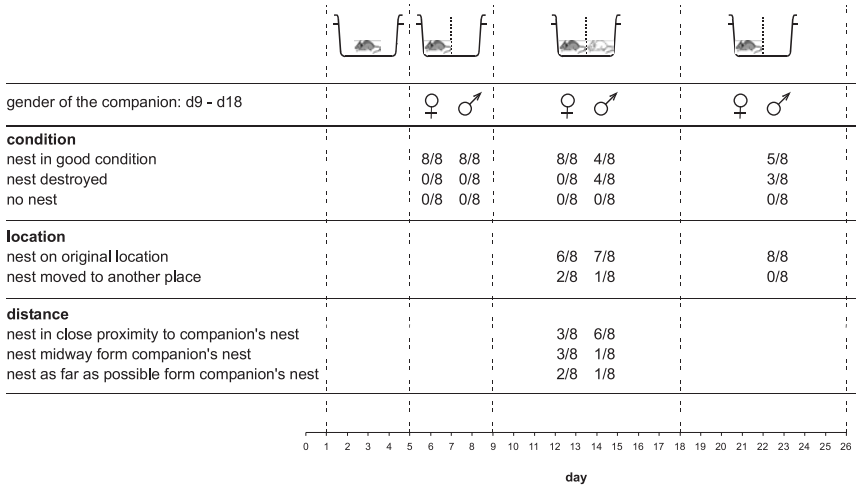


FIGURE 3 Nest building activities of transmitter-bearing male mice.

whereas in male:male pairings the nests were mostly close to the opponent's nest. In both groups, nests were rarely moved to another location.

DISCUSSION

The housing of a male mouse with sensory contact to an unfamiliar female induced temporary alterations (1 day) in physiological and behavioral parameters. Thereafter (over the next 9 days), values of the parameters measured were in general not significantly different from those seen in individual housing, although HR and ACT levels remained slightly elevated.

In contrast, sensory contact of two unacquainted adult males caused dramatic changes in physiology and behavior, with almost no habituation during the 10-day exposure period. In particular, HR was permanently and significantly increased; even after removal of the male companion, normalization was delayed. BT exhibited significant elevation over 9 days. ACT was also above that observed in single housing but showed a clear return toward baseline values during long-term intermale sensory contact, suggesting that physical exercise and bodily movements were not the main cause of the high HR values. In addition, body weight was significantly reduced (see Figure 2), and nest building was impaired (see Figure 3).

It is remarkable that the daily levels of HR and BT detected during long-term intermale sensory contact in this study far exceeded the values found in Spani et al.'s (2003) study, in which the researchers investigated adult male mice under single and unrestricted pair housing conditions using an identical telemetric method. Although in our former recordings the higher HR under single housing compared with paired housing was statistically significant, this difference appeared marginal compared with the elevation of HR levels detected under the trans-grid sensory intermale contact observed in this study. From these results, we conclude that the housing of unfamiliar, mature male mice with sensory contact through dividers induces a considerable degree of social stress in the long term, which is potentially even more severe than the stress incurred by isolation in single housing.

In general, the amplitudes of HR changes are in line with the progress of neuroendocrine stress parameters described by Veenema, Sijtsma, Koolhaas, and de Kloet (2005), who concluded that, compared with group housing with a female, individual housing led to a mild elevation of stress hormone levels (corticosterone and ACTH); however, markedly raised stress hormone levels and reduction in body weight were described when males had sensory contact with another male. This supports our interpretation that the results from the male:male housing with grid divider experiment indicated social stress resulting from sensory contact with unfamiliar males. This has been postulated as an expression of aggression due to territorial behavior. A model of daily, intermittent fighting, combined with sensory contact, documented the dependence of a broad range of physiological distur-

bances on territorial claims/rights (Bartolomucci, 2005). In addition, only sparse evidence, based on autonomic function and pathohistological alterations of the myocardium, was found that males were able to adapt to such chronic stress (Bartolomucci et al., 2003; Costoli et al., 2004), which is in accordance with the lack of habituation observed in our experiments.

The correlation between the ownership of a territory, communicated with scent marks (Hurst & Beynon, 2004; Nevison, Armstrong, Beynon, Humphries, & Hurst, 2003) and aggressive behavior in the presence of other males, is well known (Liebenauer & Slotnick, 1996). The remaining urinary scent marks on the divider grid and its vicinity could explain the delayed decline in telemetric measurement values after removal of the male opponent in our experiment. It might be expected that some values (HR) might have returned to normal faster if the mice had been provided with a fresh cage or were allowed to occupy the foreign territory by removal of the divider.

An analysis of the factors responsible for overt territorial identification indicated that our experimental males had a long history (from a previous, unrelated study) of almost all factors that enhance aggressive behavior. The animals were of an advanced age; for almost the whole of their respective lives, they had been housed in pairs with a female, which allows them a wide range of social behaviors (grooming) as well as the possibility of mating. Before the experimental phase, they underwent three, short periods (14 days) of individual housing to enable recovery from surgery, always followed by rehousing with a female for a longer time span (several weeks). We hypothesize that they had developed a distinctive territorial authority and highly aggressive traits that caused them to react strongly when confronted with an opponent (Brain, 1975; Brain, Benton, & Bolton, 1978). Therefore, extrapolating the responses of the group of males in this experiment to very young males (weanlings) or sibling groups is problematic.

In light of our current findings, the strong changes and the lack of habituation observed on sensory contact with an unfamiliar male, but not female, can be explained as signs of persistent intermale aggression. In contrast, when providing an adult male with sensory contact to a female, the parameters measured increased significantly only during the first day, suggesting that the animals habituated to the novel situation within 24 hr, with values thereafter declining to the approximate range of single housing.

We must mention that trans-grid sensory contact with a female also resulted in elevated HR compared with both individual housing and unrestricted pair housing with a female. This slight discrepancy may be due to the fact that we used ovariectomized females in the pair-housing paradigm in Spani et al.'s (2003) study but naïve adult females in the current sensory contact experiments. We hypothesize that HR in mature male mice provided with long-term female contact may stabilize at different levels depending on whether the female is sexually active or inactive. This could explain the elevated HR levels found with long-term

trans-grid sensory male:female contact. Experiments with the use of ovariectomized females for sensory contact or unrestricted group housing with fertile females would be required to test this hypothesis.

The results of the male:female sensory contact experiment imply that companion housing of two unfamiliar, adult, sexually active mice of different genders with grid dividers cannot adequately compensate for housing in compatible groups (breeding pairs) in which social interactions such as grooming, sleeping together, and other behavior can occur. However, on the basis of our data a beneficial effect cannot be excluded, and this housing model may require further investigation (for behavioral parameters).

Conversely, we conclude that housing mature, unfamiliar, male mice with sensory contact through a grid divider demonstrated no beneficial effect but instead led to distress and potential impairment of well-being. In our opinion, this housing form is therefore not to be recommended for unfamiliar, mature male mice.

ACKNOWLEDGMENTS

This study was conducted on behalf of the Cantonal Veterinary Department, Zurich, Switzerland. Housing and experimental protocols were approved under License No. ZH 82/2004 and were in accordance with Swiss Animal Protection Law.

REFERENCES

- Bartolomucci, A. (2005). Resource loss and stress-related disease: Is there a link? *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, *11*(5), RA147–RA154.
- Bartolomucci, A., Palanza, P., Costoli, T., Savani, E., Laviola, G., Parmigiani, S., & Sgoifo, A. (2003). Chronic psychosocial stress persistently alters autonomic function and physical activity in mice. *Physiology & Behavior*, *80*, 57–67.
- Beitia, G., Garmendia, L., Azpiroz, A., Vegas, O., Brain, P. F., & Arregi, A. (2005). Time-dependent behavioral, neurochemical, and immune consequences of repeated experiences of social defeat stress in male mice and the ameliorative effects of fluoxetine. *Brain, Behavior, & Immunity*, *19*, 530–539.
- Brain, P. F. (1975). What does individual housing mean to a mouse? *Life Sciences*, *16*, 187–200.
- Brain, P. F., Benton, D., & Bolton, J. C. (1978). Comparison of agonistic behavior in individually-housed male mice with those cohabiting with females. *Aggressive Behavior*, *4*, 201–206.
- Brain, P. F., & Hui, S. E. (2003). Variability in patterns of intra-specific biting attack in commonly used genetic lines of laboratory mice. *Scandinavian Journal of Laboratory Animal Science*, *30*, 113–127.
- Chida, Y., Sudo, N., & Kubo, C. (2005). Social isolation stress exacerbates autoimmune disease in MRL/lpr mice. *Journal of Neuroimmunology*, *158*, 138–144.
- Costoli, T., Bartolomucci, A., Graiani, G., Stilli, D., Laviola, G., & Sgoifo, A. (2004). Effects of chronic psychosocial stress on cardiac autonomic responsiveness and myocardial structure in mice. *American Journal of Physiology—Heart & Circulatory Physiology*, *286*, H2133–H2140.

- D'Arbe, M., Einstein, R., & Lavidis, N. A. (2002). Stressful animal housing conditions and their potential effect on sympathetic neurotransmission in mice. *American Journal of Physiology—Regulatory Integrative & Comparative Physiology*, *282*, R1422–R1428.
- Dong, E., Matsumoto, K., Uzunova, V., Sugaya, I., Takahata, H., Nomura, H., et al. (2001). Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 2849–2854.
- Guidotti, A., Dong, E., Matsumoto, K., Pinna, G., Rasmusson, A. M., & Costa, E. (2001). The socially-isolated mouse: A model to study the putative role of allopregnanolone and 5alpha-dihydroprogesterone in psychiatric disorders. *Brain Research—Brain Research Reviews*, *37*, 110–115.
- Hurst, J. L., & Beynon, R. J. (2004). Scent wars: The chemobiology of competitive signalling in mice. *Bioessays*, *26*, 1288–1298.
- Jennings, M., Batchelor, G. R., Brain, P. F., Dick, A., Elliott, H., Francis, R. J., et al. (1998). Refining rodent husbandry: The mouse. Report of the Rodent Refinement Working Party. *Laboratory Animals*, *32*, 233–259.
- Kramer, K., & Kinter, L. B. (2003). Evaluation and applications of radiotelemetry in small laboratory animals. *Physiological Genomics*, *13*, 197–205.
- Kramer, K., van Acker, S. A., Voss, H. P., Grimbergen, J. A., van der Vijgh, W. J., & Bast, A. (1993). Use of telemetry to record electrocardiogram and heart rate in freely moving mice. *Journal of Pharmacological & Toxicological Methods*, *30*, 209–215.
- Kudryavtseva, N. N., & Avgustinovich, D. F. (1998). Behavioral and physiological markers of experimental depression induced by social conflicts (DISC). *Aggressive Behavior*, *24*, 271–286.
- Kudryavtseva, N. N., Bakshtanovskaya, I. V., & Koryakina, L. A. (1991). Social model of depression in mice of C57BL/6J strain. *Pharmacology, Biochemistry & Behavior*, *38*, 315–320.
- Liebenauner, L. L., & Slotnick, B. M. (1996). Social organization and aggression in a group of olfactory bulbectomized male mice. *Physiology & Behavior*, *60*, 403–409.
- Nevison, C. M., Armstrong, S., Beynon, R. J., Humphries, R. E., & Hurst, J. L. (2003). The ownership signature in mouse scent marks is involatile. *Proceedings of the Royal Society of London—Series B: Biological Sciences*, *270*, 1957–1963.
- Spani, D., Arras, M., Konig, B., & Rulicke, T. (2003). Higher heart rate of laboratory mice housed individually vs in pairs. *Laboratory Animals*, *37*, 54–62.
- Van Loo, P. L. P., de Groot, A. C., Van Zutphen, B. F. M., & Baumans, V. (2001). Do male mice prefer or avoid each other's company? Influence of hierarchy, kinship, and familiarity. *Journal of Applied Animal Welfare Science*, *4*, 91–103.
- Van Loo, P. L. P., Van de Weerd, H. A., Van Zutphen, L. F., & Baumans, V. (2004). Preference for social contact versus environmental enrichment in male laboratory mice. *Laboratory Animals*, *38*, 178–188.
- Van Loo, P. L. P., Van Zutphen, L. F., & Baumans, V. (2003). Male management: Coping with aggression problems in male laboratory mice. *Laboratory Animals*, *37*, 300–313.
- Veenema, A. H., Sijtsma, B., Koolhaas, J. M., & de Kloet, E. R. (2005). The stress response to sensory contact in mice: Genotype effect of the stimulus animal. *Psychoneuroendocrinology*, *30*, 550–557.
- Voikar, V., Polus, A., Vasar, E., & Rauvala, H. (2005). Long-term individual housing in C57BL/6J and DBA/2 mice: Assessment of behavioral consequences. *Genes, Brain, & Behavior*, *4*, 240–252.
- Wu, W., Murata, J., Hayashi, K., Yamaura, T., Mitani, N., & Saiki, I. (2001). Social isolation stress impairs the resistance of mice to experimental liver metastasis of murine colon 26-L5 carcinoma cells. *Biological & Pharmaceutical Bulletin*, *24*, 772–776.