ENVIRONMENTAL ENRICHMENT AND BEHAVIORAL CONSEQUENCES OF PERIPHERAL IMMUNE STIMULATION

by

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1. INTRODUCTION

Environmental enrichment (EE) is any change in housing conditions or environment that facilitates enhanced sensory, cognitive, and motor stimulation relative to standard housing conditions (Nithianantharajah & Hannan, 2006). The current literature indicates that experimental EE has wide-reaching effects within the central nervous system (CNS), including effects on adulthood neurogenesis (Kempermann et al., 1997), improved outcome in the face of neurodegenerative disease, recovery of function after traumatic brain injury (Nithianantharajah & Hannan, 2006), and diminished stress reactivity (Mattson et al., 2004). Evidence suggests that enrichment also leads to changes in synaptic plasticity, resulting in changes in learning and memory in a variety of behavioral testing paradigms (Pizzorusso et al., 2007; Schrijver et al., 2001).

Early EE can impact many facets of an organism's existence, including significant alterations in immune function. Moreover, the brain and the immune system interact with one another in a variety of ways (Dantzer & Kelly, 1989), and immune challenges may interfere with learning and memory (Pugh et al., 1998; Barrientos et al., 2002). Therefore, EE may diminish the learning decrements commonly associated with immune activation by altering the nature of the immune response itself, by compensating for the immune-related deficit through improved neural plasticity, or via a combination of the two mechanisms.

Although some research has examined the effect of EE on adaptive immunity (e.g., B and T cell function), there remains a dramatic lack of information pertaining to its effects on the innate immune system, including the immune response and behavioral sequelae that ensue following exposure to a bacterial endotoxin. Since EE can permanently alter the developmental trajectory of an organism, further exploration of the relationship between EE

and immunity will expand our knowledge of developmental effects of EE and elucidate the potential role of environmental stimulation upon immune responses, central proinflammatory cytokine (i.e., interleukin-1β, interleukin-6, and tumor necrosis factor α expression) and resultant alterations in cognitive processes.

1.1. Environmental Enrichment

What is called EE varies across laboratories; however, common features include group-housing in a large cage, tunnels, nesting materials and toys (van Praag et al., 2000). Such additions to the environment, can lead to significant changes in neural structure and function. For example, within the central nervous system alone, there are many changes induced by environmental enrichment, such as increased brain weight, thickness of the cortex, and increased numbers of synapses, dendritic connections, and numbers of hippocampal neurons. (Escorihuela et al., 1995; Leggio, et al., 2005). More specifically, exposure to environmental enrichment results in 15% more neurogenesis in the dentate gyrus of the hippocampus, compared to mice housed in standard cages (Kempermann et al., 1997). Further, the majority of observed enrichment-induced neurogenesis appears to be localized to the hippocampus, and not in the olfactory bulb, which may explain a portion of the potential learning and memory benefits of EE (Brown et al., 2003).

Long-term potentiation (LTP) is a prominent neurobiological model of learning mechanisms. Hebb postulated the concept of neuronal plasticity which was developed further by Lømo and colleagues (Bliss & Lømo, 1973). The principle behind LTP is that associations between two stimuli are strengthened through the frequent firing of appropriate neural connections. Likewise, Sastry and Goh (1984) found that the weakening of neural connections occurs through the infrequent firing of inappropriate connections or long-term

depression (LTD). Mice that are exposed to an EE exhibit increased LTP and LTD in the anterior cingulate cortex (ACC) and an increased NR2B/NR2A NMDA receptor subunit ratio that is associated with an increase in plasticity (Shum et al., 2007). Rats show improvements in both hippocampal LTP and LTD induction prior to EE exposure (Artola et al., 2006). Therefore, researchers hypothesized that animals exposed to EE would perform better on hippocampus-dependent tasks such as the Morris water maze. As hypothesized, rats exposed to EE showed better performance in the Morris water maze and other hippocampus-dependant tasks compared to controls both aged and young (Escorihuela et al., 1995).

One obvious caveat of this line of research is EE's limited ecological validity when compared to the natural environment. It remains unclear whether EE actually represents enrichment or if we are merely modeling an animal's natural environment. Only one study has compared EE in free-range versus captive animals, and it showed that free-range black-capped chickadees have more hippocampal neurogenesis over a six week period when compared to captive animals housed in an "enriched environment" (Barnea & Nottebohm, 1994). It appears that enriched, rather than standard laboratory environments, might more closely represent natural conditions.

However, the degree to which laboratory enrichment mimics natural conditions does not diminish the considerable importance of EE research. Although EE may merely be replicating the environment of free-range animals, we can still learn a great deal about the role of environmental factors in disease progression.

1.2. Environmental Enrichment and Immunity

The immune system can be divided into two branches: innate and adaptive. The innate immune response is the first to react, is very general in scope, and protects against a

broad spectrum of pathogens. Furthermore, the innate immune system activates the adaptive immune system. The adaptive immune response is slower to respond, more antigen-specific, and employs antibodies and T-cell receptors that respond to the specific physical structure of a pathogen (Parham, 2005). Research indicates that EE exerts effects on immune function; however, research in this area has primarily focused on the more specific, adaptive immune response, as opposed to the immediate innate immune response. For example, animals in EE conditions showed lower levels of immunoglobulin G1 (IgG1), higher ratios of IgG2a/IgG1, and increased percentages of CD8+ cells compared to animals in standard conditions, indicating positive effects of EE on the adaptive immunity (Marashi et al., 2003). Additionally, EE enhances natural killer (NK) cell activity, leading to presumably enhanced host resistance to viral infection and carcinogenesis (Benaroya-Milshtein et al., 2004). Although this limited research has examined EE's effects on adaptive immunity, more information is needed about potential changes to the innate immune system following exposure to an enriched environment.

1.3. Cytokines

Cytokines are chemical messengers by which immune cells, and other cells, communicate with one another. Key cytokines are thought to play a major role in the effect the immune system has on cognition. Peripheral cytokines enter central nervous system (CNS) via three major pathways (Dantzer, 2004). First, cytokines can enter the brain through sites lacking a blood-brain barrier (BBB), such as the circumventricular organs (Konsman et al., 1999). Second, cytokine production can be induced centrally by peripheral cytokines stimulating the vagus nerve (Wan et al., 1994). Finally, cytokines can enter through active transport into the brain across the BBB.

Cytokines are typically divided into two categories: proinflammatory cytokines, which include interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α), and anti-inflammatory cytokines, such as interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-10 (IL-10) (Nomura et al., 2000). In times of infection, proinflammatory cytokines activate immune cells, induce an inflammatory response leading to greater tissue permeability, and induce a febrile response (Dantzer, 2004). Effects of proinflammatory cytokines such as IL-1β may be modulated by other factors. For example, evidence indicates that corticotropin-releasing factor may attenuate the behavioral effects of lipopolysaccharide (the degraded cell wall of gram-negative bacteria) due to diminished release of cytokines within the hippocampus (Kohman et al., 2007). Additionally, it appears that prostaglandin inhibition can attenuate the endotoxin-induced spatial learning deficits seen in the Morris water maze (Shaw et al., 2005)

Proinflammatory cytokines have many positive effects such as cytokine therapy which is used to treat hepatitis C, various cancers, and human immunodeficiency virus (HIV). Despite these positive effects, cytokines are most notable for their negative effects, such as exacerbating neurodegenerative disease, causing detrimental effects on memory, and their possible role in depression. An unfortunate side effect of cytokine therapy, specifically therapy with interleukin-6 (IL-6), is depression (Anisman & Merali, 2003). Further, cytokines are implicated in aggravating neurodegenerative diseases such as Alzheimer's, HIV-related dementia, and prion diseases (Ader, 2007; Perry, 2005). Additionally, cytokines play a role in learning and memory deficits through their deleterious effects on acquisition (Oitzl et al., 1993; Aubert et al., 1995; Sparkman et al., 2005) and memory consolidation (Pugh et al., 1997; Barrientos et al., 2002). Cytokines clearly have a modulatory effect on

cognition, so the possible interaction EE might have on these cognitive processes is relevant and could potentially further our knowledge of how plasticity and learning are impacted by immune events.

1.4. Lipopolysaccharide

Because work with replicating pathogens such as viruses or bacteria could be potentially dangerous for experimental subjects and could introduce extra variability into an experiment, many experiments that involve proinflammatory cytokine production use LPS as an alternative. LPS is a ubiquitous component of the cell wall of Gram-negative bacteria, and triggers an immune response through the toll-like receptor-4 complex (Pasare & Medzhitov, 2004). Once bound, immune cells respond to LPS by releasing proinflammatory cytokines (Dantzer, 2004). Administration of LPS leads to "sickness behavior" which consists of decreased social exploration, anhedonia, decreased sexual behavior, decreased feeding behavior, and decreased locomotor activity (Borowski et al., 1998; Yirmiya, 1996). These changes in an organism's behavior are not inflexible changes; rather, they represent a change in motivational state that is adapted to the needs of the current situation, to aid in recovery from illness (Dantzer, 2004).

1.5. Immune Influences on Learning and Memory

One of the best examples of the interplay between the brain and the immune system is the conditioning of certain aspects of the immune response to LPS through the conditioned taste aversion (CTA) paradigm. Typically, CTA involves exposure to a novel flavored solution, after which the animal is injected with some noxious substance, and on subsequent exposures the animal avoids the solution. CTA is an example of "one trial learning" and produces very stable and prolonged conditioning. Several symptoms or

features of the acute phase response to LPS can be conditioned by CTA, such as fever, sleep alterations (Bull et al., 1994), plasma iron concentrations (Exton et al., 1995), and anorexia (Exton et al., 1995). In addition to the conditioning of immune responses, the immune system can also have deleterious effects on learning and memory.

The effects of LPS on the CNS typically develop two to four hours after exposure and can last as long as 24 hours. During this time, the release of cytokines can produce noticeable deficits in learning and memory. Administration of LPS (or cytokines) negatively affects performance on a variety of behavioral paradigms, such as two-way active avoidance, Morris water maze, and autoshaping, among others (e.g., Aubert et al., 1998; Pugh et al., 2001; Sparkman et al., 2005). Such cognitive effects are not surprising, because of the high density of IL-1 receptors in the hippocampus, is a brain structure that is widely known for its role in learning and memory (Schneider et al., 1998).

Pugh et al. (1998) demonstrated that LPS administration impairs memory consolidation in contextual (hippocampus dependant), but not auditory fear conditioning (not hippocampus dependent). The same deficits were also seen with central administration of IL-1β, and the effects appear to be specific to tasks that depend on the hippocampus, such as contextual fear conditioning (Rachal-Pugh et al., 2001). Barrientos et al. (2002) further demonstrated that IL-1β specifically blocks learning of the context, and that latent exposure to the context prior to IL-1β administration can ameliorate these effects.

In addition to memory consolidation deficits, exposure to cytokines can impair memory acquisition as well. In an autoshaping task, animals injected with LPS during acquisition of a lever-pressing task showed less lever pressing than control animals, indicating that these animals failed to associate the lever with the administration of a food

pellet (Aubert et al., 1995). Sparkman et al. (2005) administered LPS four hours prior to two-way active avoidance conditioning. Two-way active avoidance conditioning box consists of two compartments. At the start of the trial the compartment housing the mouse is illuminated. Once this light turns on the mouse must learn to cross to the other compartment in order to avoid the onset of a mild shock. The animals that received LPS showed a decreased number of avoidance responses to the shock, compared to control animals. Since these animals showed no meaningful decrements in latency to cross, and an increased number of inter-trial interval crossings these results do not appear to be due to motor deficits. Collectively, these data indicate that the fewer avoidance responses were not due to fatigue, but rather to a diminished ability to acquire the association between the light and ensuing shock.

Clearly immune activation can influence the CNS and lead to cognitive impairments in various hippocampus-dependent testing paradigms, including the Morris water maze (Kohman et al., 2007), autoshaping (Aubert et al., 1995), contextual fear conditioning (Pugh et al., 1998), and two-way active avoidance conditioning (partially hippocampus-dependent) (Sparkman et al., 1995). Because many individuals suffer from the cognitive effects of long-term inflammatory conditions or treatment with cytokine-based therapies, further research examining the potential mechanisms or means of attenuating these effects is warranted. Additionally, the effect of environment on the acquisition and trajectory of these conditions is underrepresented in the current literature and this area of research deserves further investigation.

1.9. Summary and Hypotheses

To further examine the effects of EE on the innate immune system following

exposure to immune challenge, we will test whether post-weaning EE leads to protection against sickness behavior and improved learning. We hypothesize that enriched animals will show diminished sickness behavior after endotoxin exposure versus control animals housed in standard cages. Furthermore, we hypothesize that exposure to EE will lead to fewer learning and memory deficits following exposure to an immune challenge.

2. METHODS

2.1. Subjects

Subjects were male, four- to five-month old C57BL/6J mice bred at the TCU vivarium from breeding stock purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were housed and treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), and in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Texas Christian University.

2.2. Environmental Enrichment and Housing

Subjects in the experimental group were housed in groups of four, in an enriched environment consisting of a large (18.75cm x 47.5cm x 20cm) polycarbonate rat breeder cage with wire-mesh walkways leading to food and water, and toys which were rotated weekly. Subjects in the control group were housed in groups of four, in standard (12.5cm x 15cm x 25cm) polycarbonate mouse cages. For both groups, lights were turned on at 0600 and off at 1800, and had food and water available *ad libitum*.

2.3. Experiment 1: Environmental Enrichment & Sickness Behavior

Treatment Conditions

The design for Experiment 2 was a 2 x 2 factorial design with two between-groups factors: 1) LPS treatment: Saline, LPS (50 μg/kg) and 2) Housing Condition: Environmental Enrichment or Standard Housing, for a total of four treatment groups (see Table 1 below).

Table 1. Experimental design of Experiment 1.

Burrowing N=77	LPS (50 μg/kg)	Saline
Environmental Enrichment	n=21	n=15
Standard Housing Conditions	n=20	n=21

Behavioral Testing Procedures and Apparatus

In order to measure sickness behavior, we utilized a highly sensitive burrowing test designed by Deacon (2006). Burrowing occurred in 200 mm long, grey plastic tubes with an open end into which the animal can climb into and a lower end closed by a plastic plug. The open end was raised 30mm by bolting two machine screws through it, each close to the end of the tube.

Subjects were given two practice trials to improve burrowing ability and diminish variability between animals. During practice trials, entire cages of animals were allowed to burrow together so that social facilitation might enhance the burrowing behavior. For practice, cagemates were placed in a testing cage together with a burrow filled with 200 g food pellets at 16:00 and left to burrow until 10:00 the following morning. After two practice trials, individual baseline testing commenced for an additional two nights. The goal of an individual baseline was to properly allocate mice into treatment groups that were counterbalanced for burrowing ability so that a treatment effect might be more readily detected. Based on the amount burrowed at the two-hour measure, subjects are ranked in ascending order and assigned to treatment groups sequentially and alternately. During individual baseline trials, subjects were placed, individually, into a testing cage with a burrow filled with 200 g food pellets at 16:00. The amount of food burrowed was measured at two time points: 18:00 to obtain a more sensitive two-hour measure, and at 10:00 the next

morning to obtain an overnight measure. Mice burrowing fewer than 5 g were removed from the study due to unreliable burrowing. The testing day was performed identically to the individual baseline day with two-hour and overnight burrowing measures recorded and analyzed.

2.4. Experiment 2: Environmental Enrichment and Two-Way Active Avoidance Conditioning

Treatment Conditions

The design for Experiment 1 was a 2 x 2 factorial design with two between-groups factors: 1) LPS Treatment: Saline or LPS (250 μg/kg) and 2) Housing Condition: Environmental enrichment or Standard housing, for a total of four treatment groups (see Table 2 below). Intraperitoneal injections (i.p.) of LPS (*Escherichia coli* serotype 0111:B4, Sigma, St. Louis, MO) was diluted in sterile saline and given four hours prior to testing on the first day of testing. Control animals received an equivalent volume of sterile saline solution four hours prior to testing on each the first day. Throughout the experiment the animals were inspected visually and weighed daily.

Table 2. Experimental design of Experiment 2.

Two-way active avoidance N=76	LPS Day 1 (250 μg/kg)	Saline
Environmental Enrichment	n=20	n=17
Standard Housing Condition	n=21	n=18

Behavioral Testing Procedures and Apparatus

Four hours prior to the first day of testing, subjects received an i.p. injection of 250

μg/kg of LPS or an equivalent volume of sterile saline. Avoidance learning was assessed using eight identical Gemini II shuttlebox units manufactured by San Diego Instruments (San Diego, CA), capable of fully automated active and passive avoidance conditioning. Inside the apparatus, a partition with a hole at floor level separates the box into two equal compartments. At the beginning of each day's testing session, the animal was given a 5-min acclimatation period in the apparatus and allowed to move about freely. The animal's location was sensed via infrared photocells located in each compartment. The discriminative stimulus (S_D) was a light coming on at the end of the chamber, and the S_D interval was 5-s. Footshock (0.4 mA), the punisher, was then delivered through an electronic scrambler. During the footshock, the S_D remained on. If the animal crossed into the opposite compartment, both the light and the shock were turned off and a 20-s intertrial interval (ITI) began. During the ITI, the mouse could move freely between compartments. Any such movements did not affect the ITI. Mice could learn to avoid the footshock by crossing to the opposite compartment as soon as the light was turned on, or could escape the shock by crossing to the opposite compartment once the shock began. Each mouse was given 52 trials a day for 5 days. Each trial was classified as an avoidance response (crossing to the other side before the onset of shock), an escape response (crossing to the other side after the onset but during administration of the shock), or a null response (remaining in the original compartment and receiving 5-s of shock). In addition, latency to avoid the shock was recorded for the avoidance responses, latency to escape was recorded for the escape responses, and a general measure of response latency (collapsed across the two) was calculated. Examination of the latency measures, in conjunction with the escape and avoidance measures, enabled elucidation of potential freezing behavior, locomotor

impairment, or diminished motivation. To evaluate overall response efficiency, we calculated the subject's response efficiency score which incorporated both cued and uncued crossings (i.e., [avoidance responses/(avoidance responses+escape responses+ITIs)*100]). Lastly, the number of ITI crossings was recorded as a measure of non-cued or random crossing activity. Between the individual testing sessions, the apparatus was cleaned with Odormute™ (Ryter Corp., Madelia, MN) and the light levels within the outer room were minimized to make the CS more salient. A white noise generator and 1-inch acoustic foam (Auralex, Co., Indianapolis, IN) were placed in the outer room to minimize sound transfer.

Statistics

The behavioral data were analyzed using standard analysis of variance (ANOVA) procedures (Statview 5.0, SAS, Cary, NC). The alpha level used for all statistical analyses was 0.05. Significant omnibus effects were followed by Fisher's PLSD post hoc tests.

3. RESULTS

3.1. Experiment 1: Burrowing

The first measurement of burrowing was taken two hours after the subject was placed in the cage with the burrowing tube. There was a main effect of LPS treatment on the amount of food burrowed ($F(1,73)=48.545$; $p<0.0001$, see Figure 1), however there was no effect of housing condition on burrowing behavior ($F(1,73)=1.702$; *ns*, see Figure 2) and no Treatment X Housing interaction ($F(1,73)=0.649$; *ns*, see Figure 3). Ultimately, the two hour time point did not prove to be a sensitive measure of burrowing differences between the two housing groups because there was an almost complete suppression of burrowing in the animals given LPS.

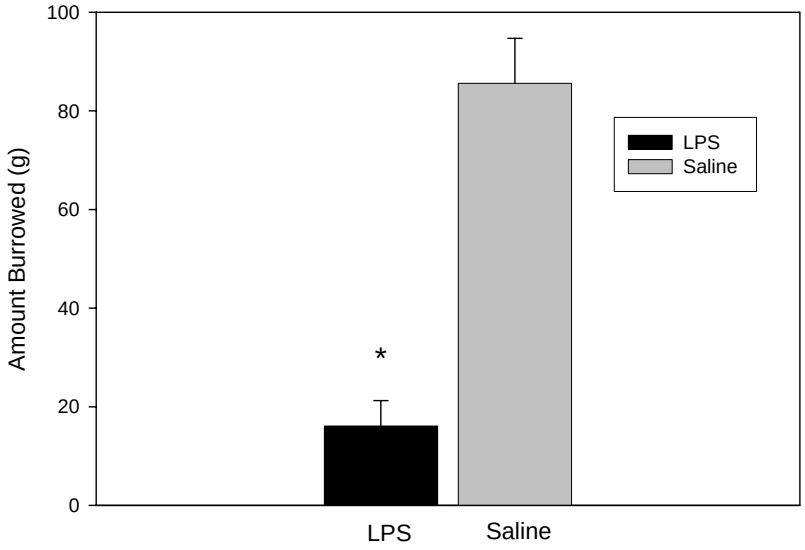

Figure 1. LPS-treated animals burrowed significantly less in two hours than saline-treated animals. * indicates a significant difference, *p's*<0.0001. Error bars reflect standard error of the mean (SEM).

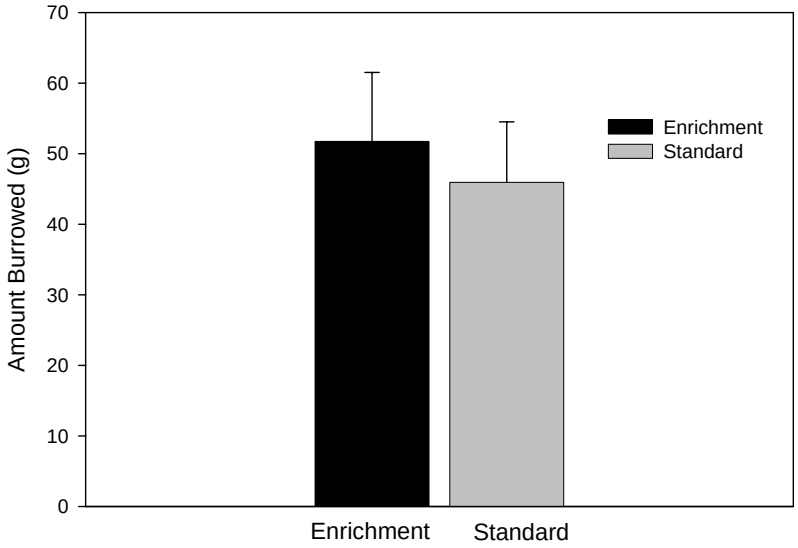

Figure 2. EE animals did not burrow significantly more when compared to animals housed in standard conditions. Error bars reflect SEM.

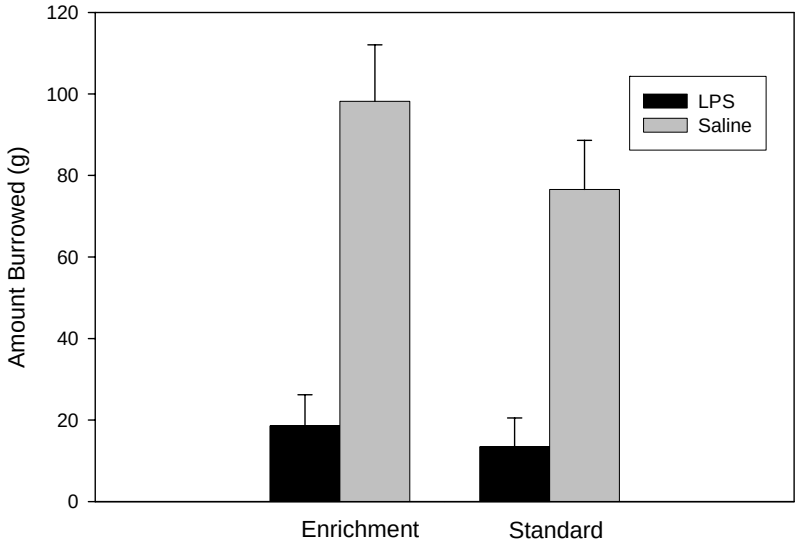

Figure 3. Animals administered LPS burrowed significantly more when compared to animals administered saline. However, EE animals administered LPS did not burrow significantly more when compared to control animals which were also administered LPS. Error bars reflect SEM.

When we measured burrowing behavior overnight we found a greater distinction between groups. A significant main effect of LPS ($F(1,73)=13.387$; $p<0.05$, see Figure 4), showed subjects administered LPS burrowed significantly less when compared with saline controls. There was no significant main effect of Housing Condition on burrowing behavior ($F(1,73)=0.012$; $p=0.91ns$, see Figure 5), and no Treatment X Housing interaction ($F(1,73)=13.387$; $p<0.05$, see Figure 6).

The absence of an effect of EE on burrowing, suggested that EE did not affect LPS-induced sickness behavior. Therefore, we decided to further examine the possible relationship of EE's role in cytokine-mediated alterations in a learning task.

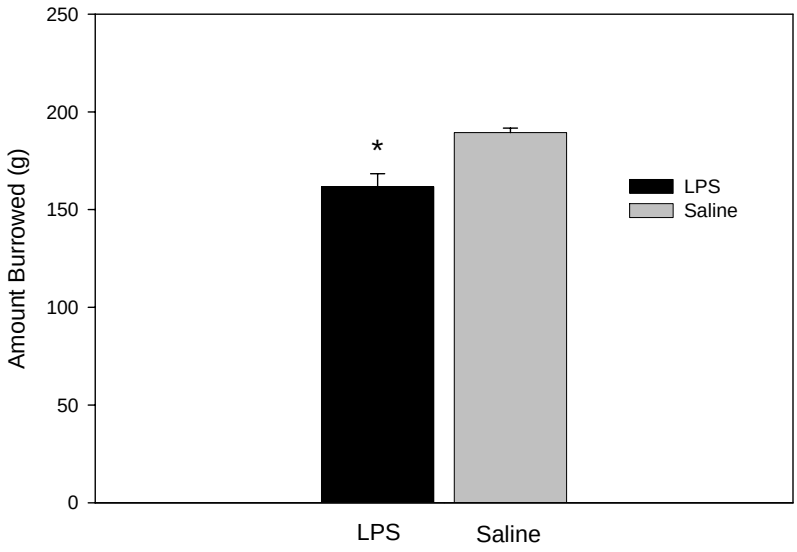

Figure 4. LPS-treated animals burrowed significantly less overnight than saline-treated animals. * indicates a significant difference, *p's*<0.05. Error bars reflect SEM.

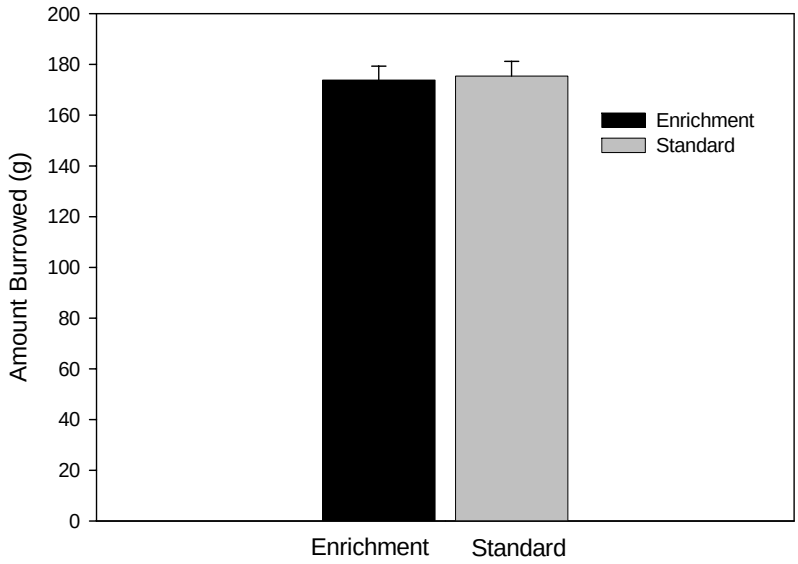

Figure 5. EE animals did not burrow significantly more when compared to animals housed in standard conditions. Error bars reflect SEM.

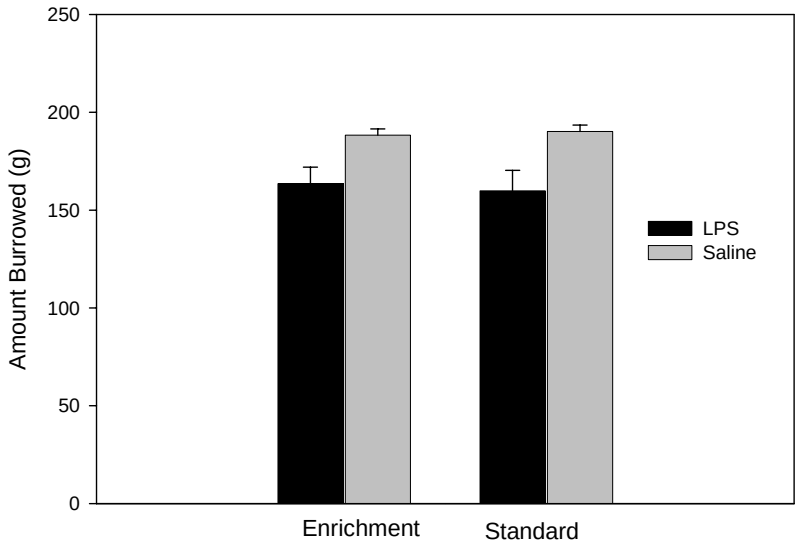

Figure 6. Despite the main effect of LPS treatment, there was no interaction between housing condition and LPS treatment. Error bars reflect SEM.

3.2. Experiment 2: Two-Way Active Avoidance Conditioning

Subjects administered LPS showed significantly fewer avoidance responses when compared with saline controls ($F(1,72)=4.305$; $p<0.05$, see Figure 7). Additionally, we found a significant LPS x Day interaction ($F(4, 288)=2.847$; $p<0.05$, see Figure 8) and post hoc tests revealed a significant difference between LPS and saline performance on Day 2 ($F(1, 74)=2.890$; $p<0.05$). Furthermore, there was a significant effect on the Housing Condition x Day interaction ($F(4, 288)=3.291$; $p<0.05$, see Figure 10), but upon further examination Fisher's post hoc test showed no significant differences by day. Mice raised in an EE showed no evidence of improved cognitive function when exposed to an immune challenge as compared to control subjects in a standard housing condition ($F(1,72)=1.093$; *ns,* see Figure 9). However, LPS x Housing Condition by day interaction was not significant ($F(4,288)=0.226$; $p=0.92$*ns*, see Figure 11). There was no interaction between the effect of

LPS treatment or housing condition. We observed similar results for the number of escape responses (*data not shown*).

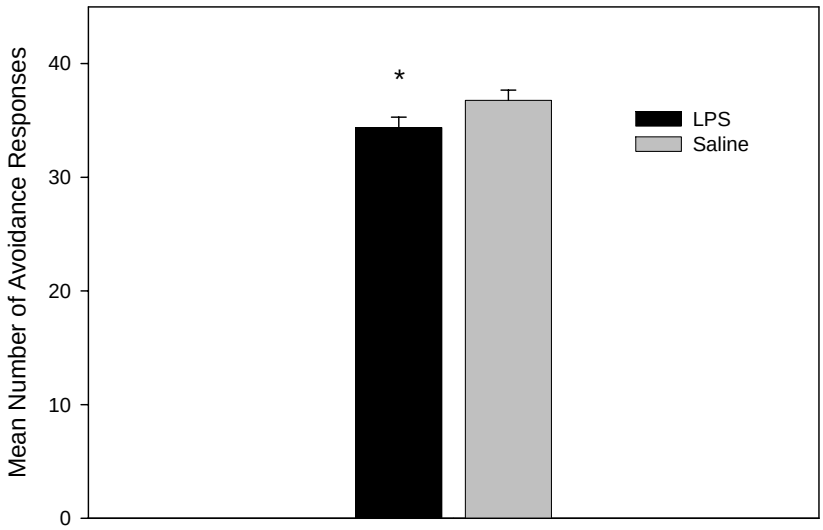

Figure 7. LPS-treated animals performed significantly fewer avoidance responses than saline-treated animals. * indicates a significant difference, *p's*<0.05. Error bars reflect SEM.

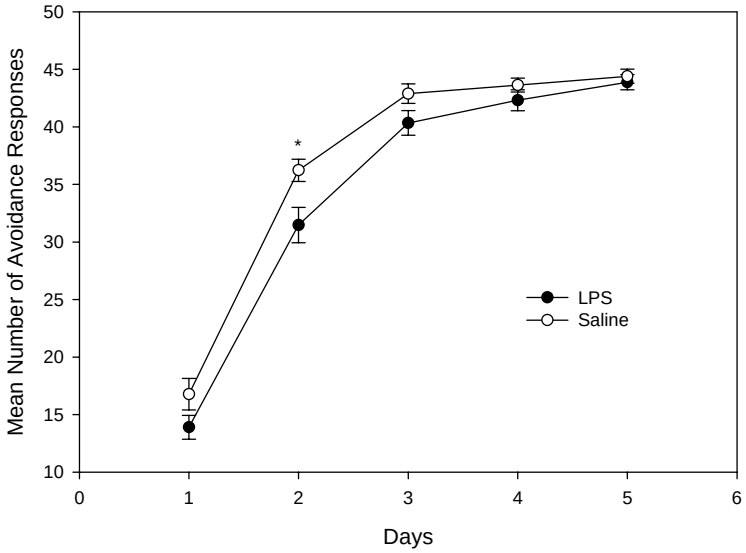

Figure 8. Saline animals performed significantly more avoidance responses on day 2. * indicates a significant difference, *p's*<0.05. Error bars reflect SEM.

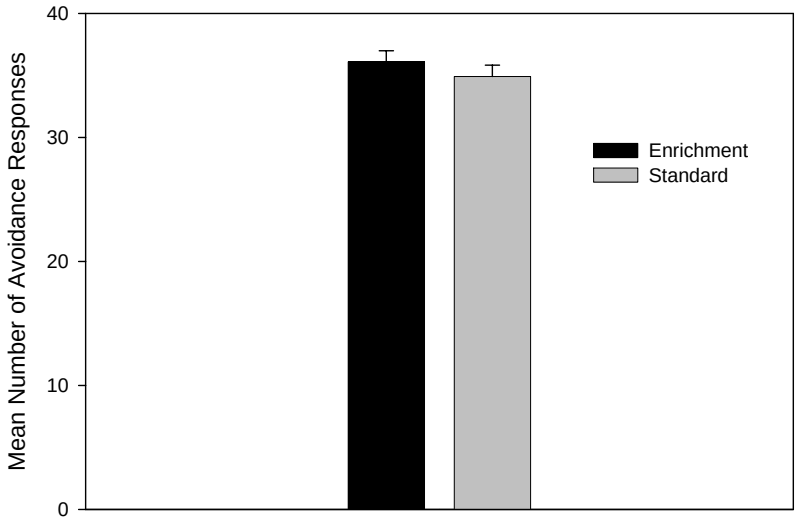

Figure 9. Subjects housed in an enriched environment showed no significant differences in the mean number of avoidance responses when compared with controls. Error bars reflect SEM.

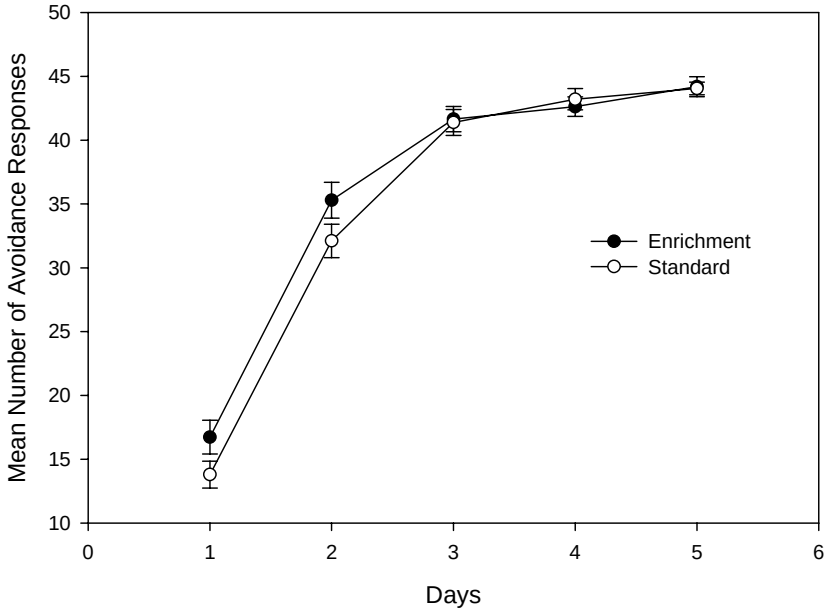

Figure 10. There was a significant omnibus *F* but post hocs revealed no significant differences by day. Subjects housed in an enriched environment showed no significant differences in the number of avoidance responses when compared to subjects housed in a standard environment. Error bars reflect SEM.

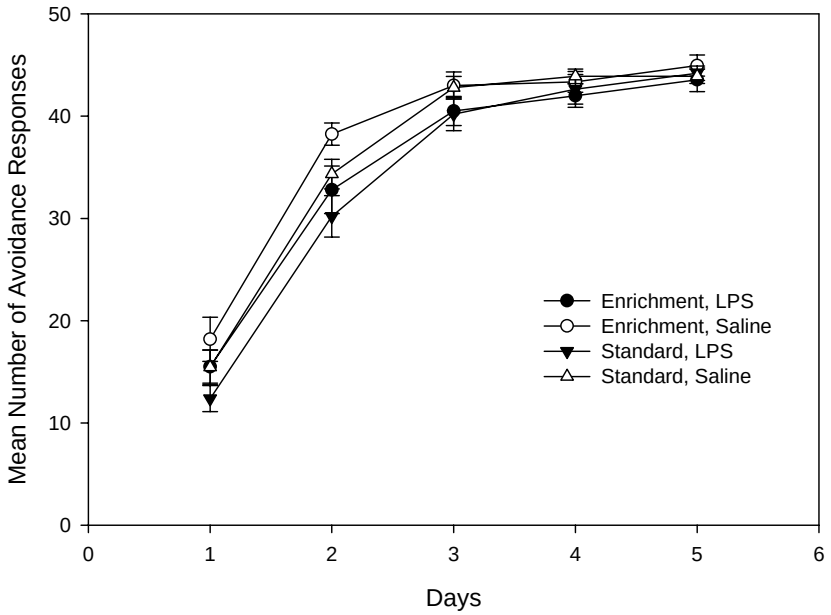

Figure 11. The subjects showed a modest effect of LPS treatment; animals administered LPS performed fewer avoidance responses compared to those administered saline. Across testing days, we found no interaction between the mean number of avoidance responses performed by subjects housed in EE and administered LPS compared to those given LPS and housed in a standard environment. Error bars reflect SEM.

When we examined the measure of response efficiency which we defined as [avoidance responses/(avoidance responses+escape responses+ITIs)*100]), there was a main effect of LPS treatment ($F(1, 72)=5.190$; $p<0.05$, see Figure 12), which demonstrates that animals administered LPS showed a diminished ability to learn the S_D-CS relationship. Once again, there was no main effect of housing ($F(1, 72)=0.051$; $p=0.82$*ns*, see Figure 13) on response efficiency, or interaction across testing days; LPS x Housing Condition x Day ($F(4, 288)=0.245$; $p=0.91$ *ns*, see Figure 14).

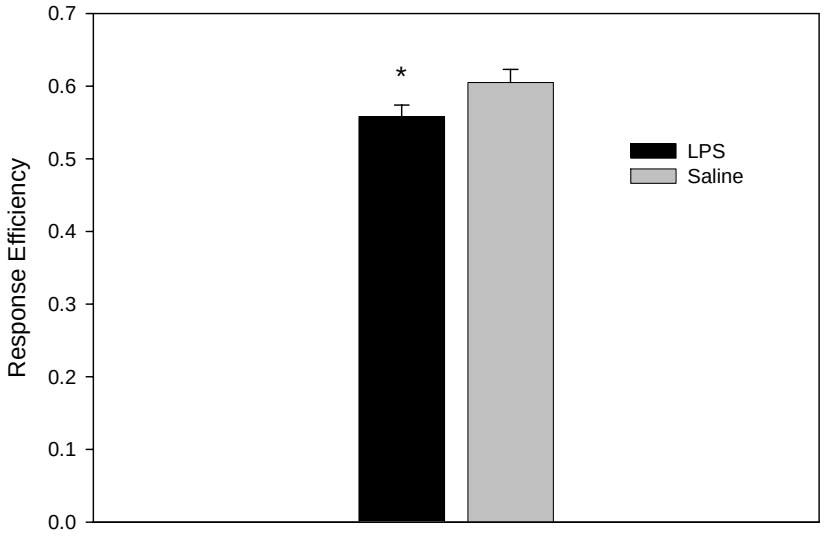

Figure 12. Subjects administered LPS showed diminished response efficiency compared with subjects that were administered saline. Error bars reflect SEM.

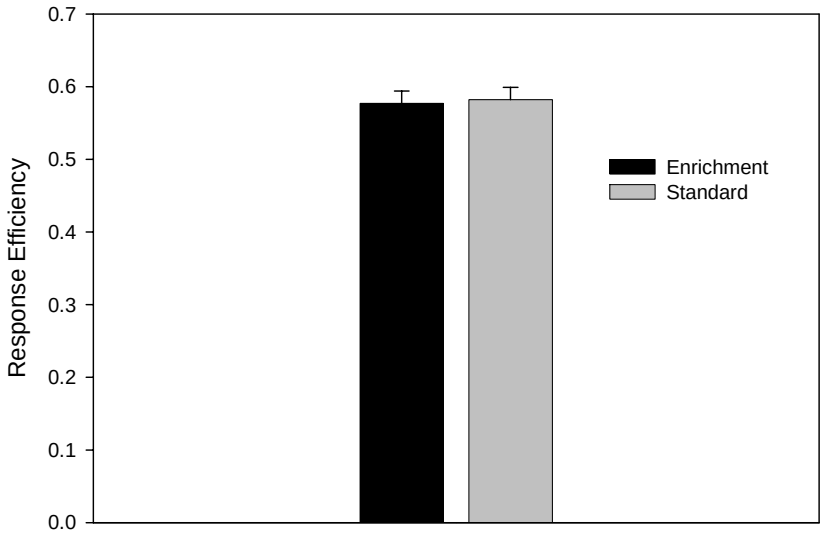

Figure 13. Subjects housed in an enriched environment showed no significant differences in response efficiency when compared with controls. Error bars reflect SEM.

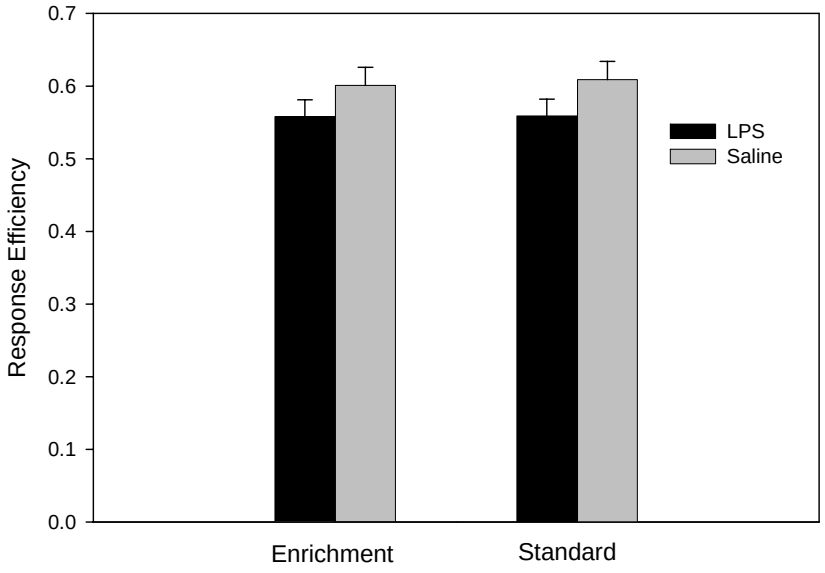

Figure 14. There is a main effect of LPS treatment on response efficiency. We found no interaction between housing condition and LPS treatment. Error bars reflect SEM.

4. Discussion

The proposed experiments were designed to examine the possible beneficial effect of EE on learning and memory after a challenge to the peripheral innate immune system. We hypothesized that prior exposure to an EE would ameliorate the deleterious effects of endotoxin exposure on sickness behavior and cognition via the innate immune system. The current data do not support our hypothesis; there was no interaction between EE and function of the innate immune system. We used burrowing as a measure of sickness behavior, and there were no differences in burrowing behavior between animals raised in EE and standard housing when exposed to LPS. Moreover, subjects raised in an enriched environment did not differ significantly in the amount burrowed when compared to those raised in standard conditions. We devised a second experiment to examine any possible effect of housing condition on learning and memory using two-way active avoidance conditioning and found similar results. Specifically, we found no significant differences across groups that might

indicate an interaction between these two effects. Surprisingly, we found that the subjects housed in an enriched environment showed no evidence of improved learning in our selected cognitive task.

It is widely known that EE leads to changes both in neural structure and function. Many studies show better learning in a wide variety of behavioral tasks after exposure to EE (Leggio et al., 2005; Escorihuela et al., 1995). Specifically, research is unclear about the relationship between EE and learning in the shuttle box paradigm (Escorihuela et al., 1994). The present studies further examine this relationship by demonstrating that EE may not affect learning in the shuttle box paradigm. One limitation of this study is that the cognitive testing only occurred in one paradigm, and therefore the choice of a single behavioral test may have failed to reveal any possible effect of EE on learning and memory. Future studies should examine the mediating effect of EE in response to an innate immune challenge using a broader range of behavioral tests, including the Morris water maze. The use of different behavioral tests could better assess the potential effect of EE on different types of memory, such as working versus reference memory.

Undoubtedly, EE exerts effects on immune function; however, research in this area has primarily focused on the more specific, adaptive immune response as opposed to the immediate innate immune response. The goal of this research was to determine if there was any interaction between the EE and function of the innate immune system. Upon completion of the present experiments, a relevant study by Kentner and colleagues (2008) was published which corroborates a portion of our results. Similar to our second experiment, Kentner et al. examined whether environmental enrichment would diminish cytokines and sickness behavior after exposure to LPS. In this study, subjects were group-housed in an enriched

environment for eight hours per day for the duration of the experiment. Subjects received a 150 μg/kg injection of LPS or an equivalent volume of saline. Two hours following the injection, sickness behaviors (i.e., piloerection, lethargy, sleep, and ptosis) were assessed on a scale of one to five. Subsequently, tail blood was collected to measure peripheral cytokine mRNA using real-time polymerase chain reaction (RT-PCR). Twelve days later the injection and assessment procedure was repeated, the animals were sacrificed, and brains were collected to measure central cytokine levels. Upon analysis, a modest effect of sickness behavior was observed; EE subjects administered LPS exhibited less piloerection than subjects administered LPS and housed in standard conditions. No other effect of EE was seen in the other measures of sickness behavior including lethargy, sleep, and ptosis. The measures of peripheral cytokine mRNA showed no significant difference between enriched and control subjects. Similarly, cytokine levels in the ventral tegmental did not show significant differences. Although the results from this study did show a modest difference on one measure of sickness behavior, overall, the data seem to indicate no substantial effect of EE on the innate immune system, the only other work we are aware of that has examined the effects of EE on cytokine-induced behaviors. The current work is consistent with these results and further demonstrates this effect in a different measure of sickness behavior and also in a learning test.

Although these data partially confirm the findings of Kentner et al. (2008), there are limitations inherent to this research. For example, the efficacy of our environmental enrichment procedure was not confirmed. The environment in which we housed our animals could be inadequate to produce the beneficial effects of enrichment such as hippocampal neurogenesis. We tested learning and memory using only one type of cognitive task, two-

way active avoidance. It is possible, as demonstrated by Escorihuela and colleagues (1994), that environmental enrichment may not affect the acquisition of this task. Two-way active avoidance may only assess a single type of learning and, as such, may fail to capture the full scope of possible learning deficits, and may not be sensitive to the benefits of EE (see Escorihuela et al., 1994). Additionally, this paper used burrowing as our sole measure of sickness behavior. Future experiments should also assess sickness behaviors such as hunched posture, anhedonia, and lethargy.

Despite these limitations, the present experiment is the first to examine the effect of EE and the innate immune response on learning and memory after exposure to an immune challenge. We found that EE appears not to interact with shuttlebox learning and that EE does not ameliorate the effect of LPS on sickness behavior. Future experiments should further characterize EE's potential relationship with the adaptive branch of the immune system.

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ABSTRACT

ENVIRONMENTAL ENRICHMENT AND BEHAVIORAL CONSEQUENCES OF PERIPHERAL IMMUNE STIMULATION

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Early environmental enrichment (EE) can impact many facets of an organism's existence, including significant alterations in neural and immune function. The brain and the immune system interact with one another in a variety of ways and immune challenges may interfere with learning and memory. We examined the possible role of EE in abrogating the negative effects of an innate immune challenge on learning and memory and sickness behavior.

We hypothesized that enriched animals will show diminished sickness behavior in a burrowing task after endotoxin exposure (lipopolysaccharide or LPS at a dose of 50μg/kg) versus control animals housed in standard cages. Furthermore, we hypothesized that exposure to EE will lead to fewer learning and memory deficits in a shuttlebox task following exposure to LPS (250 μg/kg). We found that EE appears not to protect against LPS-induced learning deficits and that EE does not ameliorate the effect of LPS on sickness behavior.