Effects of low-dose lipopolysaccharide and age on spatial learning in different Morris water maze protocols

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Abstract

Objectives: Animals administered lipopolysaccharide exhibit dose-related sickness behaviors (decreased food intake, weight loss, and cognitive changes). While research has demonstrated that spatial learning is impaired following a lipopolysaccharide immune challenge, the results differ depending on the methodology used to evaluate spatial learning. Additionally, few studies have evaluated the effects of low-dose lipopolysaccharide on spatial learning. Therefore, we assessed spatial learning, food intake, and weight changes in adult and aged rats after a low-dose lipopolysaccharide immune challenge in the Morris water maze using two water temperatures.

Methods: Adult (5–6 months) and aged (22 months) male Brown-Norway rats were administered either 50 or 100 µg/kg lipopolysaccharide or saline, and then tested in the Morris water maze for 5 days, rested for 7 days, and later underwent 2 days of retention tests. Probe trials were conducted at the end of initial and retention testing.

Results: Low-dose lipopolysaccharide administration did not result in food intake or weight changes. While the aged experimental group took longest to improve directional heading error in both cold and warm water, heading error was greater in cold water. Behavioral testing revealed an apparent age and water temperature effect on swim time. Retention and probe trial results showed that aged experimental animals had the worst performance in cold water.

Conclusion: We conclude that while low-dose lipopolysaccharide did not result in typical sickness behaviors (decreased food intake or weight), spatial learning and memory were impaired in the aged experimental group. These results have important implications for the care of elderly individuals experiencing mild to moderate infections.

Introduction

The role of infections in older individuals and the effects on cognition is an area of considerable research. In both humans and animals, infections result in a constellation of responses called sickness behaviors. In humans, these responses may include anorexia, decreased activity, fever, a loss of interest in normal activities, or the inability to concentrate.[1,2] Even a mild infection, such as a common cold, results in performance and mood changes. For example, individuals with an upper respiratory tract illness were shown to be slower at encoding new information and less alert.[3] A mild infection can also lead to an inability to participate in normal activities such as work or school. More than 70% of patients who consulted a physician due to lower respiratory tract infection reported an inability to participate in normal or social activities for at least 1 week, with work or school absences averaging 4 days.[4] There is evidence that infections may have a greater effect in the brain of the aged.[5] For example, adults with upper respiratory tract infections exhibited decreases in the speed of memory processing, but this was

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more pronounced in aged adults who were also less alert.[6] In older adults, hospitalization for pneumonia, irrespective of illness severity, was associated with new impairments in daily living activities, an increase in moderate-to-severe cognitive impairment,[7] or a shorter time for the development of dementia.[8] Individuals hospitalized with severe sepsis also had an increase in limitations in daily living activities and moderate-to-severe cognitive impairment.[9] While many individuals return to normal activity following an infection, responses to even mild infections should be studied and addressed. Research directed at preventing infections or providing earlier treatment for infections may help limit disability and/or functional impairment, especially in the elderly.

Animal models are useful in increasing the understanding of the behavioral changes occurring during infections in humans. Lipopolysaccharide (LPS), an endotoxin, is administered to animals to induce an immune challenge that mimics an infection in humans. In response, animals that become sick decrease activity and/or food intake,[1] behaviors that are greater and/or prolonged in aged animals.[10,11] Effects of an LPS challenge have also been shown to be dose-related with the highest doses resulting in the greatest weight loss.[12,13] However, cognitive problems, specifically spatial learning deficits, occur following administration of high-dose LPS[14,15] or low-dose LPS,[16] while aged animals also display long-term memory deficits following an immune challenge with *Escherichia coli*.[17] To date, few studies have evaluated the effects of low-dose LPS administration and age on spatial learning. In this study, we induced low-grade inflammation in adult and aged rats by administering low-dose intraperitoneal (ip) LPS.

Spatial learning, which is a form of hippocampal-dependent learning and memory, is the ability to acquire information about a place in order to navigate through the environment; it involves searching and exploring. In both humans and animals, the ability to navigate or wayfind is critical for normal functioning.[18] In this study, the Morris water maze (MWM) was used to assess each animal's ability to learn. Most studies examine spatial learning using water temperatures between 20°C and 22°C.[19] We initially tested animals in the MWM using water at 20°C ± 1°C. Because the body temperature in the adult and aged animals decreased after testing (data not shown), we decided to evaluate animals in either cold water (20°C ± 1°C) or warm water (35°C ± 1°C). Examination of studies with different MWM water temperatures reveals that the results vary even with subtle differences in water temperature. For example, while examining parameters affecting motivation in the MWM, Gibertini[20] found that the spatial learning performance, assessed by swim latency, in mice given interleukin-1β (IL-1β) was not different from control animals in cold water (18°C), but was impaired in warmer water (23°C). Shaw et al.[16] found that in animals administered 100 μg/kg LPS and evaluated in the MWM using 20°C water, the animals receiving a single injection of LPS displayed increased swim latencies on day 4 and swam the least direct route to the hidden platform and a longer distance on day 5 compared to control animals or animals administered daily LPS. In contrast, mice administered a single injection of 250 μg/kg LPS, and evaluated in 20°C water in the MWM, displayed increased swim latencies on days 1 and 3, while mice administered daily injections showed this deficit on days 1 to 4.[21] Older animals administered daily LPS (1-year-old mice compared to 2-month-old) displayed increased swim latencies, in comparison with older animals receiving a single injection, but no differences in distance swam.[22] In another study, aged animals administered *Escherichia coli* and assessed in 26°C water, while initially showing increased swim latencies, did improve their swim latency by the end of trials.[17] Although some of the differences in the results of these studies can be explained by variations in protocols, there is clearly a research gap requiring further study.

Despite existing studies, few have evaluated the effects of low-dose LPS on spatial learning in rats. Furthermore, the results with spatial learning vary depending on the MWM methodology used for evaluation. The aims of this pilot study were twofold: (1) to assess effects of low-dose LPS on cognition (spatial learning) and physical sickness responses (food intake and weight changes) in adult and aged rats and (2) to investigate the effects of cold and warm water temperatures on spatial learning indices.

Methods

Adult (5–6 months) and aged (22 months) male Brown-Norway rats from Harlan Sprague Dawley Inc. were used. Animals were kept in individual cages on a reverse 12-h light-dark cycle so that testing occurred during the animals' active period. The temperature in the animal room was controlled at 25°. Standard rodent food and water were provided. The protocol was approved by the University of Michigan Animal Care and Use Committee.

Spatial navigation protocol

Spatial learning, using place navigation, was assessed using the MWM.[23,24] Place learning is simulated using a large tub with fixed external environmental cues. The animal is required to locate a hidden platform permanently positioned 1 cm under the surface of the water and 15 cm from the tub wall in the same quadrant; to learn, animals must use a fixed array of external cues providing spatial information located outside of the water tub. While water temperatures of 20°C–22°C are standard,[19] our initial pilot testing revealed that animal temperature decreased after MWM testing. As a result, we decided to evaluate animals in either 20°C ± 1°C (cold water [CW]) or 35°C ± 1°C (warm water [WW]). Each animal was then placed in a holding box for a minimum of 2 min between trials.

Because sickness responses, such as fever, occur at different times in adult and aged animals after LPS administration,[25,26]

testing in the MWM was initiated at different times in adult (1.5 h) and aged (5 h) animals. Testing involved four trials per day for 5 days which is sufficient for healthy animals to reach asymptotic performance and to allow for examination of learning curves.[27] Following initial learning testing, there were 7 days of rest and 2 days of retention (memory) tests. Spatial learning was evaluated by recording directional heading error (DHE) and swim time latency. DHE was computed by recording the initial heading position of the animal and comparing that to the most direct path to the platform to calculate the degree of difference. This was recorded by the primary investigator after an inter-rater reliability of .90 was established. The time in seconds to reach the platform was recorded as swim time latency. If the animal did not reach the goal in 180 s, it was removed from the water and placed on the platform.

Animals that learn the goal location swam directly and rapidly to the platform. Probe or memory trials are used to assess whether the animal learned the location of the platform. In this study, probe trials were conducted at the end of initial (day 5) and retention (day 2) testing. During probe trials, the animal can swim freely for 30 s with the platform removed. In the absence of the goal platform, an animal that has learned the location will begin searching the local area. The animals' path was recorded and traced to calculate the percent of the distance traveled in the correct quadrant.

LPS

LPS (50 or 100 μg/kg) from *Escherichia coli* (serotype 0111:B4; Sigma–Aldrich Corporation, St. Louis, MO) or 0.9% normal saline were administered ip. The procedure to reconstitute LPS was described previously.[15] Sample sizes were the following: CW—50 μg/kg LPS ($n=1$ per age group), 100 μg/kg LPS ($n=1$ per age group), or saline ($n=2$ per age group); WW—100 μg/kg LPS (adult: $n=2$; aged: $n=4$) or saline (adult: $n=2$; aged: $n=4$). Because only one animal in each age group received either 50 or 100 μg/kg LPS in CW, those animals were combined into one experimental group for the purpose of analysis.

Statistical analysis

SAS Proc Mixed (SAS Institute, Cary, NC) was used to analyze the data as described previously.[15] Models with homogeneous error variances among the groups were also considered with the best fit determined using Model fit statistics. Using the Mixed procedure, the fixed effects of experimental status (control vs experimental), age (adult vs aged), day (days 1 to 5; day 5 vs retention days), water (cold vs warm) and the interactions between experimental status, age, day, and water were examined, while allowing the variances of the random measurement errors to vary across the groups. All possible interactions were tested and those found not significant were dropped from the final model.

The swim time latency and DHE data collected across four trials were averaged by day using IBM SPSS 22.0.2 (Chicago, IL). Initial models revealed that error residuals were not normally distributed, so the swim time and DHE responses were log transformed. The transformed variables were used in all SAS analyses, while the actual data were used in all figures. The probe trial data were calculated as the percentage of the distance traveled in the goal quadrant compared to the other three quadrants. T-tests or Mann–Whitney *U*-tests were performed to assess whether there were differences in group means.

Results

Food intake

Food intake and weight were used to determine the presence of sickness behaviors from LPS injection. There were no differences in average food intake at baseline before LPS injections between the adult and aged groups ($p=.64$) tested in CW. However, there were significant differences in food intake between the adult and aged groups ($p=.003$) tested in WW, with aged animals consuming less food prior to LPS injections. Analysis of food intake following LPS administration revealed that there was not an experimental or age effect or effect of days. However, there were significant differences in mean food intake across the CW and WW conditions for all the groups ($F(1, 16)=11.52, p=.003$). There was also a significant interaction between water temperature and days post-LPS injection ($F(4, 72)=5.88, p=.0004$), suggesting that the pattern of food intake across the post-injection days varied from CW to WW. Post hoc comparisons revealed that there were significant differences in food intake on the second day post-LPS administration between animals placed in CW or WW. The four groups tested in CW generally consumed more food ($p<.0001$) (Figure 1).

Weight

Prior to LPS administration at baseline, there were no differences in weight between the control and the experimental groups in the adult ($p=.23$) or aged ($p=.93$) groups. Analysis revealed significant differences in mean weight loss among the groups across the days based on age ($F(1, 15)=9.33, p=.008$) and across the days for all groups ($F(4, 76)=3.27, p=.015$), but there was no experimental effect. However, there was a significant interaction between water temperature and experimental status ($F(1, 15)=9.88, p=.0067$), suggesting that the pattern of weight loss varied between the control and the experimental groups across the CW and WW conditions. Post hoc comparisons confirmed that while there were differences in weight loss between the control and the experimental groups across the days in CW ($p=.029$), the experimental groups either lost less weight or lost weight for fewer days. However, there were no differences between the
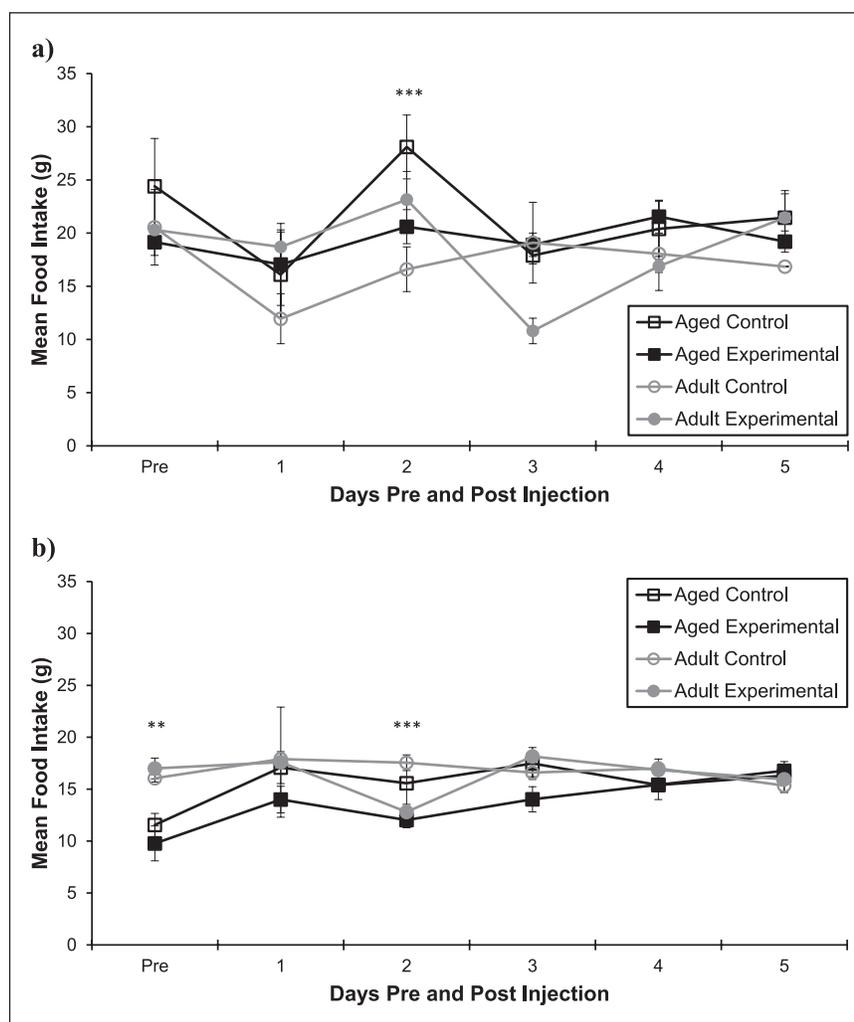
Figure 1. Effect of lipopolysaccharide on mean food intake across the days for aged and adult rats tested in (a) cold water and (b) warm water. Error bars represent ±SE (standard error of the mean). **$p < .01$ for the aged groups (experimental and control groups combined) in comparison with the adult groups on the pre-injection day in warm water (b). ***$p < .001$ for all four groups comparing cold and warm water on post-injection day 2. Cold water, $n = 2$ per group; warm water, $n = 4$ per aged groups, $n = 2$ per adult groups.

groups in WW ($p = .69$). The difference in weight loss within the experimental groups between water conditions approached significance ($p = .07$) with experimental groups losing more weight in the WW condition compared to the CW condition (Figure 2).

Spatial learning

Repeated measures analysis revealed significant differences in mean DHE for all groups across the 5-days post-injections ($F(4, 76) = 7.02$, $p < .0001$), indicating that DHE improved over time. There was no effect of age, experimental status, or water temperature. While the DHE improved over the 5-days post-LPS, examination of the learning curves revealed differences as the degree of error varied among the four groups in both water conditions, although not statistically significant (Figure 3). The aged experimental group displayed the

poorest DHE in CW and took longest to improve DHE in both CW and WW. In CW, the aged experimental group displayed an increased DHE on day 2 post-LPS administration and then had decreases in DHE parallel with the other groups. In WW, although both aged control and experimental groups displayed an increased DHE on the second day post-LPS, the DHE of the experimental group remained highest of all four groups on day 3. The learning curves of the adult groups were similar in CW and the adult experimental group displayed a better DHE than the control group in WW, although not statistically significant (Figure 3).

To examine memory, DHE on the last day of testing was compared to performance on the two retention days. Analysis revealed that while there were no differences between the groups based on experimental status ($p = .89$) or water ($p = .80$), indicating that animals learned the location of the platform, examination of the learning curves revealed
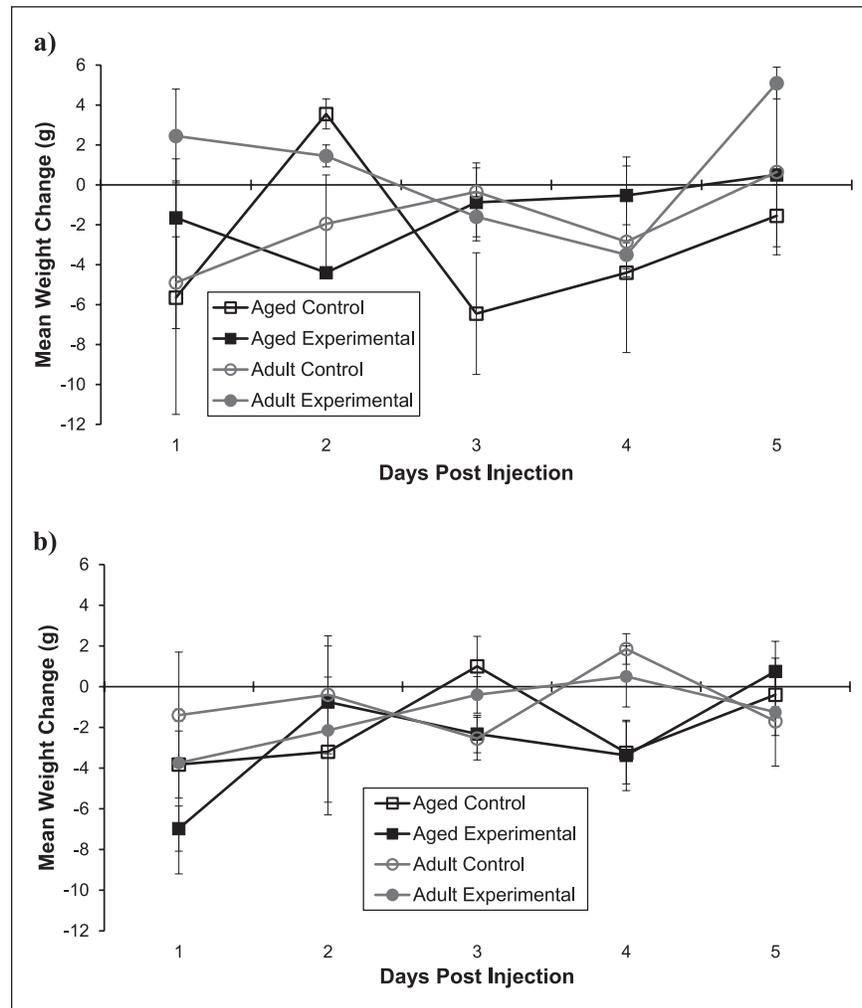
Figure 2. Effect of lipopolysaccharide on mean weight loss across the days for aged and adult rats tested in (a) cold water and (b) warm water. Error bars represent ±SE (standard error of the mean). Cold water, $n = 2$ per group; warm water, $n = 4$ per aged groups, $n = 2$ per adult groups.

differences between the groups. The aged experimental group's DHE worsened in the CW condition over the retention days while improving in the WW. In contrast, both adult groups' DHE increased in CW on the first retention day before improving on the second day, while in WW, their DHE on the first retention day was similar to the last testing day before increasing on the second retention day.

Analysis of swim time latency revealed significant differences in mean swim time across the 5 days following LPS injections for all groups ($F(4, 76) = 17.07, p < .0001$), indicating swim time improved over time, and among the groups across the days based on age ($F(1, 16) = 13.23, p = .002$), suggesting improvement was different between the age groups. Analysis also indicated that swim latency improvement differed based on water temperature ($F(1, 16) = 5.66, p = .03$). There was no effect of experimental status. Post hoc comparisons revealed a significant difference in swim latency between days 1 and 2 compared to days 3 to 5 ($p = .006$), indicating that swim latency improved in all groups by day 4. While

examination of swim latency learning curves revealed that all four groups improved in both water conditions, the aged experimental group exhibited the highest latency and took longest to improve in WW, although not statistically significant (Figure 4). In fact, the aged experimental group displayed the highest swim time latency in WW on day 3. A Mann–Whitney *U*-test revealed significant differences in swim time latency on trial day 3 in WW between the aged experimental ($Md = 36.4, n = 4$) and control groups ($Md = 13.5, n = 4, z = -2.30, p = .021$) but not in the adult groups or CW.

Evaluation of the swim time latency over the retention days in comparison with the last test day revealed differences in mean swim time latency across the 3 days for all groups ($F(2, 36) = 8.18, p = .0012$) and among the groups based on age ($F(1, 15) = 26.75, p = .0001$). This analysis also revealed significant interactions between age and water ($F(1, 15) = 6.52, p = .022$), and day and water ($F(2, 36) = 4.17, p = .023$). Evaluation of swim latency learning curves revealed that in CW, the aged groups, especially the experimental
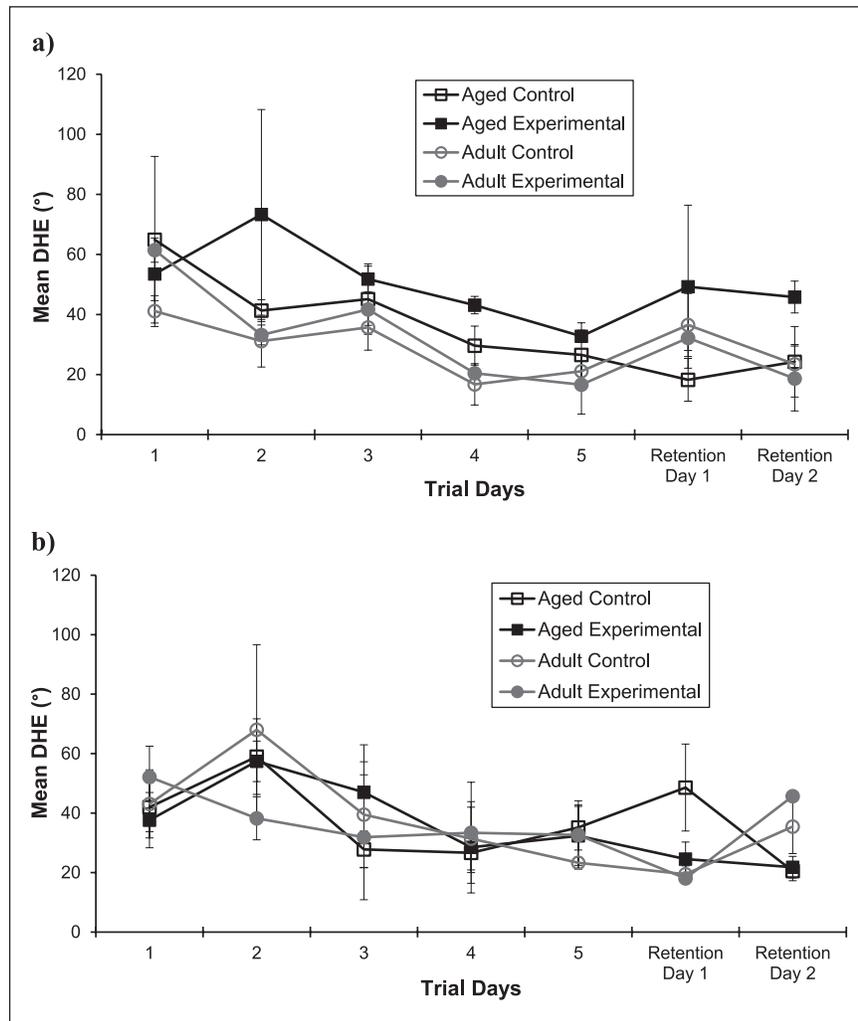
Figure 3. Effect of lipopolysaccharide on mean directional heading error in adult and aged rats tested in (a) cold water and (b) warm water. Error bars represent ±SE (standard error of the mean). Cold water, $n = 2$ per group; warm water, $n = 4$ per aged groups, $n = 2$ per adult groups.

group, displayed greater swim latency than the adult groups. In WW, the swim latency was similar and improved in all four groups by the last day. Post hoc comparisons confirmed the significant differences in swim time latency between the aged and adult groups in CW ($p = .001$) but not WW ($p = .18$). There also were significant differences in mean swim latency in WW only over the retention days between the last trial day and retention day 2 ($p = .0034$), and between the first and second retention day ($p = .024$), a finding that confirms the improvement in swim latency in all four groups over the retention days in WW.

Analysis of the percentage of the distance traveled in the correct quadrant in the probe trials revealed that there was a significant effect of time across the groups ($F(1, 19) = 7.26$, $p = 0.01$), indicating all groups improved their performance over time. There was no effect of age, experimental status, or water temperature. While there were no statistically significant differences in the distance traveled in the correct

quadrant, examination of Figure 5 shows that there were differences in the patterns among the groups. In the CW condition, the aged experimental group traveled the least percent of distance in the correct quadrant and the distance was similar from probe trials 1 to 2. During probe trial 2 in CW, although there was no statistically significant difference, the aged experimental group traveled less in the correct quadrant compared to the control group ($M = 35.5 \pm SE = 10.57$ vs 72.2 ± 13.44, respectively). The adult experimental group traveled the greatest distance in the correct quadrant in probe trial 1 with a slight decrease in probe trial 2, although not statistically significant (Figure 5). In WW, the performance of the aged experimental group was similar or slightly improved in probe trials 1 and 2 compared to their results in CW while the adult experimental group performance was worse in both. When comparing the performance of the experimental groups, the aged experimental group, in comparison with the adult group, traveled the least percent of the
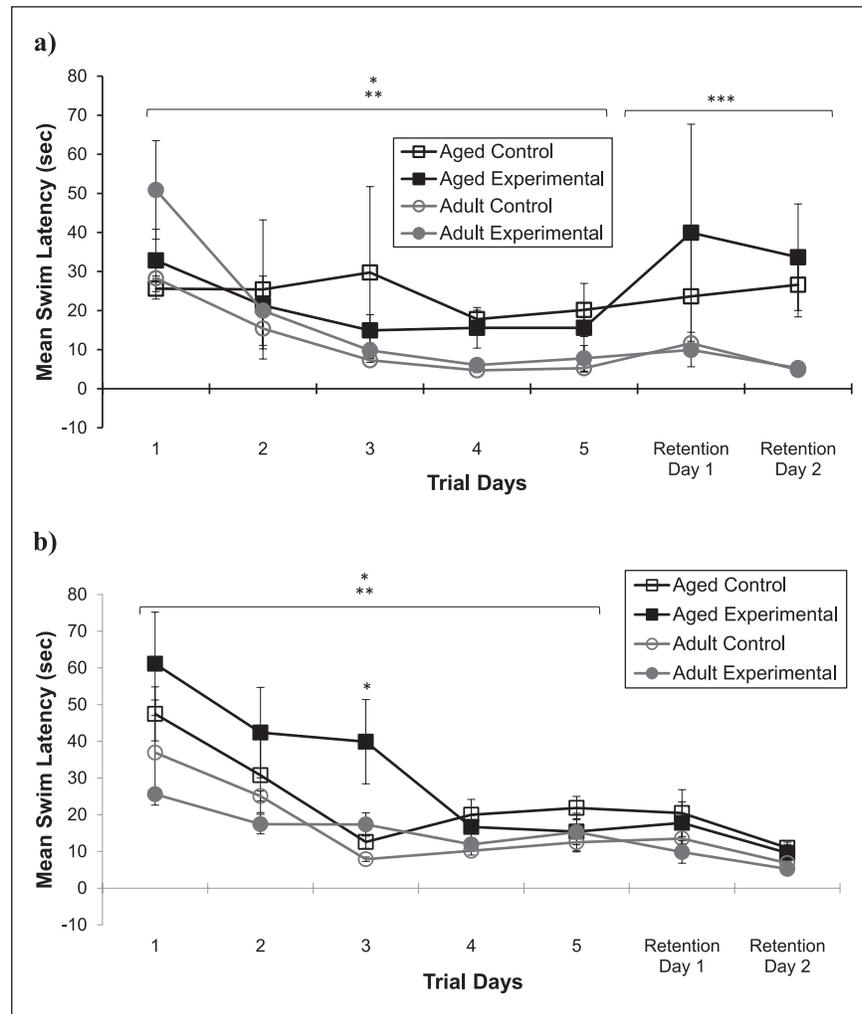
Figure 4. Effect of lipopolysaccharide on mean swim latency in adult and aged rats tested in (a) cold water and (b) warm water. Error bars represent ±SE (standard error of the mean). Trial days 1–5: main effects—*p < .05 for water and **p < .01 for age (control and experimental groups combined) for days 1–5 over both water conditions; *p < .05 for aged experimental and control groups on post-injection day 3 in (b) warm water. Retention days: ***p < .001 for aged groups (control and experimental combined) in comparison with adult groups in (a) cold water. Cold water, n = 2 per group; warm water, n = 4 per aged groups, n = 2 per adult groups.

distance in the correct quadrant in both probe trials and water conditions. The mean difference between the two experimental groups approached significance during probe trial 1 in CW ($t = -3.835$, $df = 2$, $p = 0.062$).

Post hoc comparisons of the means revealed that there were significant differences in the percentage spent in the correct quadrant across the groups and water conditions between the first probe trial in comparison with the second probe trial ($p = .01$). The overall mean percentage increased from 40.74 ± 3.14 versus 52.59 ± 3.14 from the first to second probe trial.

Discussion

Although there were differences in the patterns of food intake and weight loss for animals tested in both water temperatures, low-dose LPS had no effect on food intake or

weight, and the animals did not demonstrate evidence of sickness. However, water temperature influenced food intake with all four groups consuming more food in CW on some days. While exposure to cold has been shown to result in increased food intake,[28,29] the amount of time animals spent in cold water was minimal. These results with low-dose LPS are similar to other studies where LPS administration resulted in a dose-related weight decrease, with the highest doses resulting in the greatest weight loss.[12,13] In other words, with an LPS dose as low as those used in this study, the results of other studies would lead us to expect the animals to demonstrate little to no weight loss.

While the analysis of DHE did not reveal an experimental effect of low-dose LPS or differences based on water temperature, the small sample sizes could have limited the ability to detect experimental effects. However, assessment of the DHE patterns indicates that low-dose LPS may affect the
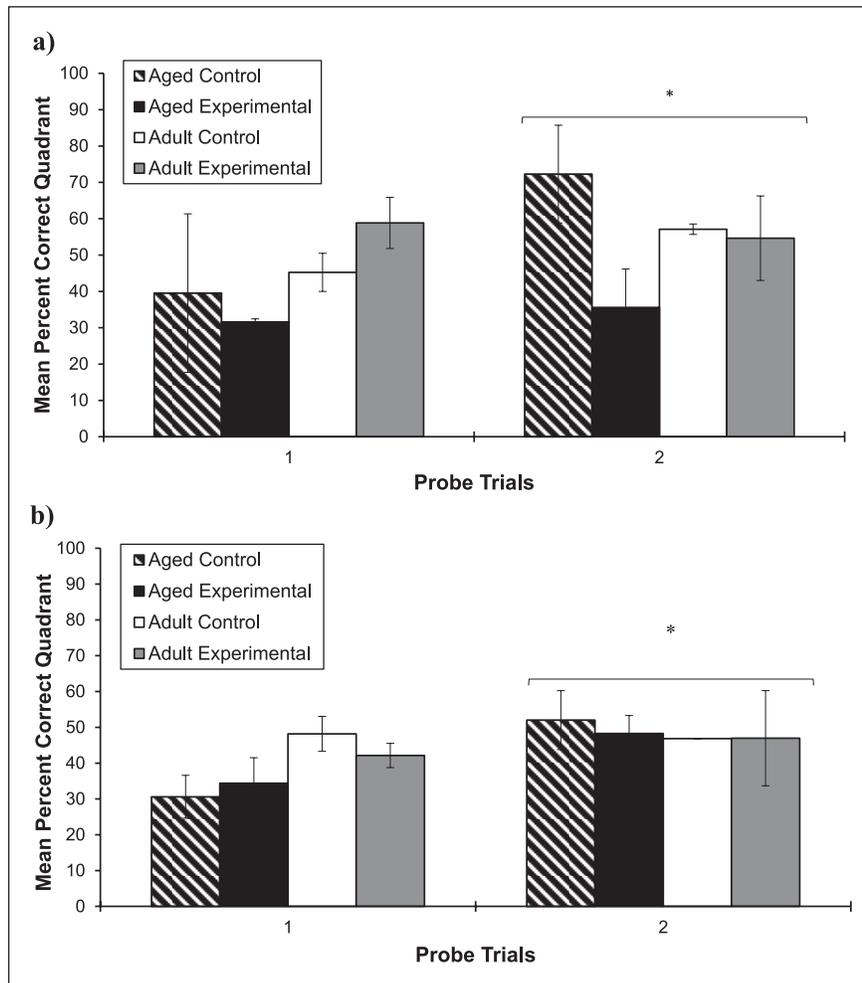
Figure 5. Effect of lipopolysaccharide on mean percent of distance traveled in the correct quadrant in each probe trial (1 and 2) in adult and aged rats tested in (a) cold water and (b) warm water. Error bars represent ±SE (standard error of the mean). *p < .05 across the groups and water conditions for the second probe trial in comparison with the first. Cold water, n = 2 per group; warm water, n = 4 per aged groups, n = 2 per adult groups.

DHE of aged animals in both water temperatures. Additional research with a larger sample size is needed to explore the effects of low-dose LPS further.

Although the differences in the swim latencies were not statistically significant, the aged experimental animals displayed the greatest swim latency and took longest to improve in WW. This is an interesting result as most studies finding increased latencies are using a colder water temperature.[16,21] While Gibertini[20] found that adult animals given an IL-1 β challenge displayed longer latencies in water at 23°C and shorter swim time latencies in colder water (18°C), the water temperatures for both groups were less than the WW temperature in our study. Our results with the pattern of swim time latency in aged animals in WW are similar to others who found that while swim time latency was increased initially in older animals in CW (26°C), there was no effect of *Escherichia coli* and performance improved.[17] However, their swim latency times were lower. In our study, sample sizes were small, and a larger sample size may improve the

ability to detect an experimental effect with DHE or swim time latency.

Interestingly, while experimental animals did not display classic appetite and weight loss sickness behaviors, the aged experimental animals exhibited a trend toward difficulty learning, as indicated by elevated DHE and swim time, and they still displayed difficulties with spatial memory (retention), especially in the CW condition. This indicates that the animals were generally able to encode their memory but had difficulty with memory consolidation even while not overtly sick. The probe trial performance of the adult experimental group in WW also indicates difficulty with spatial memory. The question of why the aged experimental group did not display sickness behaviors, such as decreased food intake, but did have spatial learning deficits is an interesting one. This result underscores the fact that cognitive and physical components and attributes of sickness behaviors exist, but suggests that each may function independently in some circumstances. In other words, while the components often may

be observed moving in parallel, they do not necessarily travel together. This influences the design and analyses of further research as well as interpretations of results. Increased swim latency, in the absence of weight loss, was also noted by others.[14] However, because large LPS doses (400–800 µg/kg of body weight) were administered to 8-week-old mice, the results may not be comparable to this study. Nevertheless, that study demonstrated that, in the absence of weight loss, animals (mice) might still have learning difficulties. The DHE and probe trial results with aged experimental animals in the CW condition need further investigation. The ability to discern an experimental effect could have been restricted by the small sample size.

Water temperature may be an important factor for future investigation when evaluating spatial learning following an immune challenge, especially in aged animals. The performance of animals in this study, as indicated by various spatial learning indices, varied in different water temperatures. Water temperature has been noted to have different effects on animal performance.[30] Indeed, lower water temperature has been noted to result in delayed learning because animals may be attempting to conserve energy.[31] Others suggest that water temperature near ambient room temperature (20°C–22°C) does not impair performance and that aged animals should be warmed between trials.[18,19] While the animals in this study were warmed between trials, there were still different effects noted indicating that further investigation may be warranted.

The results of this study have important implications for the care of individuals experiencing mild to moderate infections. Cognitive changes may occur without the presence of other sickness responses and may not be based on illness severity. These results have implications especially for the elderly who may be more at risk of the development of cognitive changes following an infection. This finding shows that clinicians must be vigilant in preventing and detecting infection. More research is necessary to identify early biomarkers of infection so that earlier diagnosis and treatment may occur, especially for older individuals.

In summary, aged animals exhibited spatial learning deficits in the absence of other signs of infection. This may be an important clinical translation of this basic research. Cognitive and physical responses functioned independently: cognitive deficits occurred while animals were not sick. The results support the anecdotal knowledge that the first sign of infection in the elderly may be a decrease in cognitive function that precedes increased temperature and weight loss. Atypical presentations can lead to delayed diagnosis and/or treatment.[32] Future studies are needed to examine the mechanisms for the aged animals in this study resisting the effects of low-dose LPS on food intake and weight but not memory.

Animal welfare

This study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

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