Different Sickness Responses in Adult and Aged Rats Following Lipopolysaccharide Administration

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Abstract

Objective: Immune challenges result in sickness responses such as decreased activity, fever, and spatial learning deficits. While these responses occur simultaneously, they are not usually evaluated concurrently or for an extended time. The purpose of this study was to examine how an immune challenge affected activity and temperature responses in animals tested concurrently in the Morris water maze (MWM) over 5 days and how aging interacts with such responses. **Method:** An accepted model of aging, adult ($n = 10$; 5–6 months) and aged ($n = 7$; 22 months) male Brown-Norway rats were implanted with a telemetry device (Mini Mitter, Oakmont, PA) to continuously monitor temperature and activity following an immune challenge. These animals were injected with either 250 $\mu\text{g/kg}$ lipopolysaccharide (LPS) or 0.9% sodium chloride and then assessed in the MWM for 5 days. **Results:** Temperature responses varied by age. Initial temperatures decreased in both experimental groups followed by an increase (fever) in the adult group, while the temperatures of the aged animals remained decreased. Although both age groups were sedentary at baseline, activity decreased after LPS only in the adult group. **Conclusion:** An LPS immune challenge resulted in age-dependent temperature and activity changes. There was an absence of fever and no effect on activity in aged LPS-treated animals. These results may suggest the need to assess a broader spectrum of sickness responses when monitoring elderly individuals for infection and not rely on the presence of fever. Activity may not be a sensitive indicator of sickness in some aging models.

Keywords

body temperature, illness behavior, lipopolysaccharide, male, motor activity

Infections activate the immune system, which releases pro-inflammatory cytokines like interleukin-1 β and interleukin 6 that mediate a pattern of responses termed sickness behaviors, such as decreased food intake, weakness, malaise, reduced activity, and fever (Kelley et al., 2003). These cytokines also elicit a nonspecific immune response, the acute-phase response, which is characterized by systemic reactions including changes in acute-phase proteins (Roth & Blatteis, 2014; Roth, Rummel, Barth, Gerstberger, & Hubschle, 2009). Fever, an important sign of infection, is considered an adaptive part of the acute-phase response (Roth & Blatteis, 2014; Roth, Rummel, Barth, Gerstberger, & Hubschle, 2006). While most individuals with an infection experience an increase in temperature, 20–30% of the elderly diagnosed with a severe infection have an absent or blunted temperature response, which is one of the factors that can result in delayed diagnosis and/or treatment of infection contributing to poorer health outcomes (Gavazzi & Krause, 2002). Indeed, Büla, Ghilardi, Wietlisbach, Petignat, and Francioli (2004) found that the occurrence of an infection was related to the subsequent decline in nursing home residents' ability to function independently. If infections could be diagnosed and treated earlier, it

might prevent or diminish declines in independent-living abilities.

Animal models are useful for simulating infections seen in humans. Lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria, is administered to activate animals' immune systems and mimic an infection that results in sickness behaviors such as decreased activity (Kelley et al., 2003). Brown-Norway rats are nocturnal. Both temperature and activity are higher during the night and lower during the day (Refinetti, 2010; Wrotek, Jedrzejewski, Potera-Kram, & Kozak, 2011). While nighttime temperatures have been

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shown to be similar in adult and aged animals, the results related to daytime temperatures are contradictory, with aged animals displaying both lower (Foster, Conn, & Kluger, 1992) and higher temperatures (Gordon et al., 2014), depending on the study. In comparison to adult animals, motor activity has been shown to be decreased in aged mice (Godbout et al., 2005) and rats (Gordon et al., 2014) at baseline.

While research has shown that LPS administration causes decreased motor activity (Abraham & Johnson, 2009; Kozak, Conn, & Kluger, 1994) and temperature changes (Roth & Blatteis, 2014; Roth et al., 2006), both responses can differ based on other factors such as age and/or ambient room temperature. Following LPS administration, aged animals have displayed a range of temperature responses. While studies have shown that some aged rats exhibit a temperature increase similar to that of adult rats, others develop hypothermia or have no temperature change (Wachulec, Peloso, & Satinoff, 1997). In rooms with ambient temperatures of 21–24°C, adult rats' temperatures increased, while aged rats' temperatures did not vary from baseline (Buchanan, Peloso, & Satinoff, 2003), increased slightly (Peloso, Florez-Duquet, Buchanan, & Satinoff, 2003), or took 3 hr to increase and then remained similar to adult rats' temperatures (Foster et al., 1992). In rooms with an ambient temperature of 31°C, the temperature increase in aged rats did not differ from that of adult rats (Buchanan et al., 2003; Peloso et al., 2003). When investigators placed animals in a thermal gradient (10–40°C), the peak temperatures in aged rats were greater and occurred later than the adult rats' peak temperatures, with the aged rats selecting warmer room temperatures (Florez-Duquet, Peloso, & Satinoff, 2001). However, the length of time over which researchers have evaluated these responses after injection varies, and animals are not consistently followed until temperature or activity return to baseline. In a study in which researchers did monitor animals for several days after an immune challenge, aged animals exhibited initial hypothermia followed by a fever lasting 3 days, while young animals exhibited an early fever lasting 1 day (Barrientos, Watkins, Rudy, & Maier, 2009). Taken together, these results suggest that aged animals have some limitations, based on ambient room temperature, in their ability to mount a fever.

Following LPS administration, motor activity decreases in a dose- and time-related manner in mice (Kozak et al., 1994). However, the decrease in motor activity is sustained in aged animals following LPS administration compared to adult animals (Abraham & Johnson, 2009; Godbout et al., 2005). While these findings suggest that changes in activity and temperature following an LPS immune challenge may differ between adult and aged animals, most studies have evaluated both responses for 24 hr or less.

The present investigation was a substudy of a larger investigation examining the effects of LPS administration on sickness responses including activity and temperature, concurrently with spatial learning as assessed by Morris water maze (MWM) performance (Kupferschmid & Therrien, 2018). Prior researchers have noted cognitive changes assessed in the MWM in both adult (Shaw, Commins, & O'Mara, 2001) and aged animals

(Barrientos et al., 2006) following an immune challenge. However, minimal literature exists on how an immune challenge affects activity and temperature responses with animals tested concurrently in the MWM or how aging interacts with such responses. While research has shown that the temperature of aged animals differs based on the ambient room temperature, little is known about how water temperature affects animal temperature after an immune challenge. Most studies use a water temperature of 20–22°C in the MWM (Vorhees & Williams, 2014). Pilot testing with standard water temperature of $20 \pm 1^\circ\text{C}$ (Vorhees & Williams, 2014) resulted in decreased body temperatures in animals following the trials (data not shown). This finding was confirmed by study results showing that body temperature decreased in healthy adult animals tested in the MWM in cold water (20°C) for 45 s (Iivonen, Nurminen, Harri, Tanila, & Puolivali, 2003) and in aged animals after they swam for 60 s in 21°C water (Lindner & Gribkoff, 1991). In the latter study, body temperature remained lower in the aged animals compared to the young animals 24 hr after the MWM test. Based on these findings, the water temperature we used for the present study was $35 \pm 1^\circ\text{C}$. We chose this temperature because it was close to baseline body temperature of these animals but not extreme enough to affect spatial learning (Vorhees & Williams, 2014). In the present study, we examined the temperature and activity responses (sickness behaviors) for 5 days after an immune challenge in adult and aged animals concurrently training in the MWM.

Materials and Method

Animals

With a 50% survival rate at 32 months, the Brown-Norway rat is a well-known aging model (Turturro et al., 1999). We used 17 male Brown-Norway rats purchased from Harlan Sprague Dawley Inc. in the present study. Adult rats were 5–6 months old ($n = 10$) and aged rats were 22 months old ($n = 7$). The animals were housed individually in cages with access to standard rodent food and water. The animal room was maintained at 25°C on a reverse 12-hr light–dark cycle. The University of Michigan Animal Care and Use Committee approved all procedures.

Experimental Protocols

Mini Mitter surgery. We monitored core body temperature and motor activity using VitalView software (Mini Mitter, Oakmont, PA). A calibrated, battery-operated radio transmitter was aseptically implanted intra-abdominally in each animal under isoflurane anesthesia. Animals were handled a minimum of 3 days prior to surgery and allowed 7 days to recover after surgery. Activity was detected by changes in the position of the implanted transmitter, which was monitored by an individual receiver board placed under each animal's cage and recorded as a pulse of activity. Temperature and motor activity data were collapsed into hourly averages.

LPS protocol. We administered one intraperitoneal injection of LPS from *Escherichia coli* (serotype 0111: B4; Sigma-Aldrich Corporation, St. Louis, MO) or saline (0.9%) NaCl to the animals, depending on group, 3 hr after lights off: 250 µg/kg LPS (adult $n = 6$; aged $n = 4$) or saline (0.9%) NaCl (adult $n = 4$; aged $n = 3$). Our selection of dose was based on pilot work demonstrating that animals exhibited sickness responses such as decreased food intake and weight loss at this dose (Kupferschmid & Therrien, 2018).

MWM testing. MWM testing was conducted at different times for the two age groups. Testing was started 1.5 hr after LPS injections for adult rats and 5 hr after injections for the aged rats. The timing difference was based on findings that temperature changes following an immune challenge differ between adult and aged animals (Florez-Duquet et al., 2001). MWM testing was initiated at times when animals were expected to have developed a fever. Water temperature was $35 \pm 1^\circ\text{C}$. Animals were allowed a maximum of 3 min to find the platform, could remain on the platform for 30 s, and were allowed 2 min between the four trials each day. We dried the animals and placed them on towels in between trials. We tested animals in the MWM at the same time each day for 5 days.

Data Analysis

Temperature and motor-activity data were aggregated into hourly means. Hourly averages were analyzed separately for the 12-hr dark and light phases. Temperature and motor activity were examined for 24-hr preinjection and 5 days postinjection for each phase.

We used IBM SPSS 22.0 to conduct the initial exploratory analysis of temperature and activity. Because the residual errors in the hourly activity variable were not normally distributed, we transformed the variable using a square root transformation and used the transformed variable in all analyses. Activity charts were constructed using the actual data.

For data analysis, we used SAS Proc Mixed (Version 9.4) to fit a linear mixed model with heterogeneous error variances (SAS Institute, NC). We described the process we used to choose each model in a previous article (Kupferschmid & Therrien, 2018). We considered different models to determine whether allowing for heterogeneous error variance improved the model fit and used model-fit statistics to determine the model with the best fit. To analyze the patterns post-LPS injections, we assessed variables for 5 days. We used a mixed model of variance (analysis of variance) to examine the effects of the between-subject group factors based on experimental status (control or experimental) and age (adult or aged) and the within-subject factor based on time (hours postinjection). We also examined two- and three-way interactions between group and time to assess whether patterns over the 5-day postinjection period were similar between the groups. When indicated, we conducted post hoc comparisons of the means. We considered a value of $p < .05$ significant.

Figure 1. Mean hourly temperature at baseline in adult and aged rats. Error bars represent $\pm$ SE (standard error of the mean). Dark horizontal bar indicates the dark period in a 12:12-hr dark–light cycle. * $p < .05$ for adult and aged groups at hour 7.

Results

Temperature

Baseline animal temperatures (1 day before LPS injections) were similar between the adult and aged groups across the dark and light phases, with temperatures slightly higher during the dark phase ($M = 37.49 \pm$ standard error of the mean [SEM] = $.01^\circ\text{C}$) compared to the light phase ($M = 37.20 \pm$ SEM = $.02^\circ\text{C}$). Repeated measures analysis revealed no significant differences in mean hourly temperature based on age during the dark ($p = .42$) or light phases ($p = .3$). There was a significant interaction between time and age-group, $F(11, 163) = 4.03$, $p < .0001$, during the dark phase only. While the temperature in the adult group was higher than in the aged group during hours 6–8, post hoc comparisons revealed that adult animals had a significantly higher temperature at hour 7 only ($p = .01$; Figure 1).

During the first 24 hr after LPS injections, temperatures in rats in the adult experimental group decreased for 3 hr (1.4°C) before beginning to increase. While not significant, 9–15 hr after LPS injections, the adult experimental group's temperature increase was 0.4 – 0.56°C greater than their controls, peaking 12 hr after injections at 0.56°C . Compared to the aged controls, temperature in the aged experimental rats decreased at hours 3 and 8 (by 0.73°C and 0.71°C , respectively) after injections and remained below their control group's temperature during the dark and light phases. Repeated measures analysis revealed that there were no significant differences in mean hourly temperature based on experimental status or age during the dark phase. There were significant interactions between time and age-group, $F(11, 130) = 4.64$, $p < .0001$; time and experimental status, $F(11, 130) = 3.30$, $p = .0005$; and between time, age-group, and experimental status, $F(12, 130) = 1.97$, $p = .03$. Post hoc analysis confirmed the significant decrease in temperatures in the experimental groups overall (age groups combined) 3 hr after LPS injections (hour 6; $p = .0005$) and in the adult experimental group compared to the control group 3-hr post-LPS injections (hour 6), which was 1 hr after the conclusion of MWM training ($p = .0001$). This result was

Figure 2. Mean hourly temperature 24 hr after lipopolysaccharide or saline injections in adult and aged rats. Error bars represent $\pm$ SE (standard error of the mean). Arrow indicates time of injection. Dark horizontal bar indicates the dark period in a 12:12-hr dark–light cycle. *** $p < .001$ for experimental groups overall in comparison to control groups and for adult experimental group in comparison to control group at hour 6 during the dark phase.

followed by a significant increase in temperatures in the adult experimental group in the final 6 hr of the dark phase, comparing hour 6 to hours 7–12 ($p = .002$) and hour 7 to hours 8–12 ($p = .01$), which corresponded with the temperature increase in the experimental group compared to the control group. During the light phase, there were significant interactions between time and age-group, $F(11, 161) = 1.88$, $p = .044$, and age and experimental status, $F(1, 13) = 7.74$, $p = .015$. Post hoc comparison did not reveal significant relationships of interest (Figure 2).

During the second 24-hr postinjection period, the adult experimental group's temperatures were higher than were the adult control group's prior to and after the MWM through the dark phase and the first half of the light phase. The aged experimental group's temperatures were lower than the aged control group's temperatures through the dark phase, but were similar during the light phase. Temperatures in all four groups decreased after the animals returned from the MWM. While repeated measures analysis revealed a main effect of age, $F(1, 13) = 4.63$, $p = .05$, during the dark phase, as the temperatures in the aged groups were lower than those in the adult groups, there was not an experimental effect. There were significant interactions between time and age, $F(11, 161) = 6.95$, $p < .0001$ and age and experimental status, $F(1, 13) = 7.68$, $p = .015$. Post hoc analysis confirmed that the temperatures in the adult experimental group were significantly increased across the hours of the dark phase ($p = .014$) compared to those of the aged experimental group. Temperatures in the aged groups were significantly lower than those in the adult group at hour 10 ($p < .0001$), which was 1 hr after the conclusion of MWM testing in the aged animals. During the light phase, there was not a main effect of age or experimental status but there was a significant interaction between time and age, $F(11, 154) = 4.25$, $p < .0001$. There was a trend toward significance in the interaction between time and experimental group, $F(11, 154) = 1.77$, $p = .063$; Figure 3.

Figure 3. Mean hourly temperature 48 hr after lipopolysaccharide or saline injections in adult and aged rats. Error bars represent $\pm$ SE (standard error of the mean). Dark horizontal bar indicates the dark period in a 12:12-hr dark–light cycle. Dark phase: * $p < .05$ for adult experimental group in comparison to aged experimental group across hours 1–12; *** $p < .001$ for aged groups (experimental and control combined) at hour 10 in comparison to adult groups.

During the third 24-hr period after injections (data not shown), temperatures were similar between the experimental and control groups for the aged and adult animals. Temperatures again decreased in both age groups after the MWM testing. Repeated measures analysis revealed that there was not a main effect of experimental status or age during the dark phase. There were significant interactions between time and age, $F(11, 163) = 3.8$, $p < .0001$ and age and experimental status, $F(1, 13) = 5.7$, $p = .032$. Post hoc comparisons confirmed the decrease in temperature in the adult ($p = .02$) and aged groups ($p = .04$) following the conclusion of MWM testing. Adult experimental group temperatures were greater than those of the aged experimental group during the dark phase, though not significantly ($p = .07$). Repeated measures analysis of the temperatures during the light phase revealed a main effect of experimental status, $F(1, 14) = 24.41$, $p = .0002$; age, $F(1, 14) = 5.47$, $p = .034$; and a significant interaction between hour and age-group, $F(11, 164) = 2.28$, $p = .013$. Post hoc comparisons revealed that the temperature in the adult groups decreased over hours 14–24 compared to hour 13 ($p = .006$).

During the fourth and fifth 24-hr periods after injections, temperatures were generally similar between the control and experimental groups for both the adult and aged animals during the dark and light phases (data not shown). The temperature in the aged groups continued to decrease upon conclusion of MWM testing on Day 4, although the decrease was greater in the experimental group. Repeated measures analysis revealed that there was not a main effect of experimental status or age or an interaction between time and experimental status during the dark or light phases on either day.

Activity

Examination of motor activity counts before and after LPS administration revealed that hourly activity ranged from 0 to 18 with an hourly count of 4.7 or less accounting for 90% of all

Figure 4. Mean hourly activity at baseline in adult and aged rats. Error bars represent $\pm$ SE (standard error of the mean). Dark horizontal bar indicates the dark period in a 12:12-hr dark–light cycle. * $p < .05$ for the adult group in comparison to the aged group across the hours of the dark phase.

activity. These results confirm that this strain of rats tends to be sedentary. On the day before LPS injections, the hourly activity counts of the aged group were significantly less than those of the adult group only during the dark phase, with activity counts ranging from 0 to 11.89 in the adult group and 0 to 7.85 in the aged group ($M = 3.14 \pm \text{SEM} = .031$ vs. $M = 1.36 \pm \text{SEM} = .023$, respectively). During the light phase, hourly activity counts ranged from 0 to 13.08 in the adult group and 0 to 12.37 in the aged group ($M = 2.04 \pm \text{SEM} = .027$ vs. $M = 1.16 \pm \text{SEM} = .029$, respectively). Repeated measures analysis of baseline hourly motor activity during the dark phase showed a main effect of age, $F(1, 15) = 4.60$ $p = .048$ but no two-way interaction between time and age-group. During the light phase, there was neither a main effect of age ($p = .15$) nor an interaction between time and age-group (Figure 4).

Although hourly activity decreased slightly in all groups during the first 24-hr postinjections, repeated measures analysis did not show a main effect of experimental status ($p = .41$) or age ($p = .28$) during the dark phase. However, there was a trend toward significance in the three-way interaction between time, experimental status, and age, $F(34, 142) = 1.49$, $p = .057$. Post hoc analysis revealed that the adult experimental group significantly decreased activity comparing hour 3 ($p = .001$) to hour 5 (after LPS injections). During the light phase, there was not a main effect of experimental status ($p = .53$) or age ($p = .29$) or an interaction (Figure 5). During the dark and light phases on Days 2–5 postinjections, there was not a main effect of experimental status or age or an interaction between time and group.

Discussion

In the present study, we examined temperature and activity responses to LPS injection in animals training in the MWM. To our knowledge, this is the first study to examine these responses concurrently. The temperature response to LPS injection varied by age-group. While the temperature of both experimental groups decreased following LPS injections, the

Figure 5. Mean hourly activity 24 hr after lipopolysaccharide or saline injections in adult and aged rats. Error bars represent $\pm$ SE (standard error of the mean). Arrow indicates the time of injection. Dark horizontal bar indicates the dark period in a 12:12-hr dark–light cycle. *** $p < .001$ for the adult experimental group comparing hour 3 to hour 5.

decrease was greater and was followed by a temperature increase (fever) in the adult experimental group. The aged animals' temperatures eventually increased but not above baseline as with the adult experimental animals (i.e., the aged animals did not develop a fever).

The temperature in the adult experimental group decreased within 3 hr after LPS injections before increasing and returning close to baseline by the light phase of Day 2 postadministration. This initial temperature response differs from those observed by prior researchers who found that temperature in young rats increased within 1- to 3-hr postinjection of LPS or *E. coli* (Barrientos et al., 2009; Foster et al., 1992; Scarpace, Borst, & Bender, 1992). The delay in fever development in the present study could be the result of training in the MWM. In contrast, the aged experimental group's temperature decreased within 3 hr of LPS injections, although the decrease was less than that which we observed in the adult experimental group, and remained decreased in comparison to the control group until the light phase of the second day post-LPS administration. Our finding of an initial decrease in temperature in aged animals after LPS injection supports the work of others (Barrientos et al., 2009; Foster et al., 1992; Scarpace et al., 1992) but differs from those same studies in that we report that aged experimental animals did not go on to develop a fever. This finding is significant because Barrientos, Watkins, Rudy, and Maier (2009) also reported that temperature was elevated in aged animals for 3 days, a finding that parallels reports of persistence of some sickness responses in aged animals (Godbout et al., 2005). However, there are some differences in study protocols between the present study and that of Barrientos et al. (2009) that may account for the differences in results including training in the MWM versus no training, inclusion of between-group rather than within-group comparisons, administration of LPS versus *E. coli*, administration during the dark versus the light phase, and different rat strains. These results are important because there are few reports on the effects of LPS injection in rats over an extended period.

While the temperatures of the experimental groups generally decreased more than those of their control counterparts after MWM assessment, the decrease was significant only for the adult experimental group. These results indicate that the water temperature in the MWM does not completely account for the temperature change. Buchanan, Peloso, and Satinoff (2003) showed that aged animals develop a fever following administration of LPS in warmer environments, specifically in rooms with an ambient temperature of 31°C. The temperature of the animal room in this study (25°C) could account for the lack of temperature increase in aged animals. However, the warm water temperature (35°C) did not result in a temperature increase in the aged group. Further research is needed to characterize the temperature response in this rat strain, as it is an accepted model of aging.

We administered LPS during the dark phase to assess animals during their active period when their temperatures are typically higher. The adult experimental groups' temperatures were higher than those of the aged experimental group during the first 24-hr postinjection, and this difference became significant during the first 12 hr of Day 2 postinjections. These results show that aged animals did not develop a fever even when LPS was administered during their normal active period. Therefore, the change in temperature is likely not due to the animals' circadian patterns.

LPS generally produces temperature responses that are similar to those produced by a bacterial infection (Roth & Blatteis, 2014). The mechanism for the development of fever during infection is likely mediated by cytokines or Toll-like receptor (Dinarello, 2004). Whatever the mechanism, diminished temperature responses in aged animals have implications for the care of the elderly experiencing infections. A lack of temperature spike in elderly individuals suffering from an infection could result in delayed diagnosis and treatment for these patients (Liang & Mackowiak, 2007), which could affect outcomes. Scrutiny of elderly patients' behaviors to promote early detection of and intervention for infections is critical to providing high-quality care for this population.

Examination of baseline activity in the present study revealed that, while the hourly activity counts were minimal, activity was significantly higher during the dark phase and highest in the adult group in both phases, which is comparable to other results with Brown-Norway rats (Gordon et al., 2014). However, the hourly activity counts of both age groups were less at baseline than they were for rats of the strain (F344 × BN F1) Barrientos et al. (2009) used in a prior study. In the present study, activity was decreased following LPS injections only in the adult experimental group during part of the dark cycle on the day of LPS administration. In contrast, Barrientos and colleagues (2009) found that the activity was significantly decreased in both age groups. The results from the present study are unusual in that LPS administration typically results in activity decreases (Kozak et al., 1995), which researchers have found to be prolonged in aged animals (Abraham & Johnson, 2009; Godbout et al., 2005; Huang, Henry, Dantzer, Johnson, & Godbout, 2008). Our results may reflect the sedentary

nature of the Brown-Norway rat. The minimal motor activity we noted may not be a sensitive indicator of sickness for these animals. This finding is important because this strain is an accepted model of aging. Also, we assessed activity in their home cage and not in a novel environment that could result in a decreased motivation to explore (McCusker & Kelley, 2013). Finally, our small sample size might have limited our ability to detect an LPS effect. LPS did not affect activity during the remaining days (2–5) postinjection, which is similar to the findings of Barrientos and colleagues (2009). While some investigators have found that the LPS-induced decrease in activity is sustained in aged animals (Abraham & Johnson, 2009; Godbout et al., 2005), few have studied this response beyond 24 hr.

Conclusion

Our findings demonstrate that both adult and aged Brown-Norway rats display temperature changes in response to an immune challenge. The aged animals did not mount a fever in response to LPS injection rather they became hypothermic. Absence of fever does not equate with absence of infection in elderly individuals. Nurses need to be aware of the patients most at risk of infections and monitor them carefully for a broader spectrum of sickness symptoms, not relying solely on the presence of fever. Nurses also need to recognize which sickness behaviors are helpful and necessary and which are deleterious. For example, a lack of interest in usual activities may conserve energy, but when does a lack of fever or decreased activity affect outcomes? Understanding more fully the development and timing of such sickness behaviors will allow for the development of more appropriate interventions for this vulnerable population.

Author Contributions

Barbara J. Kupferschmid contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Barbara Therrien contributed to conception, design, acquisition, analysis, and interpretation; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Pamela J. Rowsey contributed to design, analysis, and interpretation; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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References

- Abraham, J., & Johnson, R. W. (2009). Central inhibition of interleukin-1 β ameliorates sickness behavior in aged mice. *Brain, Behavior, and Immunity*, 23, 396–401. doi:10.1016/j.bbi.2008.12.008
- Barrientos, R. M., Higgins, E. A., Biedenkapp, J. C., Sprunger, D. B., Wright-Hardesty, K. J., Watkins, L. R., ... Maier, S. F. (2006). Peripheral infection and aging interact to impair hippocampal memory consolidation. *Neurobiology of Aging*, 27, 723–732. doi:10.1016/j.neurobiolaging.2005.03.010
- Barrientos, R. M., Watkins, L. R., Rudy, J. W., & Maier, S. F. (2009). Characterization of the sickness response in young and aging rats following *E. coli* infection. *Brain, Behavior, and Immunity*, 23, 450–454. doi:10.1016/j.bbi.2009.01.016
- Buchanan, J. B., Peloso, E., & Satinoff, E. (2003). Thermoregulatory and metabolic changes during fever in young and old rats. *American Journal of Physiology—Regulatory, Integrative & Comparative Physiology*, 285, R1165–R1169. doi:10.1152/ajpregu.00238.2003
- Büla, C. J., Ghilardi, G., Wietlisbach, V., Petignat, C., & Francioli, P. (2004). Infections and functional impairment in nursing home residents: A reciprocal relationship. *Journal of the American Geriatrics Society*, 52, 700–706. doi:10.1111/j.1532-5415.2004.52205.x
- Dinarello, C. A. (2004). Infection, fever, and exogenous and endogenous pyrogens: Some concepts have changed. *Journal of Endotoxin Research*, 10, 201–222. doi:10.1179/096805104225006129
- Florez-Duquet, M., Peloso, E., & Satinoff, E. (2001). Fever and behavioral thermoregulation in young and old rats. *American Journal of Physiology—Regulatory, Integrative & Comparative Physiology*, 280, R1457–R1461.
- Foster, K. D., Conn, C. A., & Kluger, M. J. (1992). Fever, tumor necrosis factor, and interleukin-6 in young, mature, and aged Fischer 344 rats. *American Journal of Physiology*, 262, R211–R215.
- Gavazzi, G., & Krause, K. H. (2002). Ageing and infection. *Lancet Infectious Disease*, 2, 659–666. doi:10.1016/S1473-3099(02)00437-1
- Godbout, J. P., Chen, J., Abraham, J., Richwine, A. F., Berg, B. M., Kelley, K. W., & Johnson, R. W. (2005). Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. *FASEB Journal*, 19, 1329–1331. doi:10.1096/fj.05-3776fje
- Gordon, C. J., Johnstone, A. F., Aydin, C., Phillips, P. M., MacPhail, R. C., Kodavanti, U. P., ... Jarema, K. A. (2014). Episodic ozone exposure in adult and senescent Brown Norway rats: Acute and delayed effect on heart rate, core temperature and motor activity. *Inhalation Toxicology*, 26, 380–390. doi:10.3109/08958378.2014.905659
- Huang, Y., Henry, C. J., Dantzer, R., Johnson, R. W., & Godbout, J. P. (2008). Exaggerated sickness behavior and brain proinflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide. *Neurobiology of Aging*, 29, 1744–1753. doi:10.1016/j.neurobiolaging.2007.04.012
- Iivonen, H., Nurminen, L., Harri, M., Tanila, H., & Puolivali, J. (2003). Hypothermia in mice tested in Morris water maze. *Behavioural Brain Research*, 141, 207–213. doi:10.1016/S0166-4328(02)00369-8
- Kelley, K. W., Bluthé, R. M., Dantzer, R., Zhou, J. H., Shen, W. H., Johnson, R. W., & Broussard, S. R. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity*, 17, S112–S118. doi:10.1016/S0889-1591(02)00077-6
- Kozak, W., Conn, C. A., & Kluger, M. J. (1994). Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. *American Journal of Physiology*, 266, R125–R135.
- Kozak, W., Zheng, H., Conn, C. A., Soszynski, D., van der Ploeg, L. H., & Kluger, M. J. (1995). Thermal and behavioral effects of lipopolysaccharide and influenza in interleukin-1 β -deficient mice. *American Journal of Physiology*, 269, R969–R977.
- Kupferschmid, B. J., & Therrien, B. A. (2018). Spatial learning responses to lipopolysaccharide in adult and aged rats. *Biological Research for Nursing*, 20, 32–39.
- Liang, S. Y., & Mackowiak, P. A. (2007). Infections in the elderly. *Clinics in Geriatric Medicine*, 23, 441–456, viii. doi:10.1016/j.cger.2007.01.010
- Lindner, M. D., & Gribkoff, V. K. (1991). Relationship between performance in the Morris water task, visual acuity, and thermoregulatory function in aged F-344 rats. *Behavioural Brain Research*, 45, 45–55. doi:10.1016/S0166-4328(05)80179-2
- McCusker, R. H., & Kelley, K. W. (2013). Immune-neural connections: How the immune system's response to infectious agents influences behavior. *Journal of Experimental Biology*, 216, 84–98. doi:10.1242/jeb.073411
- Peloso, E. D., Florez-Duquet, M., Buchanan, J. B., & Satinoff, E. (2003). LPS fever in old rats depends on the ambient temperature. *Physiology and Behavior*, 78, 651–654. doi:10.1016/S0031-9384(03)00046-5
- Refinetti, R. (2010). The circadian rhythm of body temperature. *Frontiers in Bioscience*, 15, 564–594.
- Roth, J., & Blatteis, C. M. (2014). Mechanisms of fever production and lysis: Lessons from experimental LPS fever. *Comprehensive Physiology*, 4, 1563–1604. doi:10.1002/cphy.c130033
- Roth, J., Rummel, C., Barth, S. W., Gerstberger, R., & Hubschle, T. (2006). Molecular aspects of fever and hyperthermia. *Neurologic Clinics*, 24, 421–439. doi:10.1016/j.ncl.2006.03.004
- Roth, J., Rummel, C., Barth, S. W., Gerstberger, R., & Hubschle, T. (2009). Molecular aspects of fever and hyperthermia. *Immunology and Allergy Clinics of North America*, 29, 229–245. doi:10.1016/j.iac.2009.02.005
- Scarpace, P. J., Borst, S. E., & Bender, B. S. (1992). The association of *E. coli* peritonitis with an impaired and delayed fever response in senescent rats. *Journal of Gerontology*, 47, B142–B145. doi:10.1093/geronj/47.4.B142
- Shaw, K. N., Commings, S., & O'Mara, S. M. (2001). Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rat dentate gyrus. *Behavioural Brain Research*, 124, 47–54. doi:10.1016/S0166-4328(01)00232-7

- Turturro, A., Witt, W. W., Lewis, S., Hass, B. S., Lipman, R. D., & Hart, R. W. (1999). Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 54, B492–B501.
- Vorhees, C. V., & Williams, M. T. (2014). Assessing spatial learning and memory in rodents. *ILAR Journal*, 55, 310–332. doi:10.1093/ilar/ilu013
- Wachulec, M., Peloso, E., & Satinoff, E. (1997). Individual differences in response to LPS and psychological stress in aged rats. *American Journal of Physiology*, 272, R1252–R1257.
- Wrotek, S., Jedrzejewski, T., Potera-Kram, E., & Kozak, W. (2011). Antipyretic activity of N-acetylcysteine. *Journal of Physiology and Pharmacology*, 62, 669–675.