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FEMALE SEX AND OBESITY INCREASE PHOTOPHOBIC BEHAVIOR IN MICE

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Abstract—Migraine affects predominantly women. Furthermore, epidemiological studies suggest that obesity is a risk factor for migraine and this association is influenced by sex. However, the biological basis for this bias is unclear. To address this issue, we assessed light avoidant behavior, a surrogate of photophobia, in female C57BL/6J mice fed regular diet (RD) or high-fat diet (HFD, 60% kcal from fat). We first assessed sex differences in basal photophobia in 20–25-week-old mice and found that both obese and lean females spent significantly less time in light than their male counterparts. Next, we assessed photophobia evoked by trigeminal stimulation with intradermal capsaicin. Females at 20–25 weeks of age did not display capsaicin-evoked photophobic behavior unless they had diet-induced obesity. When we tested 8–11-week-old females to determine if the diet alone could be responsible for this effect, we found that both HFD and RD 8–11-week-old females exhibit capsaicin-evoked photophobic behavior. This is in contrast to what we have previously shown in males and indicates a sex difference in the photophobic behavior of mice. Comparison of 20–25-week-old RD mice with 8–11-week-old RD mice suggests that age or age-related weight gain may contribute to capsaicin-evoked photophobic behavior in males, but not in females. These findings suggest that obesity exacerbates photophobia in both sexes, but additional work is needed to understand the sex- and age-specific mechanisms that may contribute to photophobia and trigeminal pain.

Key words: diet induced obesity, sex, photophobia, trigeminal, migraine, capsaicin.

INTRODUCTION

Migraine is a common and debilitating disorder that affects 36 million Americans. The prevalence of migraine is particularly high, between 20% and 28%, in women during reproductive years (Lipton et al., 2001; Buse et al., 2013). Furthermore, women report migraine symptoms, such as photophobia, more often than men (Lipton et al., 2001; Buse et al., 2013). Epidemiological studies have found that obesity increases the odds of having migraine, with the strongest association among women and individuals of reproductive age (18–50 years) (Peterlin et al., 2010, 2013). Despite these findings, we do not know how obesity interacts with female sex to influence the manifestation and progression of migraine.

Overall, the studies addressing sex differences in animal models of migraine have found that females are more sensitive to stimulation of the trigeminal system, which is critical to migraine pathophysiology. This has been demonstrated with respect to susceptibility to cortical spreading depression (Brennan et al., 2007), activation of dural afferents by inflammatory mediators (Scheff and Gold, 2011), and migraine-like behavior in response to chronic dural application of inflammatory mediators (Stucky et al., 2011). Sex differences also exist in the expression levels of signaling molecules thought to be involved in migraine pathophysiology, including calcitonin gene-related peptide and its receptor components (Stucky et al., 2011) and serotonin-synthesizing enzymes (Asghari et al., 2011). How obesity may influence any of these sex differences has not been investigated. Furthermore, how these differences contribute to aspects of migraine other than headache, like photophobia, also remains unknown.

Several studies have recently elucidated mechanisms contributing to migraine-related photophobia using rodent models (Recober et al., 2009, 2010; Noseda et al., 2010; Okamoto et al., 2010; Dolgonos et al., 2011; Kaiser et al., 2012), but only one study assessed both sexes (Recober et al., 2009). While no sex differences in photophobic behavior were found in the human Receptor Activity Modifying Protein 1 (RAMP1) transgenic mice (Recober et al., 2009), this should be verified in wildtype mice and in other experimental paradigms. This is especially important since photophobia occurs more frequently in women than men and seems to change with age among migraine patients (Wöber-Bingöl et al., 2004; Buse et al., 2013; Bolay et al., 2015).

In this study, we sought to characterize light avoidance, a surrogate measure of photophobia, in female mice, with and without diet-induced obesity, a broadly accepted obesity model. This builds on our previous work in male mice where we found that obesity enhances basal photophobic behavior and lowers the

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Abbreviations: HFD, high-fat diet; RD, regular diet.

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stimulus required to evoke photophobia (Rossi et al., 2016). While light aversion is worsened in both sexes by diet-induced obesity, our current findings suggest that sex may interact with age to modulate this behavior differently in males and females across the life span.

EXPERIMENTAL PROCEDURES

Animals

Female C57BL/6J mice bred in our colony were randomly assigned to and maintained on either a regular chow diet (RD; Teklad) or a 60% high-fat diet (HFD; Research Diets Inc. #D12492) from weaning (3–4 weeks of age). To evaluate photophobic behavior before and after the induction of obesity, a cohort of mice was tested at 8–11 weeks of age and a different cohort was tested at 20–25 weeks of age. Females are sexually mature at 8 weeks of age, and do not exhibit anestrus until 49 weeks of age (Felicio et al., 1984). The weights of the mice at weaning and during testing are presented in Table 1. We chose to test two cohorts rather than one cohort at different ages to limit the injections of capsaicin to one per whisker pad to avoid desensitization of nociceptors. All mice were maintained on a standard 12-h light–dark cycle (lights on at 06:00), with food and water available *ad libitum*.

For comparison, we are showing data from male mice derived from the same colony that have been previously published (Rossi et al., 2016). The 8–11-week-old males (RD $n = 17$, HFD $n = 17$) and females (RD $n = 15$, HFD $n = 17$) consisted of three smaller batches ($n = 3$–8 mice/diet and sex in each batch) tested together each baseline and post-treatment testing day. The 20–25-week-old females (RD $n = 49$, HFD $n = 49$) consisted of five smaller batches used for baseline evaluation in Fig. 1 (RD: $n = 6$–11 mice/batch; HFD: $n = 8$–14 mice/batch). Two of these five batches (RD $n = 21$; HFD $n = 24$) also received post-treatment testing, but were not tested with males in the same day. All protocols were approved by the University of Iowa IACUC and were conducted in accordance with *The Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23, revised 1996).

Light avoidance assay

Photophobia is a key clinical feature of migraine and light avoidance in mice can be a useful tool to evaluate mechanisms underlying migraine-related photophobia (Recober et al., 2009, 2010; Markovics et al., 2011; Kaiser et al., 2012; Chanda et al., 2013). Here we assessed light avoidance in mice as previously described (Rossi et al., 2016), using a modified commercially available apparatus (Med Associates) where mice are given a choice between light and darkness. To avoid the potential anxiety-inducing effects of the clear walls and open top on the lit side, we covered the walls of the chambers with black opaque foam panels and the top with clear Plexiglas to allow illumination of that side while being enclosed like the dark side. The light source in each box was a

Table 1. Weights of different age and diet groups of female mice at weaning (3–4 weeks old) and testing

Cohort age	Diet	Weaning		Testing		*n*
		Mean	SEM	Mean	SEM	
8–11 weeks	HFD	9.54	0.46	25.49***	0.64	17
	RD	8.63	0.55	20.09	0.34	15
20–25 weeks	HFD	10.22	0.28	37.87***	0.85	49
	RD	9.78	0.21	23.11	0.44	49

*** $p < 0.0001$ HFD vs RD.

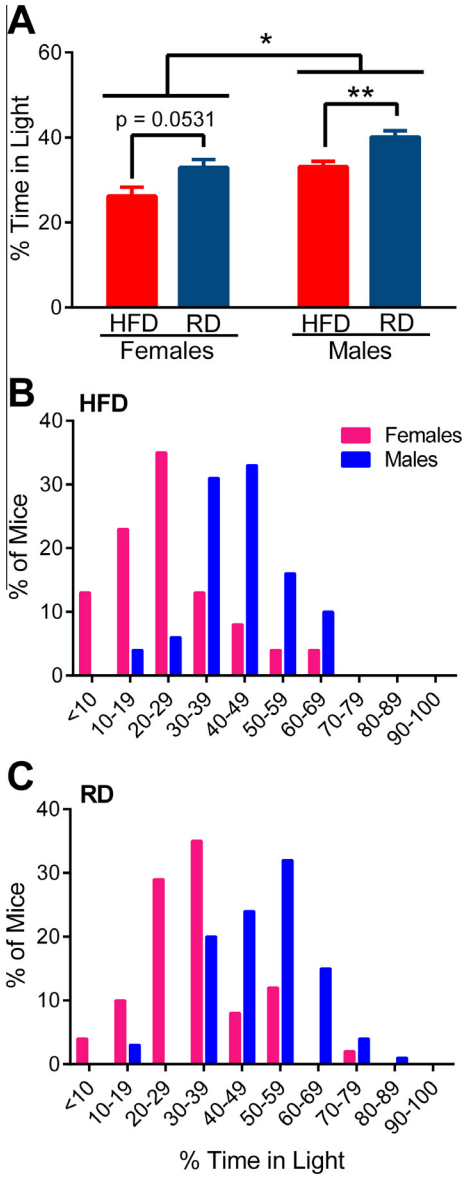

Fig. 1. Effects of sex and diet on basal time spent in light in mice at 20–25 weeks of age. (A) Baseline percentage of time spent in light for HFD (red bars) and RD (blue bars) females ($n = 49$/diet) and males ($n = 74$–80/diet). (B) Histogram stratifying the distribution of HFD female ($n = 49$, pink bars) and male ($n = 80$, blue bars) mice based on the percentage of time in light at baseline. (C) Histogram stratifying the distribution of RD female ($n = 49$, pink bars) and male ($n = 74$, blue bars) mice based on the percentage of time in light at baseline. $^{*}p < 0.05$ and $^{**}p < 0.01$ for indicated comparisons. (Male data has been previously published and is shown here for direct comparison with females (Rossi et al., 2016).)