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Evaluating fatigue in lupus-prone mice: Preliminary assessments

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ABSTRACT

Fatigue is a debilitating condition suffered by many as the result of chronic disease, yet relatively little is known about its biological basis or how to effectively manage its effects. This study sought to evaluate chronic fatigue by using lupus-prone mice and testing them at three different time periods. Lupus-prone mice were chosen because fatigue affects over half of patients with Systemic Lupus Erythematosus. Eleven $MLR^{+}/_{+}$ (genetic controls) and twelve MLR/MpJ-Fas<lpr>/J (MRL/lpr; lupus-prone) mice were tested three times: once at 12, 16 and 20 weeks of age. All mice were subjected to a variety of behavioral tests including: forced swim, post-swim grooming, running wheel, and sucrose consumption; five of the $MLR^{+}/_{+}$ and five of the MLR/lpr mice were also tested on a fixed ratio-25 operant conditioning task. MRL/lpr mice showed more peripheral symptoms of lupus than controls, particularly lymphadenopathy and proteinuria. Lupus mice spent more time floating during the forced swim test and traveled less distance in the running wheel at each testing period. There were no differences between groups in post-swim grooming or in number of reinforcers earned in the operant conditioning task indicating the behavioral changes were not likely due simply to muscle weakness or motivation. Correlations between performance in the running wheel, forced swim test and sucrose consumption were conducted and distance traveled in the running wheel was consistently negatively correlated with time spent floating. Based on these data, we conclude that the lupus-prone mice were experiencing chronic fatigue and that running wheel activity and floating during a forced swim test can be used to evaluate fatigue, although these data cannot rule out the possibility that both fatigue and a depressive-like state were mediating these effects.

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1. Introduction

Fatigue, defined for clinical use as "difficulty initiating or sustaining voluntary activities," (Chaudhuri and Behan, 2004) or "an unpleasant feeling of inability to perform physical or intellectual efforts" (Casillas et al., 2006), is a debilitating condition suffered by many as the result of chronic diseases and disorders or due to their treatment. Fatigue is fundamentally different from general muscle weaknesses (Chaudhuri and Behan, 2004) and is often distinguished as physical or mental (Casillas et al., 2006; Beiske and Svensson, 2010). Of interest to fatigue researchers and clinicians is chronic fatigue; unlike acute fatigue, chronic fatigue is not brought about by a discreet period of exertion nor is it substantially relieved by rest (Swain, 2000).

There are many examples of fatigue correlated with illness or the treatment of an illness. Individuals undergoing chemotherapy for cancer cite fatigue as the number one side effect of their treatment with 75–100% of these patients experiencing significant fatigue (Hann et al., 2000). Nearly 57% of rheumatoid arthritis (RA) patients find fatigue to be an extremely important aspect of their disease, though it often goes untreated or unnoticed by their physicians (Pouchot et al., 2008). As summarized by Zwarts et al. (2008), fatigue is a common in patients with Multiple Sclerosis (MS), Parkinson's disease (PD), and cerebrovascular disorders and it occurs as a side effect of pharmaceutical drugs such as beta-blockers. In fact, fatigue is so often an issue in patients with cardiovascular disease a recent report by Casillas et al. (2006) outlines diagnostic and management of fatigue for these patients. In 2010, Beiske and Svensson (2010) reported that the prevalence of fatigue in Parkinson's disease patients ranged from 37 to 56%.

Given fatigue's co-occurrence with many disease processes, solutions as to how to prevent and treat fatigue are sought and to this end, a much better understanding of the biological basis of chronic fatigue is needed. One way to improve our understanding of, and possibly develop treatments for this condition is to employ an animal model of fatigue. A variety of researchers have used animals to study fatigue; however, much of this work focuses on acute rather than chronic fatigue and experimentally-induced fatigue, rather than fatigue naturally occurring due to a disease state. For instance, Chao and colleagues (Chao et al., 1992) inoculated a group of female BALB/c mice by with a bacterial antigen to induce fatigue. They measured "daily voluntary exercise activity" by giving the mice access to a running wheel and recording activity in it. They also measured the latency to initiate grooming behavior after a 5-minute period of swimming. Tanaka et al. (2003; 2008) used a weight loaded forced

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swim test to both create and measure fatigue in rats. Steel rings weighing approximately 8% of the rats total body weight were attached to their leg and the length of time the rats were able to swim before sinking below the water was used as an index of fatigue. Similar to techniques employed by Chao and colleagues, others have studied fatigue in nonhuman animals by using a brief (5 min) forced swim test (Swain and Maric, 1997) and by recording of running activity and grooming behavior over the course of a number of weeks (Ottenweller et al., 1998).

Much of the previous research using animal modeled of fatigue has evaluated acute fatigue or the role of activity in inducing fatigue. While important, these studies may not adequately represent the debilitating chronic fatigue seen in humans experiencing disease. Developing an animal model which can be used to evaluate and advance the study of the biological mechanism of chronic fatigue is vital for future research on ways to help alleviate fatigue in humans. To that end, we sought to evaluate chronic fatigue in an animal model of systemic autoimmunity using a mouse model of Systemic Lupus Erythematosus (SLE). We used this disease model because of the prevalence of fatigue in SLE patients. For example, Krupp et al. (1990) reported that over 50% of patients with SLE report being affected by fatigue and fatigue is often described as one of the most disabling symptoms of the disease (Tench et al., 2000). Recently, Kellner et al. (2010) evaluated fatigue in SLE patients using the multiple dimensional fatigue inventory and SLE patients scored significantly higher on all fatigue dimensions compared to controls. Though fatigue is clearly an important factor in the lives of patients with SLE, it is not obviously related to any specific immunological state (Omdal et al., 2002) or disease activity (Wang et al., 1998), but it is associated with various psychosocial factors including depression (Omdal et al., 2003; Kellner et al., 2010).

For this study, we made use of the MRL/lpr strain of mice; these mice experience a progressive, chronic and systemic autoimmune disease that correlates with changes in behavior making them an ideal model for the study of fatigue. This strain has a genetic control, the MRL-$^{+}/_{+}$ mice, which develop an indolent form of autoimmunity late in life (after 24 wks). The MRL-$^{+}/_{+}$ mice homozygous for the *lpr* gene (MRL/MpJ-*Tnfrsf6*, or MRL/MpJ-*Fas*^{lpr}/J; referred to in this paper as MRL/lpr) develop early onset autoimmunity (6wks–16wks) (Theofilopoulos et al., 1989) and have been appropriately validated as a model for SLE in studies of the immunopathogenesis (Santoro et al., 1988) and the neuropsychiatric manifestations of lupus (e.g., Sakic et al., 1997, 2005; Tomita et al., 2001), including behavioral changes you might expect during a fatigue state. Serological markers of SLE are present in the MRL/lpr mice, including autoantibodies against native DNA and Smith antigen (Theofilopoulos and Dixon, 1985; Theofilopoulos et al., 1989). As in SLE patients, these mice also show damage to heart, kidney, brain, lung and joint tissue.

Using a spontaneous, progressive disease model will allow for the evaluation of chronic fatigue symptoms, as opposed to experimentally-induced acute fatigue. Further, to date much of the research on the biological basis of fatigue has centered on the role of proinflammatory cytokines, in particular interleukin-1β (IL-1β), tumor necross necrosis factor-α (TNF-α), and interferon (INF-α) (Swain, 2000). MRL/lpr mice have been shown to have elevated levels of central mRNA for proinflamatory cytokines including IL-1β, IL-6 and TNF-α (Tomita et al., 2001; 2004); these cytokines are hypothesized to contribute to cognitive and behavioral changes in lupus-prone mice (Santoro et al., 2007), and therefore may be contributors to a fatigue-like state seen in lupus mice.

This study sought evaluate chronic fatigue by testing mice at three different time points (12, 16 and 20 weeks of age), and by comparing lupus-prone (MRL/lpr) mice to their genetic controls (MRL $^{+}/_{+}$) mice. To distinguish the behavioral effects of fatigue from muscle weakness, depression, and other conditions which may elicit similar behavioral profiles, the current study employed a variety of measures including operant conditioning to evaluate activity, sucrose preference to evaluate anhedonia, and the forced swim test and running activity, procedures more typically used to measure fatigue.

2. Method

2.1. Subjects

Eleven male MLR$^{+}/_{+}$ (control) and twelve male MLR/lpr (lupus-prone) experimentally naïve mice were obtained from Jackson Laboratories. All mice were housed in pairs with woodchip bedding and maintained on a 12 hour:12 hour light/dark cycle. Lights turned on at 7 am and all mice were tested during the light period. Mice were given ad libitum food and water except during testing weeks at which point they were fed 2.5 g of food per day per mouse.

2.2. Behavioral tests and experimental procedure

A 2 (lupus/control) × 3 (week of testing) mixed design was utilized in which all mice were subjected to the following test conditions: forced swim, post-swim grooming, running wheel, and sucrose consumption. In addition, five lupus-prone prone and five control mice were tested on a fixed ratio 25 (FR-25) operant conditioning task in addition to the other tests. Each animal was tested at 12, 16 and 20 weeks of age.

2.2.1. Operant task

Two identical, standard mouse operant conditioning chambers were used for all sessions. The chambers (25.5 × 20.3 × 12.7 cm, Med Associates) had two retractable levers, but only one was used for this study. One house light and one food dispenser were contained on the same wall as the levers and were opposite a wall containing a panel of lights (not used). Mice were initially shaped over a period of eight days to an FR-25 schedule of reinforcement, such that eventually they were reinforced for every 25 lever presses with Bio-Serv Dustless Precision Pellets (20 mg each). After shaping, mice were run on the FR-25 schedule for four days prior to the first round of testing. For each of the two remaining testing weeks, mice were run on the FR-25 schedule for two days prior to testing.

2.2.2. Running wheel

Four metal running wheels, which were each coated in a hard plastic and measured 9 in. in diameter, were obtained from a local pet supply store. Poster board was used to close off the sides of the wheel so that the mice could not exit the running wheel on their own. A 12 function Schwinn bicycle counter was attached to each running wheel in order to measure the distance each mouse ran during a 60-minute testing session. Distance was measured (in miles) cumulatively, regardless of which direction the animal was running. Each mouse received three days of training (60-minute sessions) in the running wheel prior to the first testing period. There was no additional training periods prior to testing at 16 or 20 weeks of age.

2.2.3. Forced swim test

One 60 × 30 × 60 cm fish tank was used for all forced swim testing. Water was filled to a depth of approximately 32 cm and warmed to 22 °C. Each mouse was placed in the water for a period of 10 min and the amount of time spent floating was recorded. Floating was determined to be the cessation of all movement by the mouse. Each mouse was only exposed to the test once at each age level and no pre-exposure took place prior to any of the test sessions.

2.2.4. Grooming behavior

Immediately following the forced swim test mice were placed in an empty, dry cage and were evaluated for grooming behaviors for 10 min. Grooming behaviors included body shaking, paw licking, snout rubbing, body licking, and scratching. The latency for each mouse to initiate one of these grooming behaviors after being removed from the forced swim test was measured along with the total amount of time each mouse spent engaged in grooming behavior.

2.2.5. Sucrose consumption

Mice were separated into single cages for the administration of a sucrose solution. All mice were exposed to a 4% sucrose solution for a period of three days prior to the first test period. No training days took place prior to the second and third testing periods. Both training and testing sessions lasted 60 min and the amount of sucrose consumed by the mouse during that period was recorded in grams.

2.3. Lupus symptom evaluation

All mice were given peripheral evaluations of health at each 12, 16 and 20 week testing period. The presence of dermatitis and lymphadenopathy was evaluated by clinical inspection and severity/prevalence of symptom was rated as none (1), mild (2), moderate (3) and severe (4). Levels of blood and protein in the urine were measured using Bayer Hema-Combistix Reagent Strips for Urinalysis.

3. Results

For each variable, a 2 (lupus/control) × 3 (12, 16, 20 weeks of age) mixed ANOVA was performed. Follow up independent sample t-tests comparing lupus versus control mice at each of the three testing periods were also conducted. All data analyses were deemed significant if $p \leq 0.05$. Means ± standard deviation of all behavioral tests are reported in Table 1.

3.1. Operant task

Lupus-prone mice earned slightly more reinforcers on the operant task than control mice; however, analyses revealed no significant main effects or interactions for this variable, suggesting a similar level of operant activity in both groups of mice. Follow up t-tests showed no differences between the lupus-prone and control groups at 12, 16 or 20 weeks of age.

3.2. Sucrose consumption

Lupus-prone mice consumed less sucrose solution than control mice as averaged over the three testing weeks. A 2 × 3 ANOVA revealed a significant effect of group [$F(1,21) = 4.33$, $p < 0.05$] and a marginally significant effect of testing week [$F(2,42) = 2.81$, $p = 0.07$], but no interaction. Follow-up independent sample t-tests revealed a significant effect of group at 16 weeks of age [$t(21) = 2.35$, $p < 0.05$] with lupus mice consuming less sucrose at this time point.

3.3. Running wheel

Lupus-prone mice traveled significantly less distance in the running wheel at each testing period. An ANOVA revealed a significant effect of group [$F(1,21) = 19.50$, $p < 0.01$] and week [$F(2,42) = 13.73$, $p < 0.01$] (with less running at 16 and 20 weeks), but no interaction. Follow up independent sample t-tests indicated that lupus mice exhibited significantly less activity in the running wheel at all time points compared to controls [week 12: $t(21) = 2.62$, $p < 0.05$, week 16: $t(21) = 3.49$, $p < 0.01$, and week 20: $t(21) = 5.68$, $p < 0.01$].

3.4. Forced swim test

Lupus-prone mice spent more time floating at each testing period throughout the duration of the study. An ANOVA revealed a significant effect of group [$F(1,21) = 59.30$, $p < 0.01$] and week [$F(2,42) = 7.93$, $p < 0.01$], but no interaction. Follow up independent sample t-tests indicated that lupus mice exhibited significantly more floating at all time points [week 12: $t(21) = -6.19$, $p < 0.01$, week 16: $t(21) = -6.6$, $p < 0.01$, and week 20: $t(21) = -5.48$, $p < 0.01$].

3.5. Latency to groom

No differences in latency to groom following the forced swim test were found between lupus-prone and control mice. There was no main effect of group or interaction revealed by the ANOVA, but there was an effect of week [$F(2,42) = 4.65$, $p < 0.05$] reflecting that the latency to groom was longer at 12 weeks than at 16 or 20 weeks. Follow up t-tests comparing lupus-prone and control mice at each of 12, 16 and 20 weeks were not significant.

3.6. Time spent grooming

There was no difference in the time spent grooming for lupus-prone or control mice. An ANOVA revealed no effect of group or interaction between variables. There was an effect of week [$F(2,42) = 15.11$. $p < 0.01$] reflecting that less time was spent grooming at 12 weeks than at 16 and 20 weeks. Follow up t-tests comparing lupus-prone and control mice at each of 12, 16 and 20 weeks were not significant.

Table 1
Data collected on all behavioral measures, comparing lupus-prone and control mice at each time period (12, 16 and 20 weeks of age) and averaged over all testing periods. Data are presented as means ± standard deviation.

	Testing time period			Average across weeks	ANOVA results
	12 weeks	16 weeks	20 weeks		p values
Operant task (reinforcers)					
Control	56.60 ± 23.42	54.60 ± 19.63	56.80 ± 19.17	56.00 ± 20.74	Group n.s.
Lupus	67.40 ± 22.34	58.20 ± 13.26	70.80 ± 19.37	65.47 ± 18.23	Week n.s.
Sucrose consumption (ml)					
Control	2.5 ± 1.08	3.79 ± 2.06	2.92 ± 1.69	3.07 ± 1.61	Group < 0.05
Lupus	1.81 ± 0.66	2.07 ± 1.43	2.79 ± 1.06	2.22 ± 1.05	Week = 0.07
Running wheel (distance, miles)					
Control	0.55 ± 0.17	0.41 ± 0.14	0.47 ± 0.10	0.48 ± 0.14	Group < 0.01
Lupus	0.36 ± 0.017	0.19 ± 0.17	0.21 ± 0.12	0.25 ± 0.15	Week < 0.01
Forced swim (floating, seconds)					
Control	53.44 ± 41.33	85.91 ± 70.00	94.36 ± 60.43	77.91 ± 57.92	Group < 0.01
Lupus	217.01 ± 80.80	270.48 ± 62.14	264.94 ± 85.34	250.81 ± 76.09	Week < 0.01
Grooming (latency, seconds)					
Control	30.22 ± 26.92	16.78 ± 6.76	15.04 ± 13.03	20.68 ± 15.57	Group n.s.
Lupus	27.46 ± 18.48	22.81 ± 13.27	14.45 ± 6.09	21.57 ± 12.61	Week < 0.05
Grooming (time spent, seconds)					
Control	360.5 ± 78.83	376.0 ± 45.21	421.8 ± 31.30	389.6 ± 51.78	Group n.s.
Lupus	301.83 ± 102.30	411.17 ± 51.92	448.0 ± 62.45	387.0 ± 72.23	Week < 0.01

3.7. Lupus symptoms

Independent samples t-tests were conducted to compare lupus symptoms at each of the three time periods. Analyses revealed significant differences in lymphadenopathy when comparing lupus-prone and control mice at 16 weeks [lupus: 2.33; control: 1.09: $t(21) = -4.00$, $p<0.01$] and at 20 weeks [lupus: 2.92; control: 1.18, $t(21) = -5.38$, $p<0.01$]. Significant differences were not found for proteinuria or hematuria, but at 20 weeks lupus-prone mice were expressing higher levels of both [proteinuria, lupus: 2.64; control: 2.40; hematuria, lupus: 1.72; control: 1.00].

3.8. Correlations

Behavioral measures of fatigue, such as the forced swim test, are sometimes also used to measure depression in animals (Stone et al., 2008). Since we were interested in assessing a fatigue, rather than depression, correlations were run between the results of the running wheel test (an accepted measure of fatigue, Chao et al., 1992), the amount of time spent floating in the forced swim test (a measure of both fatigue and depression, Tanaka et al., 2003, 2008; Stone et al., 2008), and the amount of sucrose consumed (a measure of anhedonia used to evaluate depression in animals, Muscat and Wilner, 1992).

A list of all significant correlations is reported in Table 2. The distance traveled in the running wheel was found to be significantly negatively correlated with the time spent floating in the forced swim test at all testing periods, reflecting that the more time animals spent floating (and therefore the less time they spent swimming), the less distance they traveled in the running wheel. Distance traveled in the running wheel did not correlated with sucrose consumption at any week. Finally, sucrose consumption, a measure of hedonia, only correlated with floating time at week 20. At 12 and 16 weeks, there was no relationship between sucrose consumed and floating time, something we would expect if both of these tests were tapping into a depressive-like state.

To further evaluate the possible direct relationship between variables, partial correlations were conducted, allowing us to control for the effect of one variable while look at the relationships between the other variables. The relationship between distance traveled in the running wheel was correlated with floating time, while controlling for sucrose consumption, and there was a significant negative correlation at all time points [12 weeks: $r = -0.59$, $p<0.01$, 16 weeks: $r = -0.64$, $p<0.001$, 20 weeks: $r = -0.56$, $p<0.01$]; this was similar to what was seen when correlating running distance and floating duration without controlling for sucrose consumption. When controlling for running distance and correlating floating duration and sucrose consumption, there was no significant relationship at week 12 or 16; at week 20 there was a significant negative correlation, $r = -0.52$, $p<0.05$, indicating that more floating was associated with less sucrose consumption, similar to what was seen without controlling for running distance. Finally, when controlling for floating time, we did see a significant negative correlation between running distance and sucrose consumption at week 16 ($r = -.43$, $p<0.05$), but not at other time periods.

Table 2
Significant correlations achieved when correlating running distance, sucrose intake and floating duration. Running distance was negatively correlated with floating time at all testing time points. Sucrose intake negatively correlated with floating duration at 20 weeks only. No other correlations were signification. N = 23 for all correlations.

		Running distance			Sucrose
		12 weeks	16 weeks	20 weeks	20 weeks
Floating duration	12 weeks	$r = -0.53$ $p<0.01$	$r = -0.44$ $p<0.05$	$r = -0.57$ $p<0.01$	$r = -0.60$ $p<0.01$
	16 weeks	$r = -0.47$ $p<0.01$	$r = -0.64$ $p<0.01$	$r = -0.78$, $p<0.01$	$r = -0.58$ $p<0.01$
	20 weeks	$r = -0.88$ $p<0.01$	$r = -0.42$ $p<0.05$	$r = -0.62$, $p<0.01$	$r = -0.59$ $p<0.01$

4. Discussion

This study aimed to evaluate disease-associated fatigue in lupus-prone MRL/lpr mice using a battery of tests. This animal model was chosen as these mice spontaneously develop a chronic, systemic autoimmune disease allowing for the assessment of fatigue without having to experimentally induce it. By avoiding external experimenter manipulation of the mice, we were able to study chronic fatigue in a manner more representative of fatigue experienced in humans with chronic illnesses.

Consistent with previous research on fatigue-like states (Swain and Maric, 1997; Tanaka et al., 2003), the lupus-prone mice spent significantly more time floating than control mice. Additionally, the lupus-prone mice traveled significantly less distance than control mice while in the running wheels (Chao et al., 1992). The finding that lupus-prone mice spontaneously develop fatigue-like state corroborates the results of Ottenweller et al. (1998) who used mice given *Brucella abortus* in their fatigue study. In our study mice were placed in a running wheel for only 1 h per testing period, as opposed to previous studies in which mice had access to a running wheel in their home cage; this is important as it suggests that short-term access to a running wheel may be a valid manner to measure the construct of fatigue.

To rule out the interpretation that the reduction in running wheel distance or increase in floating in the forced swim test was due to the presence of disease-induced impairments in motor function, we included a motor-dependent operant task. There was no significant difference between the number of reinforcers earned (and therefore the amount of lever pressing activity) by lupus-prone mice and controls. We also saw no difference in post-swim grooming activity when comparing our control and lupus-prone mice. Based on the lack of differences in behavioral performance on these tasks, the differences seen in the forced swim and running wheel tests are unlikely due simply to a muscle or motor disability in the lupus-prone mice; however, future research should further evaluate the possible impact of muscle strength and motor activity by using a grip strength test.

We set out to evaluate fatigue in lupus-prone mice and we employed previously used measures of fatigue, including floating behavior in the forced swim test, to do so. We found that our lupus-prone mice did spend more time floating in the forced swim test than controls, which might indicate these mice are experiencing fatigue; however, one of the current challenges with interpreting behavior in the forced swim test as a measure of fatigue is that it is also widely accepted as a measure of depression. For instance, a review by Petit-Demouliere et al. (2005) confirmed the reliability and predictive validity of the forced swim test as a tool for testing antidepressants and more recently, Stone et al. (2008) used the forced swim test as a model of inducing depression. In their study, Stone and colleagues found that repeatedly exposing mice to a forced swim session (four consecutive days, plus every fourth day over a 3 week period) resulted in an increase in mouse floating that corresponded with decreased sucrose consumption and these behavioral changes were interpreted as indicative of a depressive-like state (Stone, et al., 2008). While the lupus-prone mice in our study did float significantly longer than control mice at all testing periods, there was no relationship between sucrose intake and floating time at 12 and 16 weeks of age; by 20 weeks, there was a negative correlation between sucrose consumption and time spent floating. Our mice were exposed to the forced swim test only three times, compared to Stone's mice which were exposed eight times, but in light of the results of Stone et al. (2008) future research may wish to evaluate experimentally naïve mice at 20 weeks of age to

determine if this correlation remains independent of previous exposure to the forced swim test.

Although our lupus-prone mice did show some decrease in sucrose consumption, compared to controls this was not a consistent difference, causing us to speculate that the behavioral changes we observed were likely due to fatigue and not simply to a depressive-like state. By using numerous behavioral measures, in particular sucrose consumption, forced swim floating time and distance traveled in the running wheel, we propose a possible first-step to dissociating depressive-like states from fatigue-like states in an animal model. Sucrose consumption has been used by many as a measure of hedonics in nonhuman animals and many researchers suggest that amount consumed is related to depressive-like states (DeLaGarza, 2005; Monleon et al., 1995; Muscat and Wilner, 1992). It is also generally accepted that distance traveled in a running wheel is related to fatigue (Chao et al., 1992). Forced swim activity, on the other hand, is often used to measure both fatigue (Tanaka et al., 2003, 2008) and depression (Stone et al., 2008). Since we were interested in assessing a fatigue-like state, rather than a depression-like sate, correlations were run between the results of the running wheel test, the amount of time spent floating in the forced swim test, and the amount of sucrose consumed.

Analyses showed a negative relationship between forced swim floating time and distance traveled in the running wheel at all three testing periods, a more limited negative relationship (at 20 weeks only) between forced swim floating time and sucrose consumption, and overall, no relationship between sucrose consumption and distance traveled in the running wheel (this was significant at week 16 only when controlling for floating time). The consistent negative correlations between running distance and floating duration may suggest that these two measures are tapping into the same internal construct in our animals – fatigue – and based on our findings, we are confident that our lupus mice were experiencing a fatigue-like state. In addition, the limited correlations between performance in the forced swim test and sucrose consumption, possible measures of depressive-like states, could be interpreted to mean that the behavior of our mice in the forced swim test may be due to fatigue, rather than a depressive-like state; however, our study cannot rule out that a depressive-like state might also be driving some of the behavioral changes in our mice. Regardless, we do think that the limited relationship between the distance traveled in the running wheel and the amount of sucrose consumed suggests that the running wheel activity is independent of a hedonic state of the animal.

Based on the findings from the current study, we believe that future research on chronic fatigue would benefit from the use of multiple behavioral procedures to lend support to the conclusion that behavioral differences between groups are due to the presence of chronic fatigue and not simply depression or muscle and motor deficits. This interpretation needs to be assessed with additional models of both chronic and acute fatigue, especially since in cases of chronic disease organisms might be experiencing both depression and fatigue. Future research should compare behavior in these tests using animals that might be experiencing both a fatigue- and depressive-like state, like lupus-prone mice, with animals that are more likely experiencing a "pure" fatigue state, such as animals with a weight attached to their legs (Tanaka et al., 2003, 2008). Since we used only 10 min in the forced swim test, future research might also wish to use a longer duration forced swim exposure to determine if that might impact the relationships between floating and wheel running.

As indicated above, in many patients, including those with SLE, fatigue is correlated with depression (Kellner et al., 2010; Krupp et al., 1990; Wang et al., 1998) and MRL/lpr mice have been shown to experience a depressive-like state (Goa et al., 2009). While fatigue can be independent from depression, these states are likely related in complex ways. Fatigue is one possible symptom of depression (American Psychiatric Association, 2000) and humans experiencing fatigue often also experience depression (e.g., Kellner et al., 2010). Given the relationship between fatigue and depression, it may be important for researchers to use measures that are relevant for understanding depression while also studying fatigue. Follow up work should further evaluate the distinction between depression and fatigue in nonhuman animals by administration of pharmacological agents known to block or induce depression but not fatigue. If the behaviors evaluated in our study allow researchers to distinguish fatigue from depression as we propose, a substance known to treat or induce depression but not fatigue, ought to show dissociation in this model. Research has suggested the paroxetine is effective at treating depression, but not fatigue, in cancer patients (e.g., Morrow et al., 2003) so this might be a target for such assessments.

Our data contribute to the study of chronic fatigue in animals, and provide a possible way to study fatigue associated with SLE in nonhuman animals. Proinflamatory cytokines such as Il-1β, IL-6, and TNF-α have been suggested as contributing factors in the behavioral changes elicited by lupus-prone mice (Tomita et al., 2001,2004) as well as contributing factors of a biological cause of fatigue (Swain, 2000) in many disease states. Although previous research has shown that peripheral cytokine levels did not correlate with the expression of fatigue in people with lupus (Kellner et al., 2010) this work did not rule out the possibility that brain levels of proinflammatory cytokines are correlated with fatigue in people with lupus. Follow up studies should evaluate the role of cytokines in mediating the differences in behavior seen in lupus-prone and control mice, by both measuring circulating levels of cytokines and evaluating the effects of cytokine administration, as a means of determining the biological mechanisms of fatigue and depression.

5. Conclusions

MRL/lpr lupus-prone mice appear to exhibit a fatigue-like state (as shown by decreased activity in a running when and increased floating time in the forced swim test) that we hypothesize may be independent of a depressive-like state or motor impairments. Although future research is necessary to more clearly distinguish fatigue from depression, our findings do indicate that using multiple behavioral measures when studying fatigue in nonhuman animals may be beneficial for separating fatigue from other factors that might induce behavior change. Using these behavioral measures, follow up work should evaluate the possible role of pro-inflammatory cytokines as mediators of this fatigue-like state.

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