ANALYSIS OF THE CONTRIBTUION OF DIET, EXERCISE, AND THE GUT ON THE ONSET AND SEVERITY OF UNIPOLAR DEPRESSION AND ANXIETY BETWEEN THE SEXES

By Meagan E. Hinks

A Thesis submitted

to the School of Graduate Studies in partial fulfillment of the

requirements of the degree of

Master of Science, Department of Psychology, Faculty of Science

Memorial University of Newfoundland

July 2022

St. John's, Newfoundland and Labrador

Abstract

Major depressive disorder (MDD) and anxiety disorder are two of the leading causes of global disease burden, with females being twice as likely as males to be diagnosed with the disorders. Despite this distinct sex-linked disparity of diagnosis, it is unclear what underlies the sex bias of these affective conditions. In Experiment 1, we investigated whether sex interacts with the lifestyle factors of diet and exercise to predict incidence of prior diagnosis, current treatment, and symptom severity of unipolar depression and anxiety disorder using the archival Atlantic Partnership for Tomorrow's Health (PATH) database. For Experiments 2A through 2C, we used two mouse models of depression and healthy controls to assess the role of sex and the gut microbiome on the display of depressive-like behaviours. In Experiment 1, the expected two-fold increase in MDD and anxiety disorder diagnosis was found, and diet and exercise significantly predicted MDD and anxiety disorder prevalence and severity. For Experiments 2A through 2C, it was found that gut microbial transfer from females of both healthy and depression models was sufficient to induce depressive-like behaviours, and female gut contents from depression models was most effective at inducing behavioural changes in female recipients. Together, these findings begin to elucidate the factors contributing to the sex bias in affective disorders as well as identify future avenues for alternative treatments for these conditions.

Keywords: depression, anxiety, diet, exercise, gut-brain-axis, cecal transfer

Declaration of Co-Authorship

I hereby declare the content of this thesis incorporates material that results from joint research under the supervision of Dr. Ashlyn Swift-Gallant. Yellow Martin (MSc student) performed behavioural testing and dissections for experiment 2A, assisted myself in oral gavage for experiment 2C, and assisted myself and performed behavioural testing and dissections for experiments 2B and 2C; Francine Burke (MSc student) assisted myself and performed testing and dissections for experiments 2B and 2C; Mark Corrigan (MSc student) and Derek Wan (MSc student) performed the chronic unpredictable stress paradigm on the cecum donor mice for experiment 2B under the supervision of Dr. Francis Bambico; Megan Wiseman (BSc Honours student) aided in data coding in experiment 1; Ariana Doody (BSc Honours student) aided in behavioural coding for experiment 2A. Experimental designs were made by Dr. Swift-Gallant in conjunction with myself. In all cases, primary contributions, data analysis, interpretation, and writing were performed by myself.

Acknowledgments

First of all, I would like to thank my supervisor, Dr. Ashlyn Swift-Gallant, along with members of the Swift-Gallant and Bambico labs for all their help and contributions towards my project. Thank you, Ashlyn, for offering all your patience, guidance, and expertise along my journey through my master's work at Memorial. Despite disruptions from the Covid-19 pandemic causing dynamic changes in the nature of my project time and time again, you were always willing to provide new alternatives and ideas, all the while allowing me to incorporate my own research interests throughout the process. It has been a pleasure to work with you and I look forward to continuing to follow your guidance for my PhD.

I would also like to especially thank Francine, Megan, Stephanie, and Yellow of the Swift-Gallant lab for providing their own skills and aid throughout the many days spent running statistics, behavioural testing, and dissections. Your contributions to my project have been immense and I am so grateful to have worked with such inspirational, hard-working women. Moreover, a thank you to my committee members, Dr. Jacqueline Blundell and Dr. Darcy Hallett, for extending their own expertise and providing alternative insights to my work throughout this process.

Furthermore, I would like to thank my friends and family members for their vast, never-ending support. To Caitlin and Faith, thank you for your friendship and always providing a listening ear when I'm in need, even if it's from a province away. To my parents, Yvonne Cake and Bruce Hinks, and my sister, Kaitlyn Churchill, thank you for always encouraging me to strive to do my best while providing your love and support along the way.

Table of Contents

List of Tables

Page

List of Figures

Analysis of the contributions of diet, exercise, and the gut on the onset and severity of unipolar depression and anxiety between the sexes

Affective disorders are some of the leading causes of non-fatal illness worldwide (Global Burden of Disease [GBD] 2017, Disease and Injury Incidence and Prevalence Collaborators, 2018). According to findings from the GBD database, incidence of chronic and major depressive disorders (MDD), as well as anxiety disorder, have increased by almost 50% over the past 30 years (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018; Liu et al., 2020). Among those affected, females are two times more likely to be diagnosed with either MDD or anxiety than males (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018; Knoll & MacLennan, 2017; Weissman et al., 1993). Various studies have investigated the source of this sex-linked disparity through genetic and hormonal contributions (for reviews, see Amiel Castro et al., 2021; Seney et al., 2018). However, little work has been done investigating how this sex difference could be linked to differences in lifestyle factors between males and females. Notably, sex differences have been reported in diet and exercise, two lifestyle factors known to affect the incidence of affective disorders (Craft et al., 2014; Firth et al., 2019; Kim et al., 2020). Emerging evidence suggests that such lifestyle factors may contribute to affective disorders via modulation of the microbial environment in the gut and its interaction with the brain, also known as the gut-brain axis (GBA) (Yatsunenko et al., 2012; Zhang et al., 2010).

Lifestyle Factors

Exercise

Along with the obvious physical benefits, a regular exercise regime has also been found to improve mental wellbeing (for review, see Stubbs et al., 2017; Yu et al., 2014). Implementation of a regular exercise regime has been shown to provide both anxiolytic and anti-depressive benefits to those suffering from low mood (Awick et al., 2017; Chen et al., 2014; Hallgren et al., 2018; Ströhle et al., 2009; Wipfli et al., 2011). This exercise-induced improvement in mood has been demonstrated consistently throughout the lifespan and is often used in conjunction with other therapies to improve quality of life for individuals suffering from varying ailments (Awick et al., 2017; Chen et al., 2014; Hallgren et al., 2018; Nabkasorn et al., 2005).

In work investigating the potential underlying mechanism for how exercise can improve mood, researchers have primarily focused on the endocrine changes in the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis, predominantly through either endorphin release or cortisol stabilization (Ball et al., 1987; Merenlander-Wagner et al., 2009; Nabkasorn et al., 2005; Taylor et al., 1994). Exercise has been linked to increased release of beta-endorphin, a hormone involved in the processing of painful stimuli and stress (Gerra et al., 2000; Merenlander-Wagner et al., 2009; Taylor et al., 1994). This exercise induced increase in beta-endorphin is hypothesized to ameliorate depressive symptoms through pain reduction. However, conflicting evidence posits that increased levels of beta-endorphin in depressed patients may be indicative of dysregulation of the HPA axis, with higher levels of beta-endorphin corresponding with a greater severity of symptoms (Baştürk et al., 1998; Darko et al., 1992; Goodwin et al., 1993; Lobstein et al., 1989). There is similarly varied support for exercise-induced cortisol regulation. The relationship between cortisol regulation and exercise has been found to be dependent as a threshold effect of exercise intensity, with lower intensity exercise decreasing plasma cortisol

and high intensity exercise increasing such levels (Hill et al., 2008; Ponce et al., 2019). However, regular training and psychological state prior to exercise implementation has also been found to affect this relationship, indicating that the link between exercise and cortisol regulation is dynamic and dependent on various factors, both mental and physical (Edwards et al., 2018; Tsai et al., 2014)

One often neglected factor in understanding exercise effects on affective disorders is sex differences, which may account for discrepancies in the literature. First, on average, females tend to report higher levels of exercise, although exercise is a better predictor for quality of life in men (Craft et al., 2014). There have also been reports of sex differences in the type and exertion level of exercise on mood regulation, although not all reports have been consistent. For example, Kim et al. (2020) reported that males experience significantly lower depressive symptom severity after strength training, while females showed greater benefits from walking, suggesting that the type of exercise may affect the sexes differently. Moreover, recent data has shown that females, although initially demonstrating a higher severity of both anxiety and depressive symptoms, showed a greater improvement of mood following implementation of a vigorous cardio exercise regime compared to males (McDowell et al., 2016). At pubertal onset, the HPA-axis undergoes sex-dependent changes based on corresponding surges of gonadal hormones, which may have implications for sex differences in how exercise-induced hormonal fluctuations influence mood (for review, see Sheng et al., 2021). Together, this research suggests that there is an increased importance of exercise as a protective factor for affective disorder susceptibility in females compared to males, and there may be sex differences in the underlying mechanisms (i.e., HPA-axis) that exercise acts upon to ameliorate such conditions. With these

findings in mind, more work is needed to understand how sex plays a role in the relationship between exercise and affective disorders.

Diet

Although diet quality most overtly impacts physical health, research has shown that there is a link between poor diet quality and a greater incidence of both depression and anxiety (Firth et al., 2019; Gibson-Smith et al., 2018; Yu et al., 2014). However recent work, including a meta-analysis of diet intervention studies, has demonstrated that while improvement of affect with diet quality can be observed in both sexes, females benefitted more from an improved diet than males (Firth et al., 2019). This finding suggests that diet quality could have a greater impact on susceptibility for depressive and anxiety disorders in females compared to males, akin to what is seen concerning the effects of exercise (McDowell et al., 2016). However, the majority of large-scale studies continue to ignore sex as a variable in studying diet and affective disorders (Gibson-Smith et al., 2018; Yu et al., 2014). Moreover, recent studies have suggested that adherence to not just a healthy, but a Mediterranean-style diet has pronounced improvement in wellbeing, although sex differences have not been assessed in such studies (Parletta et al., 2017; Salari-Moghaddam et al., 2019; for review, see Ventriglio et al., 2020).

Specifically, a Mediterranean diet consists of a high intake of fruits and vegetables, beans, legumes, whole grains, fish and vegetable oils, specifically olive oil, while limiting the use of added sugars, refined carbohydrates, saturated fats, and highly processed foods (Martínaz-Gonzàlez et al., 2012). Several studies have investigated the impact this diet regime has on both overall health as well as mental health (Parletta et al., 2017; Salari-Moghaddam et al., 2019). Overall, findings have consistently shown that implementation of a Mediterranean-style diet ameliorates depressive symptoms, with greater adherence resulting in more pronounced

improvements in wellbeing (Parletta et al., 2017). A proposed reason for this increased improvement is the fact that foods consumed in a Mediterranean diet are high in polyphenols, a naturally occurring micronutrient shown to have implications for mental wellbeing (Pathak et al., 2013; Westfall & Pasinetti, 2019). Interestingly, research on the effects of a Mediterranean diet on stroke risk in individuals with cardiovascular disease has shown that females demonstrated greater benefits to the intervention than males (Paterson et al., 2018). However, this apparent sex-difference in response to Mediterranean diet has not been investigated in relation to depression or anxiety.

Many studies, both human and rodent, have shown that ingesting foods specifically high in polyphenols can aid in the reduction of depressive like symptoms (Haskell-Ramsay et al., 2017; Naveen et al., 2013). Research on the action of polyphenols in relation to affective disorders have shown that they are able to modulate monoaminergic transmission within the brain as monoamine oxidase inhibitors, much like commonly prescribed antidepressants or anti-anxiety medications (Haskell-Ramsay et al., 2017; Naveen et al., 2013; Pathak et al., 2013; Westfall & Pasinetti, 2019). Efficacy of polyphenol absorption is dependent on various factors such as the bioavailability of the specific type of polyphenol being ingested, along with overall gut microbial composition (Pathak et al., 2013; Westfall & Pasinetti, 2019). Since gut microbiol composition has been found to differ between individuals in a sex-dependent manner, it is possible that the differential processing of polyphenols, among other dietary nutrients, in the gut could have an impact on the susceptibility of an individual's predisposition for affective disorders through the GBA (Chen et al., 2018; Dominianni et al., 2015; Santos-Marcos et al., 2018).

Gut-Brain Axis

One pathophysiological mechanism for affective disorders such as MDD and anxiety disorder that has gained increased interest among researchers over the past decade pertains to the link between the gut microbiome and the brain, the gut-brain axis (GBA). This link suggests that the composition of the gut microbiome could influence an individual's susceptibility to affective disorder onset and, therefore, could be a new target for treatment (Bravo et al., 2011; Liang, et al., 2018). Several studies have found differences in gut microbial composition between patients with MDD and healthy controls (Aizawa, et al., 2016; Carabotti et al., 2015; Dinan & Cryan, 2013; Kelly et al., 2016; Lin et al., 2017; Valles-Colomer et al., 2019; Zheng et al., 2016). Along with this, various studies have implemented probiotics as a potential anxiolytic to regulate the stress response through changes in the gut, with such treatment often reducing self-reported stress levels along with physiological stress-induced changes (Ait-Belgnaoui et al., 2013; Lew et al., 2019; Mohammadi et al., 2015; Yang et al., 2014). However, studies on the GBA have not been consistent in their identification of particular microbes linked to the stress response and/or low mood. Part of this inconsistency could be to the fluctuating nature of gut microbiome composition in response to lifestyle factors, and sex differences in the responsiveness of the microbial environment to such factors and how they affect the brain.

As factors such as sex and age, as well as general lifestyle factors, such as diet, exercise, and stress, can all have an effect on the diversity of the gut microbiome, it is common for microbial levels and diversity to vary from person to person (Chen et al., 2018, 2020; Dominianni et al., 2015; Konturek et al., 2011; Santos-Marcos et al., 2018; Yatsunenko et al., 2012; Zhang et al., 2010). These differences in the gut microbiome contribute to the efficiency of the digestive system, but also can affect the brain directly via the vagus nerve, or indirectly via interaction with the immune and endocrine system (for review, see Carabotti et al., 2015). While

there is increasing awareness that the responsiveness of affective disorders to lifestyle factors may be mediated via the gut-brain axis, only one study to date has investigated the potential sex-differences in microbial composition of these patients (Chen et al., 2018). Findings from this study have shown that there may be differences in microbial composition that vary by sex of the patient, with females demonstrating higher levels of Actinobacteria compared to healthy controls, while depressed males had significantly higher levels of Bacteroides (Chen et al., 2018). These findings suggest that the effects of depression on the gut microbiome may be differentially expressed in male and female patients suffering from depression compared to healthy controls (Chen et al., 2018). However, further work is needed to elucidate the underlying mechanisms resulting in this differential diversity, with more robust characterizations of lifestyle and demographic influences on the GBA.

Rodent models have often been used to understand the role of the gut microbiome on affect. Various studies have investigated the impact of the gut microbiome on depressive and anxiety-like symptoms using germ-free (GF) and specific-pathogen free (SPF) mice (Luk et al., 2018; Luo et al., 2018; Neufeld et al., 2010; Sudo et al., 2004; Zheng et al., 2016). GF mice are raised without exposure to any microorganisms, while SPF mice are free of any pathogenic organisms. In general, GF mice have been found to exhibit a decreased behavioural stress-response compared to SPF mice or controls (Luk et al., 2018; Luo et al., 2018; Neufeld et al., 2010; Zheng et al., 2016). Moreover, data has shown that treatment with a single microbe, *Bifidobacterium,* can restore anxiety-like behaviour in female SPF mice similar to that of wildtype controls, while males present a restoration of behaviours that surpass typical levels (Luk et al., 2018). This further suggests a sex-linked interaction of particular microbes within the GBA, similarly demonstrated in human studies (Chen et al., 2018; Zheng et al., 2016)

Although the exact microbial contents influencing affect are still under investigation, studies of fecal transplantation have shown that mice exposed to microbial products from either human patients or mouse models of depression exhibit a depressed phenotype (Kelly et al., 2016; Li et al., 2019; Luo et al., 2018; Pu et al., 2021; Zheng et al., 2016). For example, one key study investigated the effects of MDD fecal transfer in GF mice compared to GF and SPF treated with *E. coli* and found that only the germ-free MDD fecal transplant mice exhibited depressive-like behaviours (Luo et al., 2018). On various behavioural tests, only the germ-free MDD fecal transplant mice exhibited depressive-like behaviours (Luo et al., 2018). As the mice treated with *E. coli* bacteria did not show a corresponding increase of depressive-like behaviours as was seen in the mice who received the MDD fecal matter, this study provides evidence indicating that there are distinct microbial bacteria present in depressed subjects which may directly or indirectly affect mood (Luo et al., 2018). However, further work is needed to characterize the exact microbial contents involved in such behavioural alterations. Importantly, previous work investigating fecal transplantation and mood have strictly used male mouse models of depression (Kelly et al., 2016; Li et al., 2019; Luo et al., 2018; Pu et al., 2021; Zheng et al., 2016). Due to the sex differences in the gut microbiome as well as in the prevalence and risk for mood disorders, further work is needed to determine whether there is a corresponding change in behavioural phenotype of females undergoing such exposures.

Aims

This research consisted of two lines of study. The first analyzed the contribution of diet quality, including overall healthy diet and Mediterranean-style diet, and exercise on the likelihood of minor or major depression and anxiety disorder diagnosis and severity of symptoms in a large-scale prospective cohort study. In this study, we assessed the proportion of the sex-

linked variance in rates of diagnosis and symptom severity that can be explained by these lifestyle factors. It was hypothesized that a healthy diet, Mediterranean diet and adherence to an exercise regime would contribute to lower diagnostic rates of affective disorders and a lower severity of symptoms. Based on prior research, it was predicted that these factors would have a greater impact on diagnostic rates for females than males. Specifically, there is an apparent conflict in the literature in that females are more likely to adhere to a healthy diet and exercise regime, which have been shown to decrease vulnerability to affective disorder, but females are also more likely to be diagnosed with affective disorders than males. This discrepancy may be explained in part by the findings that females tend to benefit more from improved diet and exercise than males; as such sex-based analyses was conducted to address the questions 1) do sex differences exist in affective disorders in our cohort of participants, 2) does diet (healthy and Mediterranean) and exercise reduce the likelihood of diagnosis with an affective disorder and/or symptom severity, and 3) do such lifestyles factors interact with sex in conferring resiliency to such conditions. By understanding which factors contribute to the mental health of both males and females, sex-based recommendations on the use of diet and exercise as a potential target to ameliorate symptoms can be made, primarily for those who do not benefit from current treatment regimes. Furthermore, future work in this area can evaluate how such lifestyle factors may interact with sex differences in the underlying mechanisms contributing to the development of affective disorder (i.e., HPA-axis, gut microbiome).

For the second line of research, we used rodent models to explore how sex differences in gut microbial composition may contribute to the expression of depressive symptomology. Past research has demonstrated that microbial transplantation from depressed rodent models to healthy controls can induce a depressive-like phenotype. However, to date, this research has only

evaluated males. As such, we studied two well characterized models of depression in both sexes. Specifically, in Experiment 2A, we performed gut microbiome transplantation (i.e., cecum) from an olfactory bulbectomy (OBX) rodent model of depression in C57Bl/6 mice. This experiment was repeated in another rodent model of depression, chronic unpredictable stress (CUS), in Balb/c mice (Experiment 2B). Note, we used the mouse strain for which the depression model was most well characterized/shows the largest effect (Otmakhova et al., 1992; Sathyanesan et al., 2017). Lastly, since female mice tend to show greater depressive-like behaviours at baseline than males, we assessed in a third experiment (Experiment 2C) whether healthy control male cecum transplant to females decreases depressive-like behaviour, and whether healthy control female cecum transplant to males increases depressive-like behaviours.

Given that females present a more pronounced susceptibility to MDD, we predicted that female cecal transplantation would result in an increased depressive-like phenotype in the receivers. Moreover, we predicted that such alterations would be dependent on treatment type, with mice receiving CUS or OBX donor cecum having more pronounced behavioural alterations compared to the mice receiving wildtype cecum. Findings from this experiment would further elucidate the impact of the gut microbial products on mood using an alternative model of depression to evaluate generalizability. In all three rodent experiments, a between-subjects factorial design was used to assess the role of the gut microbiome transplantation and sex on depressive-like behaviours on several standard behavioural paradigms for depression models.

Methods

Experiment 1: Evaluating the Contributions of Sex and Lifestyle Factors to Unipolar Depression and Anxiety Prevalence and Severity

Participants

Participant data were sourced in partnership with the Atlantic Partnership for Tomorrow's Health (PATH) database, a regional subgroup of the Canadian Partnership for Tomorrow's Health study (Dummer et al., 2018). This database consists of variables, both demographic and health related, from individuals aged 30 to 75 from the Atlantic provinces of Canada. A core questionnaire was completed between 2009-2015, with a follow-up questionnaire completed between 2016-2018. Further information on participant recruitment and data collection has been previously reported (Sweeney et al., 2017). Variables pertaining to affective disorder status and lifestyle factors (i.e., diet and exercise) were obtained through the follow-up questionnaire, which assessed these variables in greater detail than the core questionnaire. Data were selected based on overall completion of questions pertaining to the variables implemented in this research. Participants with incomplete or inconclusive data were omitted from analysis for that variable (for total sample sizes, see Appendix A, Table 1). All participant data was used with consent of the participants upon completion of the questionnaire(s).

Measures

Unipolar Depression and Anxiety Diagnosis and Treatment. Participants were assessed based on both previous diagnosis and current treatment for minor and major depressive disorder and anxiety. In the questionnaire, participants were prompted to indicate whether they had ever been professionally diagnosed with either of the disorders and, if so, if they were currently undergoing treatment. Participants responding with "Prefer not to answer" or "Don't know" were omitted from analysis, while responses of "No/Not applicable" or "Yes" were coded as "0" or "1", respectively.

Severity of Depression and Anxiety Symptomology. Assessment of the severity of depressive symptoms was done via the validated, nine-item, Patient Health Questionnaire (PHQ-9) within the follow-up questionnaire of the Atlantic PATH database (Kocalevent et al., 2013; Kroenke et al., 2001). Each of the nine items of the PHQ-9 is assigned a score of 0, 1, 2, or 3, with higher scores indicating a greater severity of the associated symptom (Kroenke et al., 2001). A total score for the scale is then used to approximate an overall severity of depressive symptoms, with participants being assigned as suffering from minimal (0-4), mild (5-9), moderate (10-14), moderately severe (15-19), or severe (20-27) depressive symptoms (as previously described, Kroenke et al., 2001). Although unipolar depression and anxiety can often present through similar key symptoms, the PHQ-9 measures distinct symptoms of depression with 88% sensitivity and specificity (Kroenke et al., 2001). Due to small sample sizes in the severe category, score categorizations were modified in the present study into minimal (0-4), mild (5-9), moderate (10-14), and moderately severe to severe (15-27) levels of depression.

Assessment for level of anxiety was measured through the Generalized-Anxiety-Disorder-7 (GAD-7) scale. This scale is comprised of seven-items pertaining to anxious attitudes where respondents must indicate, on a categorical scale from 0-3, how often they experience such attitudes (i.e., from 0-“Not at all” to 3-“Nearly every day”), with higher scores indicating increased anxiety levels (Löwe et al., 2008; Spitzer et al., 2006). Similar to the specificity of the PHQ-9 for depression, the GAD-7 has been found to be distinct in its measurement of anxiety disorder and is commonly used as a validated and reliable measurement of generalized anxiety disorder (Löwe et al., 2008; Spitzer et al., 2006). The scoring of the GAD-7 is often partitioned into categories consisting of minimal (1-4), mild (5-9), moderate (10-14), and severe (15-21) symptoms. However, due to small sample sizes in the moderate to severe categories,

categorizations were modified in the present study into either minimal anxiety symptoms (0-4) or moderate to severe anxiety symptoms (5-21).

Exercise. Exercise was quantified through implementation of the International Physical Activity Questionnaire (IPAQ) – long form. The IPAQ – long form is a validated and reliable, 27-item, self-report questionnaire developed for quantification of physical activity level in adults up to 70 years of age (Craig et al., 2003; Hagströmer et al., 2006). Questionnaire items consist of various diverse measures of physical activity, from recreational activity to activity related to housekeeping (Craig et al., 2003). This allows for a broad measurement of activity level across a variety of different lifestyles (Craig et al., 2003). Activity level was determined based on corresponding metabolic equivalent (MET)-minutes per week of activity for each category. Participants were then categorized as having either low (1), moderate (2), or high (3) levels of physical activity based on their global scores of MET-minutes per week calculated within the Atlantic PATH database. Participants who failed to respond to an item were omitted from analysis.

Diet Quality. Diet quality was assessed through a 15-item Healthy Eating Index (HEI) previously developed for use with the Atlantic PATH database (see Yu et al., 2014). This HEI was developed based on recommendations made by Health Canada and other, North American-based, HEIs (Geunther et al., 2008; Health Canada, 2019). The index is comprised of five serving-based items (e.g., Milk and dairy products (serving/d)) and 10 general diet quality items (e.g., ate at least half of grain products whole grain each day). Serving-based items each offer a score ranging from 0-10, with a perfect score of 10 indicating that the recommended number of daily servings was met. Participant scores then decreased proportionately with each serving the individual was below the recommended (i.e., a score of 5 for a participant who had 1 of the

recommended 2 daily servings). In contrast to the original study which used recommended daily servings based on sex and age, the lowest serving recommendation per item was used for analysis. Furthermore, the general diet quality items were scored as "0" or "1", depending on whether the statement was applicable to the participant. Each item was then summated to obtain an overall score for the HEI, from 0 (low quality diet) to 60 (high quality diet).

For analysis, scores on the HEI were subdivided into three levels of diet quality: low to moderate (0-30), moderate to high (30-45), or high (45-60). Although the original study using the HEI subdivided scores into quintiles, the subpopulation from the database used for this study was not sufficiently large enough to conserve these divisions (Yu et al., 2014). Participants who responded "I don't know" or who failed to respond to an item were omitted from analysis.

Mediterranean-Style Diet. To obtain an estimate for the degree to which each participant's diet followed a Mediterranean-style regime, a modified form of a previously validated 14-item Mediterranean Diet adherence screener was used (Martínez-González et al., 2012). For this screener to be applied to the data available in the Atlantic PATH database, four items (i.e., #2, 6, 13, and 14) were omitted as these items were not asked of participants in the current study, and one item (i.e., #8) was modified to measure cups of green tea consumed per week in lieu of wine, due to its similar antioxidative action and high catechin content (Arts, van de Putte & Hollman, 2000; Brizuela et al., 2010; Ojebi et al., 2018).

The original Mediterranean screener consisted of primarily serving-based questions (e.g., How many fruit units (including natural fruit juices) do you consume per day?), which could be directly applied to the data available from Atlantic PATH (Martínez-González et al., 2012). Participants who met the serving criteria for each individual item were allotted a score of "1", while those who did not meet the criteria were scored as "0". However, for participants to obtain

a score of "1" for the first item in the Mediterranean screener pertaining to the primary use of olive oil as the main cooking fat, which was not serving-based, participants who responded with "olive oil" or any of the combination responses including olive oil in the Atlantic PATH questionnaire were given this point (i.e., responses of 4, 11, 12, 13, 14).

Once a total score was obtained for each participant, participants were then categorized as having either low (0-3), moderate (4-5), or high (≥6) Mediterranean scores (MedScore). These levels were determined based on the proportion of scores corresponding to each level of adherence in the original 14-item screener (Martínez-González et al., 2012). Participants who responded "I don't know" or who failed to respond to an item were omitted from analysis.

Procedure

Analysis. To assess the impact of diet quality, MedScore, and exercise level on predicting diagnosis of unipolar depression and anxiety, as well as symptom severity, multiple regression was used. When the criterion was the presence of a diagnosis (e.g., incidence of Major Depressive Disorder diagnosis across the lifetime), logistic regressions were conducted. When the criterion was severity of depression or anxiety, then OLS linear regressions were conducted. Models first tested if these lifestyles factors acted as significant predictors for unipolar depression and anxiety diagnosis and/or symptom severity, after controlling for sex. Second, these models tested the interaction between sex and each lifestyle factor to assess whether lifestyle factors play a larger role in susceptibility for females than for males. However, these interactions were only included in the model if they were significant.

Experiments 2A-2C: Evaluating the Role of Sex and the Gut Microbiome in Rodent Models of Depression

Subjects

All mice were obtained from Charles River Laboratories and underwent a seven-day acclimation period prior to intervention. Mice were housed singly on a 12:12 hour light/dark cycle, with lights on at 7:00am. Food and water were provided ad libitum.

Experiment 2A. We assessed whether gut microbiome transplantation from male and/or female rodent models of depression to control mice is sufficient to induce a depressive-like phenotype. Olfactory bulbectomy (OBX) surgeries were performed on C57BL/6 stimulus mice to induce a depressive-like phenotype (n = 6 males and 6 females). Male and female cecum contents were collected from the OBX donor mice. Two cohorts of 30 C57BL/6 mice, half male, half female, were used (N_{Total} = 60) and mice were equally split by sex into 3 treatment groups: vehicle (saline control), female OBX cecum, or male OBX cecum, creating 6 groups of 10 mice each.

Experiment 2B. For assessment of whether results from Experiment 2A are generalizable to other rodent models of depression, we conducted a replication experiment with a more ecological relevant rodent model – the CUS mouse model of depression using the Balb/C strain of mouse. Cecum contents from the CUS donor mice (n = 6-8 males and 6-8 females) were collected for treatment. Two cohorts of 30 Balb/c mice, half male, half female, were used (N_{Total} = 60). However, one male mouse was terminated prior to experiment commencement due to a medical complication (N_{Total} = 59). Mice were then equally split by sex into 3 treatment

groups: vehicle (saline control), female CUS cecum, or male CUS cecum, with one less male mouse in the vehicle treatment group.

Experiment 2C. To assess whether sex of gut microbiome contents alone (i.e., from control mice) is sufficient to influence depressive-like behaviour, we assessed the effects of cross-sex gut microbiome transplantation in otherwise unmanipulated males and females on our standard battery of tests. Two cohorts of 30 C57BL/6 mice, half male, half female, were used ($N_{Total} = 60$). Mice were equally split by sex into 3 treatment groups: vehicle (saline control), female cecum, or male cecum.

Measures

Behavioural Testing. Following cecal/saline treatment, mice were assessed for depressive-like behaviours on a behavioural test battery. This battery comprised of 4 tests of depressive-like symptomology: sucrose preference, splash, tail suspension, and forced swim tests. Each test was then scored for the corresponding behavioural indices of depression and analyzed to compare for sex and/or treatment effects using between-subjects analysis of variance (ANOVA) followed by planned comparisons for significant effects (critical alpha set at $p < .05$). For these planned analyses, comparisons were made only within sex and between treatment groups (i.e., females treated with female cecum compared to female controls) and between sexes within treatment groups (i.e., females treated with male cecum and males treated with male cecum), and only if the relevant interaction was significant or trending.

Sucrose Preference Test. The sucrose preference test (SPT) is a measure used to assess anhedonia, the decreased ability to experience pleasure, one of the core symptoms of depression (Papp et al., 1991). For this test, mice were first habituated to drinking water from two small plastic cups in their home cages (i.e., rather than from a bottle), for 18-24h. After the habituation

phase, one cup was filled with drinking water and the other cup filled with a 4% sucrose solution. Following 18 hours, the weight of each cup was recorded and deducted from the starting weight. A preference score was then recorded by subtracting the weight of the water drank from the weight of the sucrose solution. For Experiment 2A, a high sucrose preference score was characterized by a preference of 80% or above, while experiments 2B and 2C used a criterion of 70% or above. This was based on the mean sucrose preference values found for the control group mice in the respective experiments, as typical cut-off values for high sucrose preference typically vary between 65-80% (Appendix B, Tables 1, 5, 9; Cheeta et al., 1997; Eltokhi et al., 2021; Strekalova & Steinbusch, 2010).

Splash Test. In the splash test (ST), a single mouse was placed in an empty mouse cage and provided with five minutes to habituate to the new environment. Then, a viscous 20% sucrose solution was applied to the dorsal coat of the mouse. The test was then video recorded for five minutes and later scored for grooming behaviours. Grooming behaviour was used as an indication of self-care and motivational behaviour, while decreased time spent grooming indicates depressive-like behaviour (Yalcin et al., 2005).

Tail Suspension Test. In the tail suspension test mice were suspended by their tail (i.e., tail is taped to the top of a rodent cage) so that the body dangles, facing downward for five minutes. Each trial was video recorded, with immobility (measure of depressive-like behaviour) and escape behaviours (indication of motivational behaviour) measured and analyzed for both duration and frequency with Ethovision XT14 Software. Escape behaviours consist of movements of the hinds/forelimbs as well as increased number of attempts/min to reach their tail (Belovicova et al., 2017).

Forced Swim Test. Mice were placed in a transparent glass cylinder (38.0 cm height x 27.0 cm diameter) filled with water (at a temperature of 25°C +/- 0.5°C). The mice could not reach the top or bottom of the cylinder, and thus are suspended in the water for the duration of the 5-minute test. The video recordings of the trials were then analysed for duration and frequency of depressive-like behaviour (i.e., immobility) using Ethovision XT14 software (Fitzgerald et al., 2019).

Procedures

Olfactory Bulbectomy. OBX surgeries were performed on donor mice for experiment 2A between post-natal day (PND) 60-65. Anesthesia was achieved through administration of isoflurane, first in an induction chamber at 2.5%, then maintained at 2% throughout the surgery. Meloxicam was administered as a slow-release analgesic (4mg/kg). Once anesthetized, mice were mounted in a skull-flat position on a standard stereotaxic apparatus. Procedures used were modified from the OBX protocol outlined in a study by Kelly, Wrynn, and Leonard (1997). To access the olfactory bulbs, two burr holes were drilled into the cribriform plate, 1.5mm anterior to the rostral dorsal cerebral vein according to stereotaxic coordinates outlined in Paxinos and Franklin (2019). Bulbs were bilaterally aspirated through the burrs with a sterilized, 22-gauge needle attached to a vacuum-line. Ablation was confirmed visually upon removal and further consolidated visually post-mortem. After removal, haemostatic sponge was then inserted into the empty cavities to both prevent blood loss as well as regrowth, and the incision sutured shut (Kelly, Wrynn, & Leonard 1997).

Chronic Unpredictable Stress Paradigm. Donor mice for experiment 2B underwent a six-week CUS paradigm, adapted from Bambico, Nguyen, and Gobbi (2009). This paradigm consisted of a variety of physical (i.e., restraint), psychological (i.e., predator scent), and

circadian (i.e., disruption of light/dark cycle) stressors, all inescapable but non-debilitating in nature. Stressors were implemented randomly at three varied timepoints each day, morning, afternoon, and evening. At the end of each week, presence of anhedonia-like behaviour was assessed by a SPT. Once anhedonia-like behaviour was detected, the intervention ceased, and cecal contents were collected.

Cecum Collection. Cecum donor mice were overdosed with Avertin (Tribromoethanol, 40mg/kg) prior to surgery. Avertin was administered through an intraperitoneal injection in the lower right abdominal quadrant to prevent injection directly into the cecum. An incision was then made medially down the abdomen, the cecum sac removed from the intestinal tract and the cecal contents extracted. Cecal contents were collected in 1.7ml microcentrifuge tubes, combined by sex. In Experiments 2A and 2C, cecum was diluted 1:1 with 0.1M phosphate buffered saline (PBS) prior to storage due to limited donor cecum availability. Cecum was stored at -80 degrees Celsius until thawed and used for transfer.

Cecal Transfer. Cecal treatments started between PND 60-95 and cecum was administered via oral gavage using a 20-gauge ball-tipped gavage needle. Mice were treated once every other day for 12 days, for a total of six treatments. Extracted donor cecum was thawed and then diluted 1:10 with saline. Mice received 0.2mL of either diluted cecum (experimental) or saline (control) each day.

Results

Experiment 1: Evaluating the Contributions of Sex and Lifestyle Factors to Unipolar Depression and Anxiety Prevalence and Severity

Using the Atlantic PATH dataset, we found the expected sex difference such that females are approximately two times more likely to have ever been diagnosed with either major/minor depressive disorder, anxiety disorder, or to be undergoing current treatment for these disorders than males (see Appendix A, Tables 2-7). For MDD, individuals with moderate/high- or high-quality diets were less likely to have been diagnosed with MDD than those with low/moderate-quality diets, and healthy eating accounted for significantly more of the variance in diagnosis and current treatment of MDD than just sex alone (Appendix A, Table 2). Physical activity level was also found to impact incidence of past diagnosis and current treatment, with individuals characterized as having a high physical activity level being significantly less likely of having ever been diagnosed with MDD or be currently treated for the disorder than individuals with a low physical activity level, with scores on the IPAQ accounting for significantly more variance than just sex alone (Appendix A, Tables 2 & 5). Along with this, there was a significant interaction between sex and the contrast of low and high quality diets for prevalence of treatment for MDD, with females with high quality diets showing a significantly lower likelihood of currently undergoing treatment for the disorder than males with high quality diets (Appendix A, Tables 8 & 9). However, this significance is lost when looking at the effects in the overall model, suggesting that the effect of diet is strongest in the difference between low and high diet quality, but that the effect itself is not robust enough to be seen in the contrasts with low and moderate diet quality (Appendix A, Tables 8 & 9). Lastly, there were no significant effects of lifestyle factors on prevalence of diagnosis or treatment for minor depressive disorder, however physical activity level accounted for significantly more variance of minor depression diagnosis or current treatment than just sex alone (Appendix A, Tables 3 & 6).

For severity of depressive symptoms, females were more likely to be suffering from mild or moderate/severe depressive symptoms than minimal compared to males (Appendix A, Table 8). Individuals with lower quality diets (i.e., both lower healthy eating scores or Medscores) were more likely to suffer from mild or moderate/severe depressive symptoms compared to minimal than those with moderate/high- or high-quality diet scores (Appendix A, Table 8). Along with this, individuals considered as having moderate or high levels of physical activity were significantly less likely to suffer from moderate/severe depressive symptoms compared to individuals with low physical activity (Appendix A, Table 8). Furthermore, scores of either healthy eating, Medscores, or physical activity in conjunction with sex accounted for significantly more of the variance in depressive symptom severity than sex alone (Appendix A, Table 9).

As mentioned prior, females were found to be twice as likely to have been previously diagnosed with, or undergoing current treatment for, anxiety disorder than males (Appendix A, Table 4 & 7). For lifestyle factors, individuals with high quality diets were found to be significantly less likely to have ever been diagnosed with anxiety disorder or be currently undergoing treatment than those with low-moderate or moderate-high quality diets (Appendix A, Table 4 and 7). Physical activity level was also found to influence incidence of anxiety diagnosis and treatment, with individuals categorized as having moderate or high levels of physical activity being significantly less likely to be diagnosed with anxiety or undergoing current treatment than those with low levels of physical activity (Appendix A, Table 4 and 7). Diet and physical activity level was also found to account for more variance in anxiety disorder diagnosis or current treatment than just sex alone (Appendix A, Table 4).

For severity of anxiety symptoms, females were significantly more likely to be suffering from mild or moderate/severe depressive symptoms than minimal compared to males (Appendix A, Table 10). Analysis of anxiety symptom severity in conjunction with lifestyle factors showed that individuals categorized with low quality diets had a significantly higher severity of anxiety symptoms than those with moderate or high-quality diets (Appendix A, Table 10). Along with this, individuals with high levels of physical activity were significantly more likely to be suffering from less severe anxiety symptoms than those with low levels of physical activity (Appendix A, Table 10). Overall, diet and physical activity level accounted for significantly more variance in anxiety symptoms than just sex alone (Appendix A, Table 11).

Experiment 2A: Cecal Transfer from OBX-Mice to Healthy Mice

Compiled means and standard error of the means can all be found in Appendix B (Tables 1-4).

Sucrose Preference Test

Cecal transplantation from the OBX-depression model to healthy animals did not affect scores on the sucrose preference test, however a main effect of sex was found, F (1,53) = 4.076, p = .049, η^2 = 0.068, such that male mice had significantly higher sucrose preference scores than females regardless of treatment type (Appendix C, Figure 1). Similarly, chi-square analysis of the proportion of mice who had a high sucrose preference score (≥80%) compared to a low score (<80%) showed a significant main effect of sex, X^2 (2, N =59) = 7.342, p = .007, OR = 4.443, 95% CI [1.471, 13.423], with a higher proportion of males with a high sucrose preference (N = 23/31) than females (N = 11/28) (Appendix C, Figure 1).

Splash Test

In the ST, a significant main effect of sex was found for both general grooming frequency, F (1, 51) = 10.408, p = .002, η^2 = 0.158, and movement duration, F (1, 51) = 33.529, p < .001, η^2 = 0.384. For general grooming frequency, females had significantly higher grooming frequencies than males (Appendix C, Figure 2). A similar pattern was seen for movement duration, with females having a significantly longer movement duration than males (Appendix C, Figure 2). While no effects of treatment were found on this test, there was a marginal interaction between sex and treatment for back grooming frequency, F (2, 51) = 2.539, p = .089, η^2 = 0.084, and a marginal effect of sex on back grooming duration, F (2, 51) = 2.887, p = .095, η^2 = 0.049 (Appendix C, Figure 3). For back grooming frequency, male and female OBX cecum significantly decreased grooming frequencies in males compared to male controls, p = .050, Cohen's d = 0.923, 95% CI [-0.018, 1.863] and p = .015, Cohen's d = 1.159, 95% CI [0.208, 2.110], respectively (Appendix C, Figure 3). For back grooming duration, males had marginally longer grooming durations than females (Appendix C, Figure 3).

Tail Suspension Test

For the TST, a significant main effect of sex on highly mobile duration was found, F (1, 47) = 4.304, p = .044, η^2 = 0.077, with females showing a significantly longer duration of being highly mobile then males (Appendix C, Figure 4). In line with these findings, sex had an opposite effect on immobile duration, F (1,47) = 9.216, p = .004, η^2 = 0.154, with males having a significantly longer duration of being immobile than females (Appendix C, Figure 4). No other significant main effects were found, p > .05.

Significant interactions between sex and treatment condition were found for mobile duration, F (2,47) = 3.458, p = .040, η^2 = 0.125, and immobile frequency, F (2,47) = 3.596, p =

.035, $\eta^2 = 0.131$. Follow up planned analyses for mobile duration found that female OBX cecum treatment in females led to decreased mobile durations compared to female controls, $p = .022$, Cohen's $d = 1.153$, 95% CI [0.146, 2.159] (Appendix C, Figure 5). As well, females treated with female OBX cecum had significantly lower mobile durations than males treated with female OBX cecum, $p = .039$, Cohen's $d = 0.978$, 95% CI [0.032, 1.924] (Appendix C, Figure 5). For immobile frequency, female OBX cecum marginally increased frequencies in males compared to male controls, $p = .074$, Cohen's $d = 0.902$, 95% CI [-1.911, 0.107] (Appendix C, Figure 5). Moreover, female OBX cecum significantly increased immobile frequencies in males, while decreasing such frequencies in females, $p = .021$, Cohen's $d = 1.097$, 95% CI [0.145, 2.049] (Appendix C, Figure 5). No other significant main effects or interactions were found for immobile frequency, $p > .05$.

Forced Swim Test

In the FST, a significant main effect of sex on highly mobile duration was found, F (1, 51) = 4.269, $p = .044$, $\eta^2 = 0.074$, with females having a significantly longer duration of being highly mobile than males (Appendix C, Figure 6). Sex had an opposite effect on immobile duration, with males having a significantly longer duration of being immobile than females, F (1,51) = 6.940, $p = .011$, $\eta^2 = 0.117$ (Appendix C, Figure 6). A marginal effect of sex was also found in both frequency of high mobility, F (1,51) = 3.858, $p = .055$, $\eta^2 = 0.068$, and frequency of mobility, F (1,51) = 3.646, $p = .062$, $\eta^2 = 0.063$. In both cases females trended on having a higher frequency of being highly mobile or mobile compared to males (Appendix C, Figure 7). No other significant main effects of treatment or interactions were found, $p > .05$.

Experiment 2B: Cecal Transfer from CUS-Mice to Healthy Mice

Compiled means and standard error of the means can all be found in Appendix B (Tables 5-8).

Sucrose Preference Test

For the raw sucrose preference scores on the SPT, a significant main effect of sex was found F (1,40) = 20.989, $p < .001$, $\eta^2 = 0.293$, as well as a significant interaction between sex and treatment, F (2,40) = 4.190, $p = .022$, $\eta^2 = 0.117$. For the main sex effect, male mice had significantly higher sucrose preference scores than females (Appendix D, Figure 1). In line with these results, chi-square analyses revealed a marginal effect of sex on the proportion of mice with a high sucrose preference score (≥70%), with a higher proportion of males with a high sucrose preference (N = 3/23) than females (N = 0/23), $X^2 (1, N = 46) = 3.209$, $p = .073$, Cramer's $V = 0.264$ (Appendix D, Figure 1).

Post-hoc analysis on the interaction of treatment and sex showed that males treated with female CUS cecum had higher sucrose preference scores than male controls, $p = .024$, Cohen's $d = 1.181$, 95% CI [0.128, 2.234] (Appendix D, Figure 2). Moreover, female CUS cecum increased scores in males, while decreasing scores in females, $p < .001$, Cohen's d = 2.613, 95% CI [-3.883, -1.343] (Appendix D, Figure 2). Finally, male CUS cecum marginally increased sucrose preference scores in males compared to females treated with male cecum, $p = .080$, Cohen's $d = 1.907$, 95% CI [-1.945, 0.132] (Appendix D, Figure 2).

Splash Test

For the ST, a marginal interaction effect of sex and treatment was found on general grooming frequency, F (2,52) = 2.506, $p = .091$, $\eta^2 = 0.085$. Follow up planned analyses revealed that male CUS cecum significantly increased general grooming frequencies compared to male

controls, $p = .045$, Cohen's $d = 0.942$, 95% CI [-1.883, -0.002]. Along with this, female CUS cecum marginally increased grooming frequencies in males compared to male controls, $p = .057$, Cohen's $d = 0.893$, 95% CI [-1.831, 0.046] (Appendix D, Figure 3). No other significant main effects or interactions were found for general grooming duration, back grooming duration and frequency, or movement duration and frequency, $p > .05$.

Tail Suspension Test

In the TST, there were no significant main effects or interactions found for high mobility frequency and duration, mobility frequency and duration, or immobile frequency and duration, $p > .05$.

Forced Swim Test

For to FST, significant interactions between sex and treatment were found for mobile frequency, $F(2,49) = 3.913$, $p = .027$, $\eta^2 = 0.129$, mobile duration, $F(2,49) = 3.549$, $p = .036$, $\eta^2 = 0.121$, and immobile frequency, $F(2,49) = 4.635$, $p = .014$, $\eta^2 = 0.149$. Moreover, a marginal interaction of sex and treatment was found for immobile duration, $F(2,49) = 2.535$, $p = .090$, $\eta^2 = 0.088$. For mobile frequency, female CUS cecum significantly decreased frequencies in females compared to controls, $p = .042$, Cohen's $d = 0.961$, 95% CI [0.017, 1.904], while females treated with male CUS cecum showed significantly higher frequencies compared to males treated with male CUS cecum, $p = .014$, Cohen's d = 1.166, 95% CI [-2.119, -0.213] (Appendix D, Figure 4). For mobile duration, female CUS cecum marginally decreased durations in females compared to female controls, $p = .055$**,** Cohen's $d = 0.903$, 95% CI [-0.038, 1.845] (Appendix D, Figure 5). No other significant interactions were found, $p > .05$.

Analysis of interactions for immobile frequency showed that females treated with female CUS cecum had lower immobile frequencies compared to female controls, $p = .042$, Cohen's $d = 0.959$, 95% CI [0.015, 1.902], while females treated with male CUS cecum had significantly higher frequencies than males treated with male cecum, $p = .007$, Cohen's $d = 1.287$, 95% CI [-2.247, -0.328] (Appendix D, Figure 5). Interactions for immobile duration showed that females treated with female CUS cecum had marginally higher immobile durations compared to female controls, $p = .099$, Cohen's $d = 0.772$, 95% CI [-1.708, 0.165] (Appendix D, Figure 5). Moreover, females treated with male CUS cecum had marginally lower durations than males treated with male cecum, $p = .052$**,** Cohen's $d = 0.915$, 95% CI [-0.027, 1.856] (Appendix D, Figure 5). No other significant interactions were found, $p > .05$.

Experiment 2C: Same and Cross-Sex Cecal Transfer from Healthy to Healthy Mice

Compiled means and standard error of the means can all be found in Appendix B (Tables 8-12).

Sucrose Preference Test

For the raw sucrose preference scores on the SPT, an ANOVA revealed a significant main effect of treatment, $F(2,52) = 5.154$, $p = .009$, $\eta^2 = 0.156$. Post-hoc planned analyses revealed that mice that received male cecum transplantation had significantly higher sucrose preference scores than those in the control group, $p = .002$, Cohen's $d = 1.009$, 95% CI [-1.674, -0.344] (Appendix E, Figure 1). No other significant main effects or interactions were found, $p > .05$.

Chi-square analysis of the proportion of mice who had a high sucrose preference score (≥70%) compared to a low score (<70%) showed a significant main effect of treatment, X^2 (2, N

=58) = 12.534, p = .002, Cramer's V = 0.462. A follow-up series of chi-square tests found that the proportion of mice in the male cecum condition with a high sucrose preference score was significantly greater (N = 17/20) than the proportion of those in the female cecum condition (N = 10/18), X^2 (1, N =38) = 3.993, p = .046, OR = 4.533, 95% CI [0.972, 21.141] (Appendix E, Figure 1). The same trend was found when comparing the male cecum and control conditions, with a significantly higher proportion of mice in the male cecum condition having a high sucrose preference score (N = 17/20) than in the control group (N = 6/20), X^2 (1, N =40) = 12.379, p < .001, OR = 13.222, 95% CI [2.790, 62.670] (Appendix E, Figure 1). No other significant main effects or interactions were found, p > .05.

Splash Test

` In the ST, analysis revealed significant main effects of both sex, F (1,51) = 4.127, p = .047, η^2 = 0.060, and treatment, F (2,51) = 6.936, p = .002, η^2 = 0.201, on movement frequency, but no significant interaction. Analysis on movement frequency revealed that overall females demonstrated a significantly lower movement frequency than males, regardless of treatment type (Appendix E, Figure 2). Further, post-hoc analysis of treatment on movement frequency showed that mice in the control condition had significantly lower movement frequencies compared to mice in either the female, p < .001, Cohen's d = 1.221, 95% CI [-1.925, -0.517], or male cecum conditions, p = .037, Cohen's d = 0.685, 95% CI [-1.343, -0.027] (Appendix E, Figure 2). There were no significant effects on movement duration, p > .05.

For general grooming, treatment condition was found to have a significant main effect on grooming frequency, F (2,51) = 3.949, p = 0.025, η^2 = 0.132, but no significant main effect of sex and no significant interaction. Follow-up planned analyses revealed that mice in the female cecum condition had lower grooming frequencies than both controls and mice treated with male

cecum, $p = .032$, Cohen's $d = 0.725$, 95% CI [0.048, 1.401] and $p = .011$, Cohen's $d = 0.857$, 95% CI [0.182, 1.531], respectively (Appendix E, Figure 3). There were no significant effects on general grooming duration, $p > .05$.

For back grooming, treatment condition was found to have a significant main effect on grooming frequency, $F(2,51) = 2.119$, $p = .131$, $\eta^2 = 0.075$. Follow-up planned analyses revealed that mice in the female cecum condition had significantly lower back grooming frequencies than those in the control condition, $p = .045$, Cohen's $d = .676$, 95% CI [0.002, 1.350] (Appendix E, Figure 3). There were no significant effects on general grooming duration, $p > .05$.

Tail Suspension Test

In the TST, a marginal effect of sex was found on highly mobile duration, $F(1,54) = 3.717$, $p = .059$, $\eta^2 = 0.063$. Analysis revealed that females trended on having a longer duration of being highly mobile than males (Appendix E, Figure 4). There were no significant main effects or interactions found for high mobility frequency and duration, mobility frequency and duration, or immobile frequency and duration, $p > .05$.

Forced Swim Test

For the FST marginal effects of treatment were found on both immobile frequency, $F(2,54) = 2.464$, $p = .095$, $\eta^2 = 0.080$, and duration, $F(2,54) = 2.701$, $p = .076$, $\eta^2 = 0.091$. Follow-up planned analyses of treatment on immobile frequency found that control mice did not significantly differ from the cecum treatment groups. However, mice in the female cecum condition had a significantly higher frequency of being immobile than those in the male cecum condition, $p = .034$, Cohen's $d = 0.687$, 95% CI [-1.334, -0.039] (Appendix E, Figure 5).

Further, mice in the male cecum treatment group had a significantly longer duration of being immobile than those in the control group, $p = .032$, Cohen's $d = 0.695$, 95% CI [-1.343, -0.047] (Appendix E, Figure 5). There were no significant main effects or interactions found for high mobility frequency and duration, mobility frequency and duration, or immobile frequency and duration, $p > .05$.

Discussion

Overview

Our goal was to investigate potential variables that may underlie the sex-linked disparity found in the prevalence of affective disorders. Using an archival database in Experiment 1, we investigated the correlation between the lifestyle variables (diet and exercise) on affective disorder diagnosis and treatment, and how the effect of these variables may differ based on individuals' assigned sex. Next, to investigate one of the potential mechanisms linked to lifestyle variables underlying the sex differences in affective disorders, we assessed the role of the gut microbiome in two models of depression using cecal transfer in Experiments 2A-2C. Overall, evidence from Experiment 1 supported the role of lifestyle factors as predictors for resiliency and risk for affective disorders, along with symptom severity. In Experiments 2A through 2C, cecal transfer from depression models was sufficient to induce a depressive-like phenotype in otherwise healthy mice, though both donor and receiver sex greatly influenced these results, with female cecum treatment to female recipients most consistently inducing a depressive-like phenotype across experiments. Moreover, behavioural changes arising from cross-sex healthy cecum transfer demonstrated that there could be baseline sex-differences in microbial composition that may influence mood regulation in healthy animals.

Assessment of Lifestyle Factors and Sex on Affective Disorders

For Experiment 1, we assessed whether lifestyle factors of exercise, diet, as well as adherence to a Mediterranean-style diet, impacted prevalence and severity of unipolar depression and anxiety in a cohort population obtained through the Atlantic PATH database. Similarly noted through assessment of global statistics, our analysis of affective disorder status demonstrated a sex-linked disparity in prevalence of unipolar depression and anxiety, as assessed through both diagnostic rates and treatment status (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). Our findings demonstrated that females were twice as likely to have ever been diagnosed or be currently treated for minor or major depressive disorder, as well as anxiety, compared to males. As these findings are consistent with what is found in the general global population, this supports the generalizability of findings from our subset cohort from the Atlantic PATH database.

For lifestyle factors, both diet and exercise influenced the likelihood of ever being diagnosed with unipolar depression or anxiety, as well as prevalence of active treatment. Specifically, higher quality diets and greater levels of physical activity were correlated with lower incidence and severity of depression and anxiety. This further emphasizes such factors as potential protective and/or risk factors for such disorders (Firth et al., 2019; Nabkasorn et al., 2005; Stubbs et al., 2017; Yu et al., 2014).

Interestingly, the impact of a high quality diet on affective disorder status interacted with sex for prevalence of current treatment for MDD, while exercise did not interact with sex on any measure. Specifically, females with high quality diets had a lower likelihood of currently undergoing treatment for MDD than males with high quality diets. However, this significance was lost when looking at the effects of moderate quality diets in the overall model. This indicates

that a higher quality diet may have a greater protective effect overall, specifally in females more than males, supporting previous literature (for review, see Firth et al., 2019). Alternatively, this could be due to differences in lifestyle trends between males and females. For example, in one study using the UK Biobank Resource, it was found that although males tended to have a greater energy intake, females were significantly more likely to exceed the recommended intake of fats, both overall and saturated, as well as sugar (Bennett et al., 2018). Since high levels of fats and sugar have often been found to increase rates of negative affect, and our measures did not partition this out from the overall diet score, this could explain why females tend to experience affective disorders at a higher rate than males (Jacques et al., 2019; Vermeulen et al., 2017). Moreover, the majority of studies finding sex differences on the impact of exercise on affect looked at specific types of exercise, whereas the measures obtained from the IPAQ for the purpose of this study looked at general exercise level (Kim et al., 2020; McDowell et al., 2016).

An alternate possibility for this sex-linked disparity of a high quality diets effect on affective disorder resiliency may be owed to the innate differences in the gut microbiome found between the sexes and how this may impact absorption of nutrients. It is well established that overall microbial content and diversity impacts how nutrients become metabolized and absorbed (Carabotti et al., 2015; Vernocchi et al., 2020). Although the exact sex-related microbial alterations that may impact mood are still under investigation, microbial changes related to sex have been found (Chen et al., 2018; Dominianni et al., 2015; Kim, 2022). Thus, although higher quality diets are found to predict a lower likelihood of suffering from either anxiety and/or depression in both sexes, female nutrient absorption may impact this relationship between higher quality diets and the GBA.

Alternatively, sex hormones also contribute to affective disorders vulnerability and thus may be of interest/may interact with lifestyle factors, leading to sex differences in depression and anxiety. For instance, hormones, both endogenous and exogenous, are linked to mood disorder prevalence and onset (Steiner et al., 2003). For example, age of first menarche was found to negatively correlate with risk of developing depression later in life (Harlow et al., 2004). Moreover, contraceptive use has been shown to alter brain structure and activity, with such changes promoting an increase in affective disorder susceptibility (Sharma et al., 2020).

Limitations and Future Directions for Work in Human Populations

While large-scale studies of this nature are required to understand how sex and lifestyle factors contribute to mood disorders, there are also limitations to the current study that can be addressed in future research. For example, all findings are correlational in nature and, since variables obtained through the database were not all intended to be used in the way we implemented them, there could be issues with both reliability and validity of measures – this is most notable for the Medscore (i.e., see modifications/substitutions to scale items described in methods). This could explain the inconsistency of our findings with how a Mediterranean-style diet may impact mood, as was seen in previous studies (Parletta et al., 2017; Salari-Moghaddam et al., 2019). Along with this, scores on the IPAQ were not categorized into exercise type; they were based solely on overall scores of MET-minutes per week. As it has been found that type of exercise can influence the outcome of exercise-based interventions in relation to mood and sex, such a specificity may provide more robust evidence on how exercise impacts resiliency to and severity of affective disorders (Kim et al., 2020; McDowell et al., 2016). Moreover, the variables used in the Atlantic PATH questionnaire only assessed variables of current lifestyle status of diet and exercise. Thus, it is likely that the lifestyle variables used would innately act as better

predictors for incidence of current treatment rather than previous treatment status. Future work assessing sex and affective disorder status should assess the impact of lifestyle factors (i.e., with attention to type of exercise and detailed accounts of diet), sex and affective disorders in a longitudinal design as well as in an experimental setting to address these limitations.

Future research should also consider the mechanism underlying the lifestyle and sex effects on affective disorders. The gut microbiome is one mechanism that we are beginning to assess in animal models, but human work will be needed. Furthermore, as mentioned prior, alternative factors, such as hormones, should also be assessed. As variables pertaining to these factors are available through the database, such factors should be analyzed to further characterize the relationship between endogenous and exogenous factors and mood disorder susceptibility and prevalence.

Assessing the Role of the Gut Microbiome in Rodent Models of Depression

For experiments 2A through 2C, I assessed the impact of cecal transfer on manifestation of a behavioural phenotype of depressive-like symptoms using two mouse strains and models of the disease. As previous work with fecal/cecal transfer from patients or rodent models had either not considered sex as a variable or neglected to use females, we first wanted to assess if this phenomenon could be replicated in both sexes, as well as to assess whether the sex of cecal donor or cecal receiver would influence how these behavioural changes manifested. Further, cross-sex cecal transfer from healthy mice was used to assess whether there were baseline sex differences in the gut microbiota that may impact the manifestation of a depressive-like phenotype/influence sex differences on our battery of tests. Through the current study, I found further support for previous research that gut microbial transfer from rodent models of depression to healthy animals increased a depressive-like phenotype in mice. However, it was

also found that sex of the donor and recipient matter. While there are nuances to these findings, discussed in detail below, findings suggest that female mice show a greater depressive-like phenotype at baseline and gut microbial contents from female OBX or CUS donors to female recipients results in the largest increase in depressive-like behaviours. This suggests that females possess a microbial profile which is more conducive to prompting a low affect, as well as such a gut more amenable to microbial colonization that leads to lower affect than what is found in males.

Experiment 2A: Cecal Transfer from OBX-Mice to Healthy Mice

For sex effects in Experiment 2A, females showed greater depressive-like behaviours on measures of anhedonia, regardless of treatment condition, whereas males show a greater depressive-like phenotype on escape behaviours. Specifically, females showed a decreased sucrose preference, while males showed decreased levels of mobility on the tail suspension and forced swim tests. As prevalence of affective disorder status can vary by sex, the way such disorders manifest behaviourally can vary by sex as well. For example, prior work has shown that with chronic unpredictable mild stress in mice, females undergoing the stress had a pronounced decrease in sucrose preference compared to males that underwent the same paradigm (Liu et al., 2019). This stress-induced alteration in sucrose preference may be partly mediated by changes in ovarian hormones. In one study analyzing the effects of trauma in mice who underwent gonadectomy, only the females with intact gonads experienced a decrease in sucrose preference in response to stress, but not males (Pooley et al., 2018). Moreover, measures of helplessness (which we measured with tail suspension and forced swim) are not as robust as a measure of stress for females as they are for males, with these measures having been developed for use with male rodents (Dalla et al., 2007; Fernandes et al., 1999). Thus, this emphasizes the

importance of considering both sex as well as the specific behavioural manifestation being measured when analyzing depression models.

Gut microbiome transplantation from the OBX model of depression to healthy animals had an effect on depressive-like behaviours of recipients, but in many cases these effects were sex dependent. Specifically, on the splash test, OBX cecum, regardless of donor sex, induced a depressive-like phenotype (i.e., decreased grooming on splash test) in males, but not females. Conversely, for escape behaviours, as measured through mobility (i.e., tail suspension), female OBX cecum, not male OBX cecum, induced a depressive-like phenotype in female mice, but not male mice. Thus, transfer of OBX cecum was sufficient to induce depressive-like behaviours on certain measures but was dependent on donor and receiver sex as well as on the behavioural test. These findings highlight the need to include both sexes when examining the role of the gut microbiome in depression and the need for extensive batteries of tests to assess behaviour. Note that if we only included males in our study the results would support prior research that gut microbial transfer from a rodent model of depression induces a depressive-like phenotype in healthy mice (Kelly et al., 2016; Li et al., 2019; Luo et al., 2018; Pu et al., 2021; Zheng et al., 2016); however, with the inclusion of females, we can see that this is only true for male mice on grooming/self-care measures, while gut transplantation from female but not male OBX mice induced a depressive-like phenotype in female mice on escape behaviours.

While these results are of interest and begin to disentangle the effect of sex and the gut in a rodent model of depression, there are complexities to consider for future research. For example, it is not simply the sex of the donor but also of the recipient that matters. This suggests potential interactions of pre-existing microbes within the gut of each sex and how they interact with the microbes introduced via oral gavage (Carabotti et al., 2015; Lin et al., 2017; Zheng et

al., 2016). The gut is highly susceptible to factors such as diet changes, medicinal usage, as well as alterations in other lifestyle factors (Dabke et al., 2019; Dominianni et al., 2015; Konturek et al., 2011; Yatsunenko et al., 2012). However, in our controlled setting, the sexes did not differ on these variables. Thus, it is likely that other endogenous factors, such as hormones, contribute to the way that the recipient reacts to donor gut microbes. For example, there may be sex differences in the enteric nervous system that influence the way the gut responds to microbes/communicates with the brain (Carabotti et al., 2015; Li et al., 2022). Thus, future research will need to consider both sex differences in the gut microbiome contents as well as in the responsivity of the gut-brain axis to gut microbes.

Experiment 2B: Cecal Transfer from CUS-Mice to Healthy Mice

Of particular interest in the CUS cecum transplantation experiment, female CUS cecum treatment to healthy females increased depressive-like behaviours on the forced swim and sucrose preference tests. Specifically for escape behaviours, females treated with female CUS cecum, but not male CUS cecum, displayed decreases in mobility (i.e., frequency and duration), with a corresponding increase in immobility duration. While immobility frequency showed a decrease compared to controls, this is likely an artifact of switching behaviours less often/staying in an immobile state longer (i.e. leading to less immobile events, but longer duration in immobility and less duration/frequency in mobility). There are also indications on the sucrose preference test that female CUS cecum treatment decreased anhedonia in female mice – they showed lower sucrose preference compared to males treated with female CUS cecum. Together, these findings suggest that female CUS cecum is sufficient to induce a depression-like phenotype in female mice.

Conversely, neither female CUS cecum nor male CUS cecum were sufficient to induce depressive-like behaviours in male mice. Thus, since female cecum appeared to act differently in female and male mice, this could indicate an increased vulnerability of the female gut to microbial changes/influences on the brain and/or there could be sex differences in the gut, such that the depression-inducing microbes only thrive in the female but not male gut. Given that diet is controlled, it is likely that these sex differences are due to sex hormones. For example, it has been found that sex-linked differences in microbial composition appear to emerge upon pubertal onset, suggesting a role for hormones, such as testosterone, in microbial development and variation (Markle et al., 2013; Yatsunenko et al., 2012; Yurkovetskiy et al., 2013). Future work will be required to understand the underlying mechanisms leading to these sex differences, but nevertheless, similar to Experiment 2A, our results support sex differences in microbial composition and/or colonization which may impact communication within the GBA, and vulnerability to MDD.

Finally, on the splash test, treatment with male or female CUS cecum increased grooming frequency in male mice compared to male controls, which on its own would suggest a decrease in depressive-like behaviours, counter to our hypothesis and previous research. However, grooming duration did not differ between treatment groups – thus, it is more likely that CUS-cecum treated males are switching behaviors more, rather than showing more grooming/self-care than controls. This increased switching between behaviours could indicate hyperactivity, a behavioural artifact of anxiety and depression models in mice (Strekalova et al., 2005). With this in mind, behavioural frequencies should be assessed in context with corresponding durations in order to accurately extrapolate meaning from such behaviours.

Experiment 2C: Same and Cross-Sex Cecal Transfer from Healthy to Healthy Mice

This experiment was done to understand the sex effects of healthy gut microbes on depressive-like behaviours, as the other experiments with models of depression suggested that females may be showing a baseline greater depressive-like phenotype when healthy/unmanipulated. This was supported in part: For self-care/motivational behaviours, female cecum treated mice showed a greater depressive-like phenotype on measures of general grooming and back grooming frequency compared to control mice and mice treated with male cecum. However, on other measures this was not the case; for example, on the SPT, both control mice and mice treated with female cecum presented more depressive-like behaviours on anhedonic measures compared to mice treated with male cecum, suggesting that male cecum may have a protective factor rather than female cecum increasing depressive-like behaviours on this test. On other tests, the reverse was found - for the FST, male cecum treated mice showed a greater depressive phenotype on measures of immobility duration compared to female cecum mice. These results suggest that the effects of the gut microbiome on depressive-like behaviors are 1) not entirely due to depression itself; cecal transfer from healthy animals can change depression-like behaviours, and thus it may be that the gut microbes are affective mood regulation more broadly, rather than depression specifically, and 2) there are distinctions in the role of the gut microbiome/sex on self-care vs escape behaviours. This demonstrates support that there could be sex differences within the gut microbiome in healthy/unmanipulated mice that may predispose them to be susceptible to depressive-like behaviours (Dalla et al., 2007, 2010; Org et al., 2016).

Overview of Findings

Through experiments 2A through 2C, we have shown that cecal transfer from a model of depression, either OBX or CUS, was sufficient to induce a depressive-like phenotype in healthy

mice on various behavioural measures. This is consistent with previous findings using fecal and cecal transfer (Kelly et al., 2016; Li et al., 2019., 2018; Pu et al., 2021). However, our findings established that whether a depressive-like phenotype is induced is dependent upon the sex of donor and recipient, and often these changes are not seen across anhedonia, self-care and escape/learn helplessness behaviours. In Experiment 2A, both male and female OBX cecum was found to induce a depressive-like phenotype in male mice on self-care measures, while only female OBX cecum was sufficient in increasing depressive-like behaviours in female mice on measures of escape behaviours. For anhedonic measures, overall effects of sex were found, such that regardless of treatment type females showed a greater depressive-like phenotype than males. In Experiment 2B, we replicated the findings that females exhibited greater anhedonic behaviours than males, and female CUS-cecum induced depressive-like phenotype in females but not males. However, in contrast to OBX-cecum, female or male CUS-cecum did not change depressive-like behaviours among males.

Experiment 2C provided evidence that there could be baseline sex-differences within the gut microbiome of healthy animals which may influence behaviour on our battery of tests. On measures of self-care, transference of female cecum was sufficient for inducing depressive-like behaviours, while male cecum induced such a behavioural phenotype on measures of escape/learned helplessness. Furthermore, mice treated with female cecum, as well as controls, had increased anhedonia compared to male cecum treated mice. This shows that sex differences in the gut of healthy animals can change behaviour, and overall female cecal contents tend to increase depressive-like behaviours on anhedonia and self-care measures, while male cecal contents increase escape behaviours.

When taking the findings from these three experiments altogether, there are a few key consistencies. First, transfer of female donor cecum, either OBX, CUS or healthy, was sufficient for inducing depressive-like behavioural changes in recipients. Moreover, it was found that such transference was most successful and consistent in inducing such behavioural alterations when the recipients were also female. These two findings together suggest that 1) the female gut is more vulnerable to depression inducing-microbes, and 2) healthy females may possess a sex-characteristic microbial profile that could lead to instability in mood regulation, with such sex effects being amplified when depression is induced. Furthermore, although male cecum was sufficient for inducing behavioural changes in some instances, this was not consistent across depression models, and healthy male cecum had paradoxical effects on anhedonia and escape behaviours.

Limitations

Along with sex often being neglected in analysis of behaviour, behavioural measures of depression in rodents have also been developed primarily in respect to males, neglecting to determine whether such tests are valid for measuring depression in females (Fernandes et al., 1999). This could explain why males tended to exhibit decreases in mobility levels in the TST and FST, where females tended to show higher rates of escape behaviours at baseline/in a healthy state and thus may be less likely to show a dramatic change in these measures. For example, females exhibit escape behaviours in response to stressors, while males tend to show immobility (Beatty & Beatty, 1970; Colom-Lapetina et al., 2019). Thus, the definition of a stress response on the FST and TST may differ between males and females. However, the sex differences seen at baseline/in healthy animals may also be influenced by the gut microbiota – cross-sex treatment of cecal contents does seem to affect behaviours that present sex differences

in healthy mice. It is also possible that the sex differences at baseline/in healthy mice contribute to sex differences in vulnerability to depression and/or anxiety – thus, future work may consider these limitations in behavioural tests to develop more sex-specific behavioural batteries, as well as assess whether these baseline sex differences in behaviour contribute to the sex disparity in risk of affective conditions.

Further care in future work should be taken to ensure behavioural tests are measuring depression-typical behaviours rather than anxiety-typical behaviours. Although there is a high level of comorbidity between anxiety and depression, as well as a substantial overlap of symptoms, it may be best to use measures that more robustly measures anxiety, such as the elevated-plus maze, in addition to the typical battery for depressive-like behaviours. This would help in differentiating between depressive- and anxiety-like behaviour phenotypes (Belzer & Schneier, 2004; Kraeuter et al., 2019).

Furthermore, although the donor mice were assessed for a depressive-like phenotype through behavioural measures (data not shown; data collected in another lab), there remains a level of uncertainty as to whether the particular depressive paradigm was sufficient to induce changes in microbial products within the gut. Although there can appear to be alterations in behaviour due to such manipulations, individual differences can also alter such measures (Stock et al., 2000; Strekalova & Steinbusch, 2010). Thus, a more concrete measure, such as cecal analysis pre- and post-implementation of such paradigms, should be used to confirm intervention effectiveness.

Finally, it may be of interest in future work to increase our sample size to allow for smaller effect sizes to be seen. We currently report on 20 mice per treatment (10 males; 10

females), which only allows for large effects to be seen. An increase in group size may allow for more robust findings, decreasing the influence of individual differences on particular measures.

Future Directions

We have collected the blood, brains, and cecal matter of the mice from Experiments 2A-2C, and thus in future work, we plan to further characterize the physiological changes that occurred with cecal transplantation. To assess hormonal changes, an enzyme-linked immunosorbent assay (ELISA) will be run to quantify potential changes in the prominent sex hormone testosterone and stress-hormone corticosterone. As levels of testosterone reliably vary by sex, it would be worthwhile to analyze how/if levels of testosterone correlate with behavioural changes, as well as whether these levels become altered in mice receiving cecum from the opposite sex compared to a same-sex donor. Moreover, it has been found that certain microbes contain enzymatic components that can degrade circulating testosterone, resulting in an increase of depressive-like symptoms in both rodents and humans alike (Li et al., 2022). Further, assessment of corticosterone levels as a measure of HPA-axis activation would provide a greater level of certainty for the impact of cecal transfer on stress induction through the gut.

Prior work using pre- and probiotics to induce microbial changes found that levels of both gamma-aminobutyric acid (GABA) and brain-derived neurotrophic factor (BDNF) were found to vary based on administration of particular microbes (Bercik et al., 2011; Bravo et al., 2011). In such studies, changes in levels of GABA and BDNF in regions such as the amygdala and/or hippocampus were found to have corresponding changes in behaviour, notably the stress response (Bercik et al., 2011; Bravo et al., 2011; Zhou et al., 2022). Thus, assessment of these factors within mice exhibiting an increased depressive-like phenotype could provide further consolidation of how cecal transfer may induce behavioural alterations via the gut.

Finally, cecal analysis would provide a robust measure of microbial changes and further elucidate which particular alterations in the gut microbiome may promote changes in behaviour. Although various studies have evaluated such changes in both humans and rodents, there lacks consensus as to whether changes in specific microbes or overall diversity drive these behavioural alterations (Carabotti et al., 2015; Chen et al., 2018; Lin et al., 2017; Zheng et al., 2016). One microbe family of interest that has been found to consistently promote changes in depressive behaviours is *Lactobacillus* (Aizawa et al., 2016; Bravo et al., 2011; Zhou et al., 2022) A recent study using a Dcf1 knockout mouse found that administration of either *Lactobacillus murine* or *Lactobacillus reuteri* rescued wildtype behaviours altered by the Dcf1 knockout (Zhou et al., 2022). In this study, mice with the genetic knockout exhibited a disruption in GABA concentration, as well lower levels of *Firmicutes* and *Lactobacillus* within the gut (Zhou et al., 2022). Along with restoring wildtype behaviours, treatment with *Lactobacilli* was also found to return circulating GABA and GABA-receptor expression to a typical range (Zhou et al., 2022). With this in mind, further work using administration of specific microbes, such as *Lactobacilli*, to assess gut microbial mechanisms in depression vulnerability between the sexes.

Conclusions

Aligning with previous literature, sex was found to be a major predictor in the prevalence of diagnosis and active treatment for minor and major depression and anxiety in a large human sample. Diet and exercise accounted for significantly more variance in disorder prevalence than just sex alone. Diet interacted with sex in predicting whether individuals were currently undergoing treatment for MDD, with higher quality decreasing current MDD treatment in females compared to males. These findings altogether emphasize the importance of considering lifestyle factors and sex when assessing affective disorder risk, as well as when considering

individualized treatment options for those both responsive and irresponsive to current therapeutic methods.

Furthermore, Experiments 2A-2C provided a basis for the consideration of sex and its relationship to depression and the GBA using various mouse models. Overall in experiments 2A through 2C, it was found that cecal transplantation was sufficient to induce alterations in various behavioural measures of a depressive-like phenotype across mouse strains and depression paradigms. Such changes were found to be mediated by both donor and receiver sex, as well as the behaviour being measured. In general, female mice showed the greatest degree of behavioural change across the three experiments. Along with this, female cecum, regardless of donor status (i.e., OBX, CUS, healthy), was most proficient in inducing a depressive-like phenotype. This suggests that female mice may have a distinct microbial profile that is conducive to inducing depressive-like behaviours through the GBA, as well as the female gut may be more vulnerable to the depressive-inducing effects of these microbes. It was also found that sex differences in the gut contents of healthy mice contribute to sex differences on the battery of depression tests. Future work will consider measures of biological correlates to such behavioural changes, such as hormonal and microbial profiles, to further elucidate the sex-based mechanisms underlying depression risk.

References

Ait-Belgnaoui, A., Colom, A., Braniste, V., Ramalho, L., Marrot, A., Cartier, C., Houdeau, E., Theodorou, V., & Tompkins, T. (2013). Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice. *Neurogastroenterology & Motility*, *26*(4), 510–520.

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Ota, M., Koga, N., Hattori, K., & Kunugi, H. (2016). Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. *Journal of Affective Disorders*, *202*, 254–257.

Amiel Castro, R. T., Ehlert, U., & Fischer, S. (2021). Variation in genes and hormones of the hypothalamic-pituitary-ovarian axis in female mood disorders – A systematic review and meta-analysis. *Frontiers in Neuroendocrinology*, *62*.

Arts, I. C. W., van de Putte, B., & Hollman P. C. H. (2000). Catechin contents of foods commonly consumed in the Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *Journal of Agricultural and Food Chemistry, 48*(5), 1752-1757.

Awick, E. A., Ehlers, D. K., Aguiñaga, S., Daugherty, A. M., Kramer, A. F., & McAuley, E. (2017). Effects of a randomized exercise trial on physical activity, psychological distress and quality of life in older adults. *General Hospital Psychiatry*, *49*, 44–50.

Ball, R., Howlett, T., Silverstone, T., & Rees, L. (1987). The interrelationship of beta endorphin, ACTH and cortisol in depressive illness: a controlled study. *Psychological Medicine*, *17*(1), 31–37.

Bambico, F. R., Nguyen, N.T., & Gobbi, G. (2009). Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. *European Neuropsychopharmacology*, *19*(3), 215–228.

Baştürk, M., Muhtawglut, S., Karaaslan, M., Oguz, A., Şimşek, A., & Reyhancan, M. (1998). The relationship between plasma β-endorphin levels and severity of depression. *European Neuropsychopharmacology*, *8*, 151–152.

Beatty, W. W., & Beatty, P. A. (1970). Hormonal determinants of sex differences in avoidance behavior and reactivity to electric shock in the rat. *Journal of Comparative Physiological Psychology, 73,* 446-455.

Belovicova, K., Bogi, E., Csatlosova, K., & Dubovicky, M. (2017). Animal tests for anxiety-like and depression-like behavior in rats. *Interdisciplinary Toxicology*, *10*(1), 40–43.

Belzer, K., & Shneier, F. R. (2004). Comorbidity of anxiety and depressive disorders: Issues in conceptualization and treatment. *Journal of Psychiatric Practice, 10*(5), 296-306.

Bennett, E., Peters, S. A., & Woodward, M. (2018). Sex differences in macronutrient intake and adherence to dietary recommendations: Findings from the UK Biobank. *BMJ Open, 8*(4).

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K. D., Verdu, E. F., Collins, S. M., (2011). The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology, 141*(2), 599-609.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., Bienenstock, J., & Cryan, J. F. (2011). Ingestion of lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus

nerve. *Proceedings of the National Academy of Sciences of the United States of America, 108*(38), 16050-16055.

Brizuela, L., Dayon, A., Doumerc, N., Ader, I., Golzio, M., Izard, J. C., Hara., Y., Malavaud, B., & Cuvillier, O. (2010). The sphingosine kinase-1 survival pathway is a molecular target for the tumor-suppressive tea and wine polyphenols in prostate cancer. *The Federation of American Societies for Experimental Biology, 24*(10), 3882-3894.

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Annals of Gastroenterology, 28*(2), 203-209.

Cheeta, S., Ruigt, G., van Proosdij, J., & Willner, P. (1997). Changes in sleep architecture following chronic mild stress. *Biological Psychiatry 41*, 419–427.

Chen, H. M., Tsai, C. M., Wu, Y. C., Lin, K. C., & Lin, C. C. (2014). Randomised controlled trial on the effectiveness of home-based walking exercise on anxiety, depression and cancer-related symptoms in patients with lung cancer. *British Journal of Cancer*, *112*(3), 438–445.

Chen, J. J., Zheng, P., Liu, Y. Y., Zhong, X. G., Wang, H. Y., Guo, Y. J., & Xie, P. (2018). Sex differences in gut microbiota in patients with major depressive disorder. *Neuropsychiatric Disease and Treatment*, *14*, 647–655.

Chen, J. J., He, S., Fang, L., Wang, B., Bai, S. J., Xie, J., Zhou, C. J., Wang, W., & Xie, P. (2020). Age-specific differential changes on gut microbiota composition in patients with major depressive disorder. *Aging*, *12*(3), 2764–2776.

Colom-Lapetina, J., Li, A. J., Pelegrina-Perez, T. C., & Shansky, R. M. (2019). Behavioral diversity across classic rodent models is sex-dependent. *Frontiers in Behavioral Neuroscience, 13*.

Craft, B. B., Carroll, H. A., & Lustyk, M. K. B. (2014). Gender differences in exercise habits and quality of life reports: Assessing the moderating effects of reasons for exercise. *International Journal of Liberal Arts and Social Science, 2*(5), 65-76.

Dabke, K., Hendrick, G., & Devkota, S. (2019). The gut microbiome and metabolic syndrome. *The Journal of Clinical Investigation, 129*(10), 4050-4057.

Dalla, C., Edgecomb, C., Whetstone, A. S., & Shors, T. J. (2007). Females do not express learned helplessness like males. *Neuropsychopharmacology, 33,* 1559-1569.

Dalla, C., Pitychoutis, P. M., Kokras, N., & Papadopoulou-Daifoti, Z. (2010). Sex differences in response to stress and expression of depressive-like behaviours in the rat. *Biological Basis of Sex Differences in Psychopharmacology*, 97–118.

Darko, D. F., Risch, S. C., Gillin, J. C., & Golshan, S. (1992). Association of beta-endorphin with specific clinical symptoms of depression. *American Journal of Psychiatry*, *149*(9), 1162–1167.

Dinan, T. G., & Cryan, J. F. (2013). Melancholic microbes: A link between gut microbiota and depression? *Gastroenterology and Motility, 25(9).*

Dominianni, C., Sinha, R., Goedert, J. J., Pei, Z., Yang, L., Hayes, R. B., & Ahn, J. (2015). Sex, body mass index, and dietary fiber intake influence the human gut microbiome. *PLOS O, 10*(4).

Dummer, T. J. B., Awadalla, P., Boileau, C., Craig, C., Fortier, I., Goel, V., Hicks, J. M. T., Jacquemont, S., Knoppers, B. M., Le, N., McDonald, T., McLaughlin, J., Mes-Masson,

A.M., Nuyt, A.M., Palmer, L. J., Parker, L., Purdue, M., Robson, P. J., Spinelli, J. J., … Zawati, M. (2018). The Canadian Partnership for tomorrow project: A pan-Canadian platform for research on Chronic Disease Prevention. *Canadian Medical Association Journal*, *190*(23).

Edwards, J. P., Walsh, N. P., Diment, P. C., & Roberts, R. (2018). Anxiety and perceived psychological stress play an important role in the immune response after exercise. *Annals of Research in Sport and Physical Activity*, *24*, 203–204.

Eltokhi, A., Kurpiers, B., & Pitzer, C. (2021). Baseline depression-like behaviors in wild-type adolescent mice are strain and age but not sex dependent. *Frontiers in Behavioral Neuroscience, 15*.

Fernandez, C., Gonzalez, M. I., Wilson, C.A., & File, S.E. (1999). Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. *Pharmacology Biochemistry and Behavior, 64*(4), 731-736.

Firth, J., Marx, W., Dash, S., Carney, R., Teasdale, S. B., Solmi, M., Stubbs, B., Schuch, F. B., Carvalho, A. F., Jacka, F., & Sarris, J. (2019). The effects of dietary improvement on symptoms of depression and anxiety: A meta-analysis of randomized controlled trials. *Psychosomatic Medicine*, *81*(3), 265–280.

Fitzgerald, P., Yen, J., & Watson, B. (2019). Stress-sensitive antidepressant-like effects of ketamine in the mouse forced swim test. *Biological Psychiatry*, *85*(10).

GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet, 392*(10159), 10-16.

Gerra, G., Zaimovic, A., Zambelli, U., Timpano, M., Reali, N., Bernasconi, S., & Brambilla, F. (2000). Neuroendocrine responses to psychological stress in adolescents with anxiety disorder. *Neuropsychobiology*, *42*(2), 82–92.

Gibson-Smith, D., Bot, M., Brouwer, I. A., Visser, M., & Penninx, B. W. (2018). Diet quality in persons with and without depressive and anxiety disorders. *Journal of Psychiatric Research*, *106*, 1–7.

Goodwin, G., Austin, M. P., Curran, S., Ross, M., Murray, C., Prentice, N., Ebmeier, K., Bennie, J., Carroll, S., Dick, H., & Fink, G. (1993). The elevation of plasma β-endorphin levels in major depression. *Journal of Affective Disorders*, *29*(4), 281–289.

Guenther, P. M., Reedy, J., & Krebs-Smith, S. M. (2008). Development of the Healthy Eating Index-2005. *Journal of the American Dietetic Association, 108*(11), 1896-1901.

Hallgren, M., Kraepelien, M., öJehagen, A., Lindefors, N., Zeebari, Z., Kaldo, V., & Forsell, Y. (2015). Physical exercise and internet-based cognitive–behavioural therapy in the treatment of depression: Randomised controlled trial. *British Journal of Psychiatry*, *207*(3), 227–234.

Hagströmer, M., Oja, P., & Sjöström, M. (2006). The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutrition*, *9*(6), 755–762.

Harlow, B. L., Cohen, L. S., Otto, M. W., Spiegelman, D., & Cramer, D. W. (2004). Early life menstrual characteristics and pregnancy experiences among women with and without major depression: The Harvard Study of moods and cycles. *Journal of Affective Disorders*, *79*(1-3), 167–176.

Haskell-Ramsay, C. F., Stuart, R. C., Okello, E. J., & Watson, A. W. (2017). Cognitive and mood improvements following acute supplementation with purple grape juice in healthy young adults. *European Journal of Nutrition, 56*(8), 2621–2631.

Health Canada. (2019). *Canada's dietary guidelines: For health professionals and policy makers*. https://food-guide.canada.ca/en/guidelines/

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A., & Hackney, A. C. (2008). Exercise and circulating cortisol levels: The intensity threshold effect. *Journal of Endocrinological Investigation, 31*(7), 587–591.

Kelly, J., Borre, Y., Aidy, S. E., Deane, J., Patterson, E., Kennedy, P., Beers, S., Scott, K., Moloney, G., Scott, L., Ross, P., Stanton, C., Clarke, G., Cryan, J., & Dinan, T. (2016). P.4.001 Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *European Neuropsychopharmacology*, *26*, 109-118.

Kelly, J. P., Wrynn, A. S., & Leonard, B. E. (1997). The olfactory bulbectomized rat as a model of depression: An update. *Pharmacology & Therapeutics*, *74*(3), 299–316.

Kim, H., Jeong, W., Kwon, J., Kim, Y., Jang, S. I., & Park, E. C. (2020). Sex differences in type of exercise associated with depression in South Korean adults. *Scientific Reports*, *10*(1).

Knoll, A. D., & MacLennan, R. N. (2017). Prevalence and correlates of depression in Canada: Findings from the Canadian Community Health Survey. *Canadian Psychology, 58*(2), 116-123.

Kocalevent, R. D., Hinz, A., & Brähler, E. (2013). Standardization of the depression screener Patient Health Questionnaire (PHQ-9) in the general population. *General Hospital Psychiatry, 35*(5), 551–555.

Konturek, P. C., Brzozowski, T., & Konturek, S. J. (2011). Stress and the gut: Pathophysiology, clinical consequences, diagnostic approach, and treatment options. *Journal of Physiology and Pharmacology, 62(6),* 591-599.

Kraeuter, A., Guest, P. C., & Sarnyai, Z. (2019). The elevated plus maze test for measuring anxiety-like behavior in rodents. *Methods in Molecular Biology, 1916,* 69-74.

Kroenke, K., Spitzer, R. L., & Williams, J. B. W. (2001). The PHQ-9**:** Validity of a brief depression severity measure. *Journal of General Internal Medicine, 16*(9), 606-613.

Jacques, A., Chaaya, N., Beecher, K., Ali, S. A., Belmer, A., & Bartlett, S. (2019). The impact of sugar consumption on stress driven, emotional and addictive behaviors. *Neuroscience & Biobehavioral Reviews*, *103*, 178–199.

Lew, L. C., Hor, Y.Y., Yusoff, N. A., Choi, S.-B., Yusoff, M. S. B., Roslan, N. S., Ahmad, A., Mohammad, J. A. M., Abdullah, M. F., Zakaria, N., Wahid, N., Sun, Z., Kwok, L.-Y., Zhang, H., & Liong, M. T. (2019). Probiotic lactobacillus plantarum p8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study. *Clinical Nutrition*, *38*(5), 2053–2064.

Li, D., Liu, R., Wang, G., Li, Y., & Liu, T. (2022). 2C-Hydroxysteroid dehydrogenase expressed by gut microbes degrades testosterone and is linked to depression in males. *Cell Host & Microbe, 30,* 329-339.

Li, N., Wang, Q., Wang, Y., Sun, A., Lin, Y., Jin, Y., & Li, X. (2019). Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice via the gut microbiota-inflammation-brain axis. *Stress*, *22*(5), 592–602.

Liang, S., Wu, X., Hu, X., Wang, T., & Jin, F. (2018). Recognizing depression from the microbiota-gut-brain axis. *International Journal of Molecular Sciences, 19(6).*

Lin, P., Ding, B., Feng, C., Yin, S., Zhang, T., Qi, X., Lv, H., Guo, X., Dong, K., Zhu, Y., & Li, Q. (2017). Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. *Journal of Affective Disorders*, *207*, 300–304.

Liu, L., Li, J., Su, W., Wang, B., & Jiang, C. (2019). Sex differences in depressive-like behaviour may relate to imbalance of microglia activation in the hippocampus. *Brain, Behavior, and Immunity, 81*, 188-197.

Liu, Q., He, H, Yang, J., Feng, X., Zhao, F., & Lyu, J. (2020) Changes in the global burden of depression from 1990 to 2017: Findings from the Global Burden of disease study. *Journal of Psychiatric Research, 126,* 134-140.

Lobstein, D. D., Rasmussen, C. L., Dunphy, G. E., & Dunphy, M. J. (1989). Beta-endorphin and components of depression as powerful discriminators between joggers and sedentary middle-aged men. *Journal of Psychosomatic Research*, *33*(3), 293–305.

Luo, Y., Zeng, B., Zeng, L., Du, X., Li, B., Huo, R., Liu, L., Wang, H., Dong, M., Pan, J., Zheng, P., Zhou, C., Wei, H., & Xie, P. (2018). Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus. *Translational Psychiatry*, *8*(1).

Löwe, B., Decker, O., Müller, S., Brähler, E., Schellberg, D., Herzog, W., & Herzberg, P. Y. (2008). Validation and standardization of the generalized anxiety disorder screener (GAD-7) in the general population. *Medical Care*, *46*(3), 266–274.

Markle, J. G., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolle-Kampczyk, U., von Bergen, M., McCoy, K. D., Macpherson, A. J., & Danska, J. S. (2013). Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science, 339*(6123), 1084–1088.

Martínaz-Gonzàlez, M. A., García-Arellano, A., Toledo, E., Salas-Salvadó, J., Buil-Cosiales, P., Corella, D., Covas, M. I., Schröder, H., Arós, F., Gómez-Garcia, E., Fiol, M., Ruiz-Gutiérrez, V., Lapetra, J., Lamuela-Raventos, R. M., Serra-Majem, L., Pintó, X., Muñoz, M. A., Wärnburg, J., Ros, E., & Estruch, R. (2012). A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk participants: The PREDIMED trial. *PLOS One, 7*(8).

McDowell, C. P., Campbell, M. J., & Herring, M. P. (2016). Sex-related differences in mood responses to acute aerobic exercise. *Medicine and Science in Sports and Exercise, 48*(9), 1798-1802.

Merenlender-Wagner, A., Dikshtein, Y., & Yadid, G. (2009). The beta-endorphin role in stress-related psychiatric disorders. *Current Drug Targets, 10*(11), 1096–1108.

Mohammadi, A. A., Jazayeri, S., Khosravi-Darani, K., Solati, Z., Mohammadpour, N., Asemi, Z., Adab, Z., Djalali, M., Tehrani-Doost, M., Hosseini, M., & Eghtesadi, S. (2015). The effects of probiotics on mental health and hypothalamic–pituitary–adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. *Nutritional Neuroscience, 19*(9), 387–395.

Nabkasorn, C., Miyai, N., Sootmongkol, A., Junprasert, S., Yamamoto, H., Arita, M., & Miyashita, K. (2005). Effects of physical exercise on depression, neuroendocrine stress

hormones and physiological fitness in adolescent females with depressive symptoms. *European Journal of Public Health*, *16*(2), 179–184.

Naveen, S., Siddalingaswamy, M., Singsit, D., & Khanum, F. (2013). Anti-depressive effect of polyphenols and omega-3 fatty acid from pomegranate peel and flax seed in mice exposed to chronic mild stress. *Psychiatry and Clinical Neurosciences*, *67*(7), 501–508.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2010). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility*, *23*(3).

Ojelabi, O. A., Lloyd, K. P., De Zutter, J. K., & Carruthers, A. (2018). Red wine and green tea flavonoids are *cis*-allosteric activators and competitive inhibitors of glucose transporter 1 (GLUT1)-mediated sugar uptake. *Journal of Biological Chemistry, 293*(51), 19823-19834.

Org, E., Mehrabian, M., Parks, B. W., Shipkova, P., Liu, X., Drake, T. A., & Lusis, A. J. (2016). Sex differences and hormonal effects on gut microbiota composition in mice. *Gut Microbes*, *7*(4), 313–322.

Otmakhova, N. A., Gurevich, E. V., Katkov, Y. A., Nesterova, I. V., & Bobkova, N. V. (1992). Dissociation of multiple behavioral effects between olfactory bulbectomized C57B1/6J and DBA/2J MICE. *Physiology & Behavior*, *52*(3), 441–448.

Papp, M., Willner, P., & Muscat, R. (1991). An animal model of anhedonia: Attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology, 104*(2), 255–259.

Parletta, N., Zarnowiecki, D., Cho, J., Wilson, A., Bogomolova, S., Villani, A., Itsiopoulos, C., Niyonsenga, T., Blunden, S., Meyer, B., Segal, L., Baune, B. T., & O'Dea, K. (2017). A Mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: A randomized controlled trial (HELFIMED). *Nutritional Neuroscience*, *22*(7), 474–487.

Paterson, K. E., Myint, P. K., Jennings, A., Bain, L. K. M., Lentjes, M. A. H., Khaw, K.-T., & Welch, A. A. (2018). Mediterranean diet reduces risk of Incident stroke in a population with Varying cardiovascular disease risk profiles. *Stroke*, *49*(10), 2415–2420.

Pathak, L., Agrawal, Y., & Dhir, A. (2013). Natural polyphenols in the management of major depression. *Expert Opinion on Investigational Drugs*, *22*(7), 863–880.

Paxinos, G., & Franklin, K. (2019). *Paxino's and Franklin's the mouse brain in stereotaxic coordinates* (5th ed.). Academic Press.

Ponce, P., del Arco, A., & Loprinzi, P. (2019). Physical activity versus psychological stress: Effects on salivary cortisol and working memory performance. *Medicina*, *55*(5), 119.

Pooley, A. E., Benjamin, R. C., Sreedhar, S., Eagle, A. L., Robison, A. J., Mazei-Robison, M. S., Breedlove, S. M., & Jordan, C. L. (2018). Sex differences in the traumatic stress response: The role of adult gonadal hormones. *Biology of Sex Differences*, *9*(1).

Pu, Y., Tan, Y., Qu, Y., Chang, L., Wang, S., Wei, Y., Wang, X., & Hashimoto, K. (2021). A role of the subdiaphragmatic vagus nerve in depression-like phenotypes in mice after fecal microbiota transplantation from Chrna7 knock-out mice with depression-like phenotypes. *Brain, Behavior, and Immunity*, *94*, 318–326.

Pytka, K., Głuch-Lutwin, M., Kotańska, M., Waszkielewicz, A., Kij, A., & Walczak, M. (2017). Single administration of HBK-15 – a triple 5-HT_{1A}, 5-HT_7, and 5-HT_3 receptor

antagonist- reverses depressive-like behaviors in mouse model of depression induced by corticosterone. *Molecular Neurobiology, 55*(5), 3931-3945.

Salari-Moghaddam, A., Keshteli, A. H., Mousavi, S. M., Afshar, H., Esmaillzadeh, A., & Adibi, P. (2019). Adherence to the MIND diet and prevalence of psychological disorders in adults. *Journal of Affective Disorders*, *256*, 96–102.

Santos-Marcos, J. A., Rangel-Zuñiga, O. A., Jimenez-Lucena, R., Quintana-Navarro, G. M., Garcia-Carpintero, S., Malagon, M. M., Landa, B. B., Tena-Sempere, M., Perez-Martinez, P., Lopez-Miranda, J., Perez-Jimenez, F., & Camargo, A. (2018). Influence of gender and menopausal status on gut microbiota. *Maturitas*, *116*, 43–53.

Sathyanesan, M., Haiar, J. M., Watt, M. J., & Newton, S. S. (2017). Restraint stress differentially regulates inflammation and glutamate receptor gene expression in the hippocampus of C57BL/6 and BALB/C Mice. *Stress*, *20*(2), 197–204.

Seney, M. L., Huo, Z., Cahill, K., French, L., Puralewski, R., Zhang, J., Logan, R. W., Tseng, G., Lewis, D. A., & Sibille, E. (2018). Opposite molecular signatures of depression in men and women. *Biological Psychiatry*, *84*(1), 18–27.

Sharma, R., Smith, S. A., Boukina, N., Dordari, A., Mistry, A., Taylor, B. C., Felix, N., Cameron, A., Fang, Z., Smith, A., & Ismail, N. (2020). Use of the birth control pill affects stress reactivity and brain structure and function. *Hormones and Behavior*, *124*.

Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueinfar, M., Hale, T. M., & Handa, R. J. (2021). The hypothalamic-pituitary-adrenal axis: Development, programming actions of hormones, and maternal-fetal interactions. *Frontiers in Behavioral Neuroscience*, *14*.

Spitzer, R. L., Kroenke, K., Williams, J. B. W., & Löwe, B. (2006). A brief measure for assessing generalized anxiety disorder. *Archives of Internal Medicine*, *166*(10).

Steiner, M., Born, L., & Dunn, E. (2003). Hormones and mood: From menarche to menopause and beyond. *Journal of Affective Disorders*, *74*(1), 67–83.

Stock, H. S., Ford, K., & Wilson, M. A. (2000). Gender and gonadal hormone effects in the olfactory bulbectomy animal model of depression. *Pharmacology Biochemistry and Behavior*, *67*(1), 183–191.

Strekalova, T., Spanagel, R., Dolgov, O., & Bartsch, D. (2005). Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice. *Behavioural Pharmacology*, *16*(3), 171–180.

Strekalova, T., & Steinbusch, H. W. M. (2010). Measuring behavior in mice with Chronic Stress Depression Paradigm. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(2), 348–361.

Ströhle, A., Graetz, B., Scheel, M., Wittmann, A., Feller, C., Heinz, A., & Dimeo, F. (2009). The acute antipanic and anxiolytic activity of aerobic exercise in patients with panic disorder and healthy control subjects. *Journal of Psychiatric Research*, *43*(12), 1013–1017.

Stubbs, B., Vancampfort, D., Rosenbaum, S., Firth, J., Cosco, T., Veronese, N., Salum, G. A., & Schuch, F. B. (2017). An examination of the anxiolytic effects of exercise for people with anxiety and stress-related disorders: A meta-analysis. *Psychiatry Research*, *249*, 102–108.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of Physiology*, *558*(1), 263–275.

Sweeney, E., Cui, Y., Declercq, V., Devichand, P., Forbes, C., Grandy, S., Hicks, J. M. T., Keats, M., Parker, L., Thompson, D., Volodarsky, M., Yu, Z. M., & Dummer, T. J. B.

(2017). Cohort profile: The Atlantic Partnership for Tomorrow's Health (Atlantic PATH) study. *International Journal of Epidemiology, 46*, 1762–1763.

Taylor, D. V., Boyajian, J. G., James, N., Woods, D., Chicz-Demet, A., Wilson, A. F., & Sandman, C. A. (1994). Acidosis stimulates beta-endorphin release during exercise. *Journal of Applied Physiology, 77*(4), 1913–1918.

Tsai, C. L., Wang, C. H., Pan, C. Y., Chen, F. C., Huang, T. H., & Chou, F. Y. (2014). Executive function and endocrinological responses to acute resistance exercise. *Frontiers in Behavioral Neuroscience, 8*.

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E. F., Wang, J., Tito, R. Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., van Oudenhove, L., Zhernakova, A., Vieira-Silva, S., & Raes, J. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. *Nature Microbiology*, *4*(4), 623–632.

Ventriglio, A., Sancassiani, F., Contu, M. P., Latorre, M., di Slavatore, M., Fornaro, M., & Bhugra, D. (2020). Mediterranean Diet and its benefits on health and mental health: A literature review. *Clinical Practice & Epidemiology in Mental Health*, *16*(1), 156–164.

Vermeulen, E., Stronks, K., Snijder, M. B., Schene, A. H., Lok, A., de Vries, J. H., Visser, M., Brouwer, I. A., & Nicolaou, M. (2017). A combined high-sugar and high-saturated-fat dietary pattern is associated with more depressive symptoms in a multi-ethnic population: The helius (healthy life in an urban setting) study. *Public Health Nutrition*, *20*(13), 2374–2382.

Vernocchi, P., Del Chierico, F., & Putignani, L. (2020). Gut microbiota metabolism and interaction with food components. *International Journal of Molecular Sciences, 21*(10), 3688.

Weissman, M. M., Bland, R., Joyce, P. R., Newman, S., Wells, J. E., Wittchen, H-U. (1993). Sex differences in rates of depression: cross-national perspectives. *Journal of Affective Disorders, 29,* 77-84.

Westfall, S., & Pasinetti, G. M. (2019). The gut microbiota links dietary polyphenols with management of psychiatric mood disorders. *Frontiers in Neuroscience, 13*.

Wipfli, B., Landers, D. M., Nagoshi, C. T., & Ringenbach, S. (2011). An examination of serotonin and psychological variables in the relationship between exercise and mental health. *Scandinavian Journal of Medicine and Science in Sports, 21*(3), 474-481.

Yang, H., Zhao, X., Tang, S., Huang, H., Zhao, X., Ning, Z., Fu, X., & Zhang, C. (2014). Probiotics reduce psychological stress in patients before laryngeal cancer surgery. *Asia-Pacific Journal of Clinical Oncology, 12*(1).

Yalcin, I., Aksu, F., & Belzung, C. (2005). Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *European Journal of Pharmacology*, *514*(2-3), 165–174.

Yang, X., Fang, Y., Chen, H., Zhang, T., Yin, X., Man, J., Yang, L., & Lu, M. (2021) Global, regional, and national burden of anxiety disorders from 1990 to 2019: results from the Global Burden of Disease Study 2019. *Epidemiology and Psychiatric Sciences, 30.*

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehen, I., Dominguez-Bello, M. G., & Contreras, M. (2012). Human gut microbiome viewed across age and geography. *Nature, 486,* 222-227.

Yu, H., & Chen, Z-Y. (2011). The role of BDNF in depression on the basis of its location in the neural circuitry. *Acta Pharmacologica Sinica, 32*(1), 3-11.

Yu, Z. M., Parker, L., & Dummer, T. J. B. (2014). Depressive symptoms, diet quality, physical activity, and body composition among populations in Nova Scotia, Canada: Report from the Atlantic Partnership for Tomorrow's Health. *Preventative Medicine, 61*, 106-113.

Yurkovetskiy, L., Burrows, M., Khan, A. A., Graham, L., Volchkov, P., Becker, L., Antonopoulos, D., Umesaki, Y., & Chervonsky, A. V. (2013). Gender bias in autoimmunity is influenced by microbiota. *Immunity*, *39*(2), 400–412.

Zhang, C., Zhang, M., Wang, S., Han, R., Cao, Y., Hua, W., Mao, Y., Zhang, X., Pang, X., Wei, C., Zhao, G., Chen, Y., & Zhao, L. (2010). Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *The ISME Journal*, *4*(2), 312–313.

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N. D., Licinio, J., Wei, H., & Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*, *21*(6), 786–796.

Zhou, H., Wang, R., Zhang, S., Zhang, X., Zhou, H., Wen, T., & Wang, J. (2022). Depression-like symptoms due to DCF1 deficiency are alleviated by intestinal transplantation of lactobacillus murine and lactobacillus reuteri. *Biochemical and Biophysical Research Communications, 593*, 137-143.

Zhu, A., Sunagawa, S., Mende, D. R., & Bork, P. (2015). Inter-individual differences in the gene content of human gut bacterial species. *Genome Biology*, *16*(1).

Appendix A

Table 1

Total subjects, N, per group based on reporting of lifestyle factors and affective disorder status.

	Total *N*		
	Diet	MedScore	Exercise
Major Depression (diagnosis)	5519	3833	3655
Minor Depression (diagnosis)	5870	4100	3917
Anxiety (diagnosis)	5765	4000	3789
Major Depression (current Treatment)	6435	4472	4256
Minor Depression (current treatment)	6493	4525	4301
Anxiety (current treatment)	6467	4494	4277
PHQ-9 score (depressive symptom severity)	9914	7075	5731
GAD-7 score (anxiety symptom severity)	9632	6819	5862

Table 2
Logistic regression predicting incidence of Major Depressive Disorder diagnosis across the lifetime from lifestyle factors and sex.

Predictor	*n*	Odds Ratio/χ^2	95% Confidence Interval		*p*
			Lower	Upper	
MedScore		0.590			.744
Score					
Low[a]	1661				
Moderate	1630	0.889	0.659	1.200	.443
High	542	0.935	0.615	1.421	.754
Sex					
Female - Male[a]		2.239	1.576	3.180	<.001
HEI		12.526			.002
Score					
Low-Moderate Quality[a]	898				
Moderate-High Quality	3360	0.654	0.488	0.877	.005
High Quality	1261	0.520	0.360	0.751	<.001
Sex					
Female - Male[a]		2.082	1.558	2.783	<.001
IPAQ		21.070			<.001
Score					
Low[a]	292				
Moderate	1221	0.778	0.492	1.232	.284
High	2142	0.428	0.271	0.678	<.001
Sex					
Female - Male[a]		2.275	1.845	4.023	<.001

Note: Lifestyle factors are Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ). The χ^2 reported for each lifestyle factor tests the overall effect of that factor in predicting the criterion after controlling for Sex. Subject data was obtained from the Atlantic PATH database.
[a] Indicates reference group

Table 3
Logistic regression predicting incidence of Minor Depressive Disorder diagnosis across the lifetime from lifestyle factors and sex.

Predictor	*n*	Odds Ratio/χ^2	95% Confidence Interval		*p*
			Lower	Upper	
MedScore		1.068			.586
Score					
Low[a]	1758				
Moderate	1761	1.115	0.906	1.372	.303
High	581	1.048	0.782	1.405	.753
Sex					
Female - Male[a]		2.048	1.620	2.589	<.001
HEI		0.631			.730
Score					
Low-Moderate Quality[a]	925				
Moderate-High Quality	3590	1.066	0.840	1.353	.600
High Quality	1355	0.993	0.754	1.307	.961
Sex					
Female - Male[a]		2.066	1.685	2.534	<.001
IPAQ		7.245			.027
Score					
Low[a]	299				
Moderate	1317	1.211	0.818	1.792	.339
High	2301	0.913	0.622	1.339	.640
Sex					
Female - Male[a]		2.353	1.830	3.025	<.001

Note: Lifestyle factors are Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ). The χ^2 reported for each lifestyle factor tests the overall effect of that factor in predicting the criterion after controlling for Sex. Subject data was obtained from the Atlantic PATH database.
[a] Indicates reference group

Table 4
Logistic regression predicting incidence of Anxiety Disorder diagnosis across the lifetime from lifestyle factors and sex.

Predictor	*n*	Odds Ratio/χ^2	95% Confidence Interval		*p*
			Lower	Upper	
MedScore		3.660			.161
Score					
Low[a]	1744				
Moderate	1693	0.825	0.656	1.038	.100
High	563	0.836	0.603	1.159	.283
Sex					
Female - Male[a]		2.573	1.949	3.396	<.001
HEI		8.010			.018
Score					
Low-Moderate Quality[a]	922				
Moderate-High Quality	3532	0.941	0.737	1.202	.628
High Quality	1311	0.722	0.537	0.971	.031
Sex					
Female - Male[a]		2.059	1.653	2.566	.001
IPAQ		11.495			.003
Score					
Low[a]	309				
Moderate	1244	0.580	0.398	0.844	.004
High	2236	0.524	0.367	0.747	<.001
Sex					
Female - Male[a]		2.223	1.672	2.956	<.001

Note: Lifestyle factors are Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ). The χ^2 reported for each lifestyle factor tests the overall effect of that factor in predicting the criterion after controlling for Sex. Subject data was obtained from the Atlantic PATH database.
[a] Indicates reference group

Table 5
Logistic regression predicting prevalence of current treatment for Major Depressive Disorder across the lifetime from lifestyle factors and sex.

Variable	*n*	Odds Ratio/χ^2	95% Confidence Interval		*p*
			Lower	Upper	
MedScore		1.788			.409
Score					
Low[a]	1924				
Moderate	1912	0.785	0.541	1.140	.204
High	636	0.804	0.472	1.368	.421
Sex					
Female - Male[a]		2.257	1.433	3.554	<.001
HEI		14.139			<.001
Score					
Low-Moderate Quality[a]	1038				
Moderate-High Quality[1]	3939	0.717	0.305	1.688	.447
High Quality[2]	1458	1.129	0.419	3.038	.810
Sex					
Female - Male[a]		3.469	1.604	7.501	.002
Sex*HEI		4.253			.119
Sex*HEI[1]		0.718	0.281	1.836	.489
Sex*HEI[2]		0.310	0.102	0.946	.040
IPAQ		20.128			<.001
Score					
Low[a]	354				
Moderate	1430	0.668	0.390	1.143	.141
High	2472	0.329	0.190	0.569	<.001
Sex					
Female - Male[a]		2.639	1.571	4.432	<.001

Note: Lifestyle factors are Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ). The χ^2 reported for each lifestyle factor tests the overall effect of that factor in predicting the criterion after controlling for Sex. Subject data was obtained from the Atlantic PATH database.
[a] Indicates reference group

Table 6
Logistic regression predicting prevalence of current treatment for Minor Depressive Disorder across the lifetime from lifestyle factors and sex

Variable	*n*	Odds Ratio/χ^2	95% Confidence Interval		*p*
			Lower	Upper	
MedScore		0.780			.677
Score					
Low[a]	1953				
Moderate	1933	1.124	0.863	1.463	.387
High	639	1.030	0.706	1.502	.879
Sex					
Female - Male[a]		2.104	1.538	2.879	<.001
HEI		0.349			.840
Score					
Low-Moderate Quality[a]	1041				
Moderate-High Quality	3985	1.017	0.744	1.390	.915
High Quality	1467	1.092	0.766	1.558	.626
Sex					
Female - Male[a]		1.994	1.514	2.627	<.001
IPAQ		8.117			.017
Score					
Low[a]	356				
Moderate	1451	1.099	0.675	1.791	.703
High	2494	0.735	0.454	1.191	.211
Sex					
Female - Male[a]		2.455	1.702	3.541	<.001

Note: Lifestyle factors are Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ). The χ^2 reported for each lifestyle factor tests the overall effect of that factor in predicting the criterion after controlling for Sex. Subject data was obtained from the Atlantic PATH database.
[a] Indicates reference group

Table 7
Logistic regression predicting prevalence of current treatment for Anxiety Disorder across the lifetime from lifestyle factors and sex

Predictor	*n*	Odds Ratio/χ^2	95% Confidence Interval		*p*
			Lower	Upper	
MedScore		3.020			.221
Score					
Low[a]	1934				
Moderate	1924	0.781	0.593	1.028	.078
High	636	0.774	0.520	1.151	.205
Sex					
Female - Male[a]		2.619	1.850	3.709	<.001
HEI		6.440			.040
Score					
Low-Moderate Quality[a]	1041				
Moderate-High Quality	3965	0.850	0.640	1.130	.264
High Quality	1461	0.616	0.432	0.879	.007
Sex					
Female - Male[a]		2.319	1.750	3.074	<.001
IPAQ		10.218			.006
Score					
Low[a]	355				
Moderate	1434	0.586	0.380	0.905	.016
High	2488	0.490	0.323	0.743	<.001
Sex					
Female - Male[a]		2.245	1.693	3.531	<.001

Note: Lifestyle factors are Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ). The χ^2 reported for each lifestyle factor tests the overall effect of that factor in predicting the criterion after controlling for Sex. Subject data was obtained from the Atlantic PATH database.
[a] Indicates reference group

Table 8

Linear models of Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ) on PHQ-9 scores as a measure of depression severity. Subject data was obtained from the Atlantic PATH database.

Predictor	n	Estimate	Standard Error	95% Confidence Interval		df	*t*	*p*
				Lower	Upper			
MedScore	7075							
Sex								
Female–Male[a]		0.352	0.027	0.298	0.406	7071	12.860	<.001
MedScore[1]								
Moderate–Low[a]		-0.106	0.027	-0.159	-0.052		-2.996	<.001
MedScore[2]								
High – Low[a]		-0.184	0.039	-0.261	-0.106		-3.941	<.001
Healthy Eating Index (HEI)	9914							
Sex								
Female–Male[a]		0.308	0.023	0.263	0.353	9910	13.333	<.001
HEI[1]								
Moderate–Low[a]		-0.199	0.029	-0.257	-0.142		-6.784	<.001
HEI[2]								
High – Low[a]		-0.391	0.034	-0.459	-0.324		-11.362	<.001
IPAQ	5731							
Sex								
Female–Male[a]		0.265	0.031	0.205	0.326	5727	8.634	<.001
IPAQ[1]								
Moderate–Low[a]		-0.127	0.054	-0.233	-0.021		-2.347	.019
IPAQ[2]								
High – Low[a]		-0.280	0.052	-0.381	-0.178		-5.408	<.001

[a] Indicates reference group

Table 9

Comparison of regression models using either sex or both sex and Mediterranean-style diet scores (MedScore), sex and scores on a Healthy Eating Index (HEI), or sex and scores on the and International Physical Activity Questionnaire (IPAQ) as predictors for depressive symptom severity. Subject data was obtained from the Atlantic PATH database

Predictors		ΔR^2	F	df_1, df_2	*p*
Model 1	Model 2				
Sex	Sex + MedScore	0.004	13.777	2, 7071	<.001
Sex	Sex + HEI	0.013	65.176	2, 9910	<.001
Sex	Sex + IPAQ	0.008	22.270	2, 5727	<.001

Table 10

Linear models of Mediterranean diet (MedScore), Healthy Eating Index (HEI), and International Physical Activity Questionnaire (IPAQ) on GAD-7 scores as a measure of anxiety severity. Subject data was obtained from the Atlantic PATH database.

Predictor	n	Estimate	Standard Error	95% Confidence Interval		df	*t*	*p*
				Lower	Upper			
MedScore	6819							
Sex								
Female – Male[a]		0.465	0.075	0.317	0.613	6815	6.173	<.001
MedScore[1]								
Moderate – Low[a]		-0.146	0.076	-0.294	-0.002		-1.933	.053
MedScore[2]								
High – Low[a]		-0.199	0.109	-0.413	0.015		-1.819	.069
Healthy Eating Index (HEI)	9632							
Sex								
Female – Male[a]		0.446	0.064	0.321	0.570	9628	7.006	<.001
HEI[1]								
Moderate – Low[a]		-0.524	0.081	-0.682	-0.365		-6.474	<.001
HEI[2]								
High – Low[a]		-0.706	0.095	-0.892	-0.520		-7.448	<.001
IPAQ	5862							
Sex								
Female – Male[a]		0.427	0.089	0.252	0.602	5858	4.790	<.001
IPAQ[1]								
Moderate – Low[a]		-0.234	0.155	-0.538	0.069		-1.512	.131
IPAQ[2]								
High – Low[a]		-0.371	0.148	-0.660	-0.082		-2.513	.012

Table 11

Comparison of regression models using either sex or both sex and Mediterranean-style diet scores (MedScore), sex and scores on a Healthy Eating Index (HEI), or sex and scores on the and International Physical Activity Questionnaire (IPAQ) as predictors of anxiety symptom severity. Subject data was obtained from the Atlantic PATH database.

Predictors		ΔR^2	F	df_1, df_2	*p*
Model 1	Model 2				
Sex	Sex + MedScore	$7.652e^{-4}$	2.624	2, 6815	.073
Sex	Sex + HEI	0.006	29.723	2, 9628	<.001
Sex	Sex + IPAQ	0.001	3.671	2, 5858	.026

Appendix B

Table 1

Means and standard errors of the mean values for the sucrose preference test for experiment 2A. Values are split both by sex and treatment group.

		Sucrose Preference Score (%)	
		Mean	Standard Error
Male	Control	80.4	1.7
	Male	83.5	1.4
	Female	79.1	4.1
Female	Control	75.1	1.1
	Male	74.3	5.1
	Female	79.4	1.7

Table 2

Means (and standard errors) of the mean values for the splash test for experiment 2A. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	General Grooming	Frequency	7.9 (1.1)	5.3 (0.4)	5.4 (0.8)
		Duration	24.8 (3.2)	18.9 (2.0)	18.0 (4.2)
	Back Grooming	Frequency	8.4 (1.0)	6.1 (0.6)	5.5 (0.8)
		Duration	42.3 (4.5)	30.9 (3.4)	37.1 (5.1)
	Movement	Frequency	7.8 (0.7)	9.8 (0.6)	8.1 (0.6)
		Duration	148.8 (9.0)	156.4 (14.4)	165.6 (13.4)
Female	General Grooming	Frequency	9.5 (1.1)	9.3 (1.0)	8.2 (1.5)
		Duration	22.1 (2.8)	34.6 (8.1)	21.2 (2.8)
	Back Grooming	Frequency	5.4 (0.7)	5.6 (0.9)	6.1 (0.9)
		Duration	30.0 (4.0)	33.5 (2.6)	30.6 (2.6)
	Movement	Frequency	9.4 (0.6)	8.9 (0.9)	9.5 (0.8)
		Duration	219.3 (8.1)	199.3 (12.5)	214.7 (6.3)

Table 3

Means (and standard errors) of the mean values for the tail suspension test for experiment 2A. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	Highly Mobile	Frequency	400.7 (37.2)	396.5 (39.4)	335.9 (34.6)
		Duration	82.0 (11.9)	80.0 (11.1)	53.4 (7.0)
	Mobile	Frequency	567.3 (33.1)	560.8 (33.2)	607.0 (39.5)
		Duration	60.3 (3.9)	59.0 (3.1)	68.4 (4.8)
	Immobile	Frequency	412.7 (31.8)	413.3 (19.1)	486.9 (24.2)
		Duration	155.9 (13.9)	150.5 (14.1)	177.3 (10.2)
Female	Highly Mobile	Frequency	397.7 (38.9)	392.2 (43.2)	402.8 (53.2)
		Duration	84.2 (10.7)	86.8 (12.2)	92.1 (12.8)
	Mobile	Frequency	600.6 (59.7)	546.4 (38.1)	524.8 (63.5)
		Duration	66.2 (7.3)	56.8 (3.8)	54.7 (7.5)
	Immobile	Frequency	439.0 (44.0)	415.6 (24.0)	380.6 (47.3)
		Duration	137.9 (16.6)	134.2 (15.7)	129.8 (15.1)

Table 4

Means (and standard errors) of the mean values for the forced swim test for experiment 2A. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	Highly Mobile	Frequency	36.7 (12.9)	38.7 (13.6)	47.4 (13.3)
		Duration	3.9 (1.4)	4.1 (1.7)	5.3 (1.7)
	Mobile	Frequency	275.7 (46.3)	281.0 (33.4)	320.3 (29.8)
		Duration	54.4 (12.4)	57.4 (7.8)	61.5 (6.5)
	Immobile	Frequency	246.1 (37.9)	244.3 (23.1)	275.4 (28.9)
		Duration	240.6 (13.4)	238.4 (9.1)	233.2 (7.6)
Female	Highly Mobile	Frequency	75.3 (15.3)	53.6 (13.8)	59.9 (10.2)
		Duration	9.2 (2.1)	6.1 (1.7)	6.8 (1.3)
	Mobile	Frequency	392.8 (40.7)	305.5 (42.3)	355.6 (32.3)
		Duration	73.7 (6.9)	66.5 (10.4)	67.9 (7.6)
	Immobile	Frequency	324.0 (32.0)	254.1 (32.0)	299.8 (25.6)
		Duration	217.0 (8.6)	223.3 (10.8)	211.1 (6.6)

Table 5

Means and standard errors of the mean values for the sucrose preference test for experiment 2B. Values are split both by sex and treatment group.

		Sucrose Preference Score (%)	
		Mean	Standard Error
Male	Control	68.4	5.7
	Male	77.4	3.1
	Female	66.0	6.7
Female	Control	55.6	5.3
	Male	77.0	3.3
	Female	70.2	4.6

Table 6

Means (and standard errors) of the mean values for the splash test for experiment 2B. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	General Grooming	Frequency	13.4 (1.2)	14.9 (1.0)	10.8 (1.2)
		Duration	33.6 (3.9)	32.7 (2.4)	25.6 (3.2)
	Back Grooming	Frequency	10.0 (1.1)	7.4 (0.9)	6.0 (1.2)
		Duration	28.0 (3.8)	22.4 (2.0)	25.2 (3.8)
	Movement	Frequency	23.4 (2.9)	28.3 (1.2)	32.0 (3.4)
		Duration	119.1 (9.4)	137.5 (9.2)	140.4 (14.6)
Female	General Grooming	Frequency	13.8 (1.1)	13.3 (1.4)	11.2 (1.0)
		Duration	33.0 (2.1)	35.9 (7.3)	29.5 (5.8)
	Back Grooming	Frequency	7.8 (1.5)	7.8 (1.3)	6.3 (1.0)
		Duration	30.0 (4.7)	29.1 (4.2)	31.6 (4.8)
	Movement	Frequency	19.5 (1.4)	24.4 (1.5)	28.3 (3.0)
		Duration	117.8 (13.8)	127.4 (16.5)	143.7 (14.9)

Table 7

Means (and standard errors) of the mean values for the tail suspension test for experiment 2B. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	Highly Mobile	Frequency	349.0 (42.2)	436.2 (62.4)	373.9 (64.5)
		Duration	62.2 (8.9)	77.0 (14.7)	61.6 (8.3)
	Mobile	Frequency	564.1 (55.4)	610.1 (71.7)	523.0 (70.1)
		Duration	60.3 (5.8)	63.5 (8.1)	54.3 (7.4)
	Immobile	Frequency	432.0 (38.5)	492.6 (51.7)	408.8 (45.0)
		Duration	208.3 (15.3)	190.7 (20.3)	205.7 (15.7)
Female	Highly Mobile	Frequency	449.4 (70.3)	453.3 (42.8)	427.2 (42.9)
		Duration	86.9 (16.2)	91.6 (15.0)	81.7 (10.2)
	Mobile	Frequency	631.5 (80.6)	629.9 (29.3)	618.5 (42.9)
		Duration	67.6 (9.5)	64.2 (3.6)	65.7 (4.8)
	Immobile	Frequency	525.5 (57.2)	506.5 (21.4)	459.9 (21.6)
		Duration	205.2 (15.9)	174.4 (16.1)	172.8 (14.2)

Table 8

Means (and standard errors) of the mean values for the forced swim test for experiment 2B. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	Highly Mobile	Frequency	126.2 (17.7)	107 (16.6)	157 (18.5)
		Duration	19.1 (2.7)	17.4 (3.6)	28.5 (5.2)
	Mobile	Frequency	284.5 (41.2)	239.1 (28.0)	337.8 (51.8)
		Duration	36.9 (5.7)	30.4 (3.5)	42.9 (6.9)
	Immobile	Frequency	239.8 (31.5)	209.4 (24.1)	296.9 (44.4)
		Duration	232.1 (10.2)	246.1 (8.5)	220.7 (10.5)
Female	Highly Mobile	Frequency	145.2 (23.9)	103.8 (12.5)	124.9 (16.3)
		Duration	26.4 (5.8)	17.0 (2.3)	18.6 (2.9)
	Mobile	Frequency	287.9 (28.6)	216.4 (28.9)	261.2 (29.3)
		Duration	35.6 (3.4)	26.8 (3.2)	32.6 (3.5)
	Immobile	Frequency	246.1 (26.6)	187.2 (23.5)	230.1 (24.7)
		Duration	225.4 (9.9)	250.3 (6.6)	244.7 (6.6)

Table 9

Means and standard errors of the mean values for the sucrose preference test for experiment 2C. Values are split both by sex and treatment group.

		Sucrose Preference Score (%)	
		Mean	Standard Error
Male	Control	63.5	1.3
	Male	66.3	1.6
	Female	68.5	0.8
Female	Control	61.1	1.7
	Male	62.5	1.6
	Female	57.5	2.0

Table 10

Means (and standard errors) of the mean values for the splash test for experiment 2C. Values are split by sex, treatment group, behaviour type, and behavioural measure

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	General Grooming	Frequency	2.9 (0.6)	4.8 (0.8)	4.7 (0.7)
		Duration	10.1 (1.2)	14.6 (2.1)	14.0 (2.6)
	Back Grooming	Frequency	0.9 (0.5)	1.2 (0.7)	0.3 (0.2)
		Duration	2.3 (1.6)	4.4 (2.6)	0.4 (0.3)
	Movement	Frequency	23.7 (2.6)	24.5 (1.4)	23.8 (1.4)
		Duration	131.1 (14.4)	134.4 (10.4)	132.8 (14.4)
Female	General Grooming	Frequency	4.1 (0.5)	3.5 (0.5)	4.3 (1.1)
		Duration	14.1 (1.8)	12.9 (2.2)	12.3 (2.3)
	Back Grooming	Frequency	1.8 (1.1)	0.6 (0.3)	0.3 (0.2)
		Duration	8.6 (6.2)	1.5 (0.7)	0.2 (0.1)
	Movement	Frequency	22.7 (1.4)	25.0 (1.6)	27.1 (2.5)
		Duration	146.5 (10.7)	133.2 (9.3)	158.2 (13.2)

Table 11

Means (and standard errors) of the mean values for the tail suspension test for experiment 2C. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	Highly Mobile	Frequency	231.4 (66.2)	118.1 (38.6)	129.2 (39.7)
		Duration	47.2 (17.1)	18.2 (8.6)	19.9 (7.7)
	Mobile	Frequency	388.7 (47.2)	319.0 (48.1)	329.2 (49.4)
		Duration	39.3 (4.2)	34.2 (4.6)	35.1 (5.2)
	Immobile	Frequency	349.2 (39.6)	298.2 (43.5)	308.8 (47.4)
		Duration	209.3 (21.6)	240.8 (14.5)	245.8 (11.2)
Female	Highly Mobile	Frequency	161.2 (47.3)	164.1 (36.5)	125.2 (32.7)
		Duration	30.0 (12.2)	25.1 (8.9)	18.3 (5.6)
	Mobile	Frequency	303.8 (54.7)	365.4 (54.8)	285.0 (47.6)
		Duration	30.8 (5.6)	41.5 (6.7)	29.5 (5.2)
	Immobile	Frequency	282.7 (50.0)	337.7 (42.9)	261.0 (40.1)
		Duration	231.7 (17.0)	231.4 (14.2)	259.5 (10.0)

Table 12

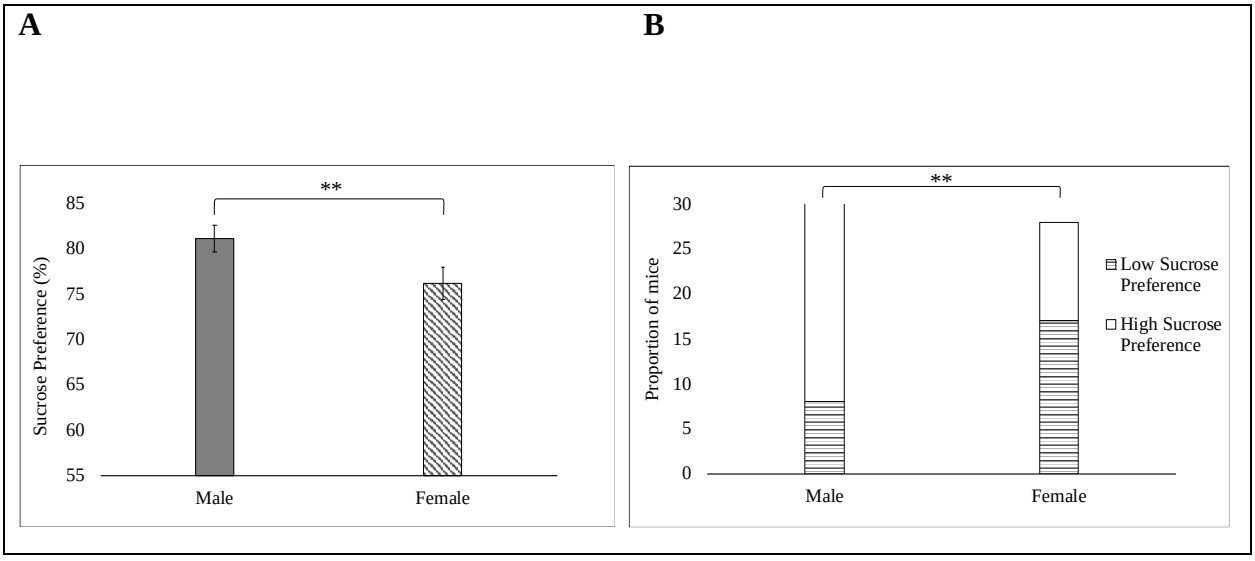
Means (and standard errors) of the mean values for the forced swim test for experiment 2B. Values are split by sex, treatment group, behaviour type, and behavioural measure.

			Treatment		
Sex	Behaviour		Control	Male	Female
Male	Highly Mobile	Frequency	41.2 (10.1)	47.6 (10.2)	51.0 (10.2)
		Duration	6.0 (1.6)	6.9 (1.5)	6.4 (1.2)
	Mobile	Frequency	123.6 (15.8)	116.7 (16.2)	146.8 (22.4)
		Duration	16.0 (2.4)	15.5 (2.3)	19.7 (3.4)
	Immobile	Frequency	101.6 (9.7)	93.2 (12.2)	121.5 (18.3)
		Duration	277.9 (4.0)	276.9 (3.6)	273.8 (4.5)
Female	Highly Mobile	Frequency	55.4 (12.7)	66.8 (10.3)	40.9 (6.5)
		Duration	7.8 (2.0)	9.8 (1.7)	5.2 (1.0)
	Mobile	Frequency	167.9 (26.5)	187.8 (25.1)	109.3 (10.5)
		Duration	21.8 (3.6)	24.1 (3.4)	14.1 (1.4)
	Immobile	Frequency	134.7 (20.5)	153.6 (20.1)	89.7 (7.4)
		Duration	270.5 (5.5)	265.2 (4.8)	280.4 (2.3)

Appendix C

Figure 1

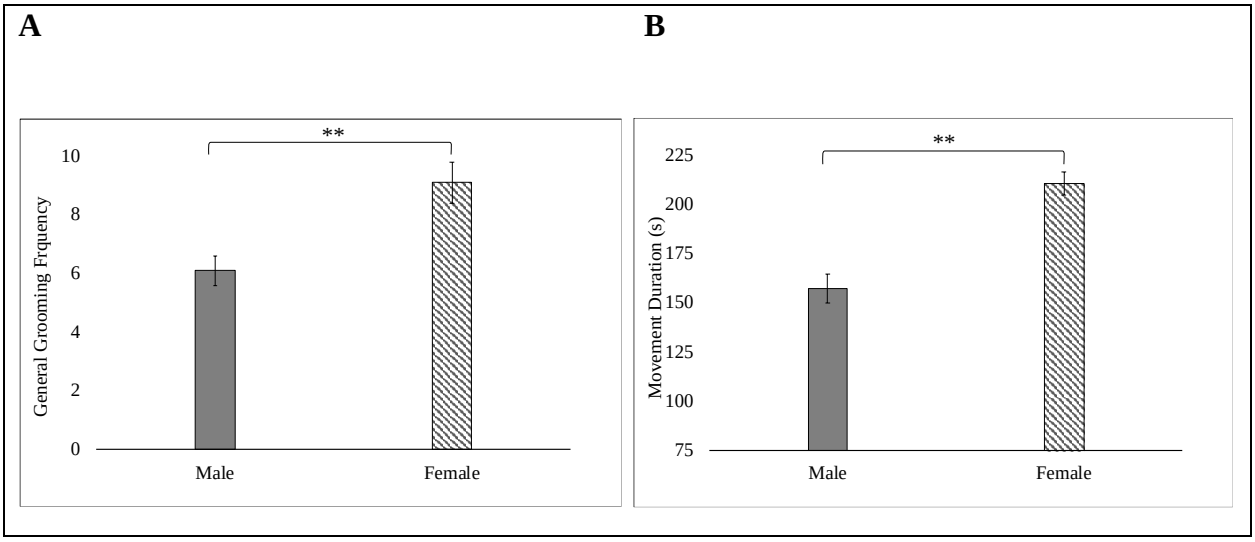
Sucrose preference scores as a function of sex and treatment condition.



Note. Data shown as mean ± standard error of the mean (SEM) of raw sucrose preference scores (**A**) and proportion of males and females with high/low scores (**B**) for the sucrose preference test. **A.** Female mice had significantly lower raw sucrose preference scores than males, $^{**}p < .05$. **B.** Males had significantly higher proportion of high sucrose preference scores compared to females, $^{**}p < .05$.

Figure 2

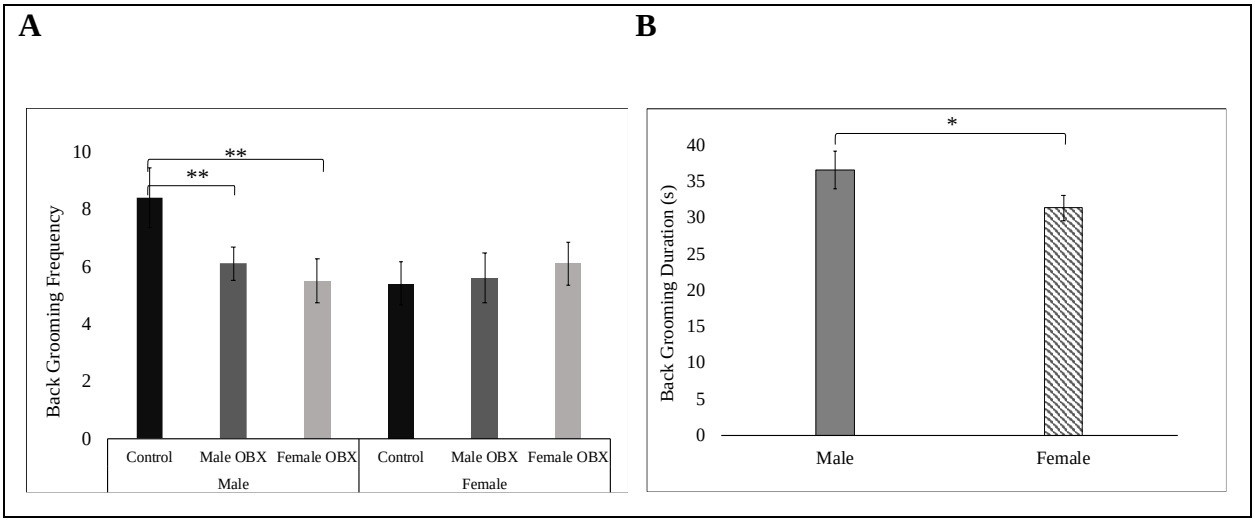
General grooming frequency and movement duration for the splash test as a function of sex.



Note. Data shown as mean ± standard error of the mean (SEM) of general grooming frequency (**A**) and movement duration (B) on the splash test. **A.** Male mice had significantly lower general grooming frequencies than female mice, $^{**}p < .05$. **B.** Male mice had significantly higher mobile durations than female mice, $^{**}p < .05$.

Figure 3

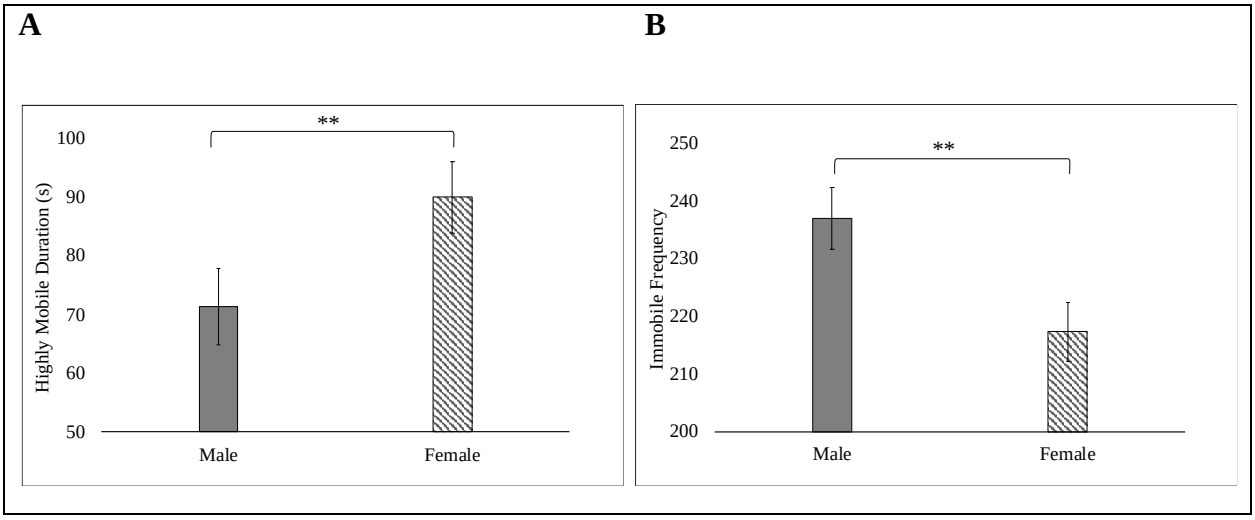
Back grooming frequency and duration for the splash test as a function of sex by treatment and sex.



Note. Data shown as mean ± standard error of the mean (SEM) of back grooming frequency (**A**) and duration (**B**) for the splash test. **A.** Female and male OBX cecum significantly decreased back grooming frequencies in male mice compared to male controls, $^{**}p < 0.05$. **B.** Males had marginally higher back grooming durations compared to females, $^{*}p < .1$.

Figure 4

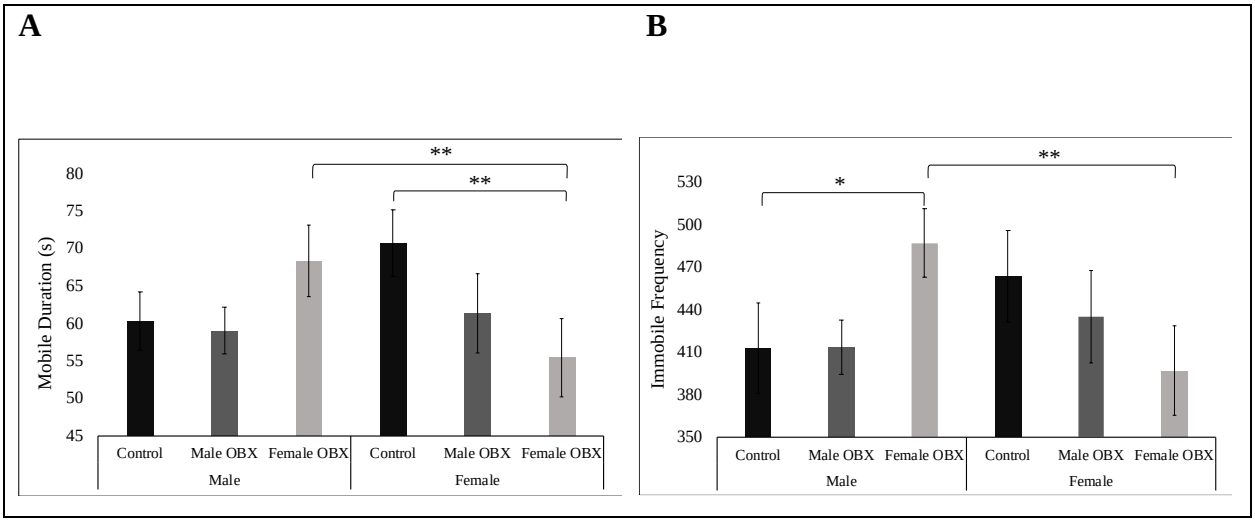
Highly mobile duration and immobile frequency for the tail suspension test as a function of sex.



Note. Data shown as mean ± standard error of the mean (SEM) of highly mobile duration (**A**) and immobile frequency (**B**) for the tail suspension test. **A.** Females had significantly greater highly mobile durations than males, $^{**}p < .05$. **B.** Males had significantly higher immobile frequencies than females, $^{**}p < .05$.

Figure 5

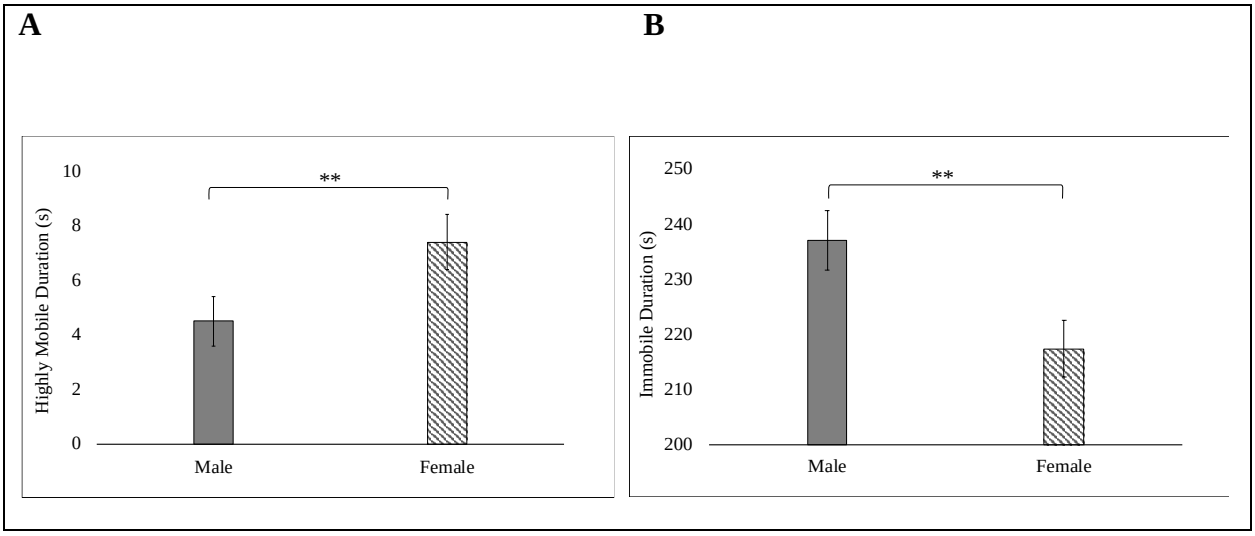
Mobile duration and immobile frequency for the tail suspension test as a function of sex by treatment condition.



Note. Data shown as mean ± standard error of the mean (SEM) of mobile duration (**A**) and immobile frequency (**B**) for the tail suspension test. **A.** Female OBX cecum significantly decreased mobile durations in females compared to female controls, while it significantly increased durations in males, $^{**}p < .05$. **B.** Female OBX cecum increased immobile frequencies in males, decreased it in females, $^{**}p < .05$. Female OBX cecum marginally increased immobile frequencies in males compared to male controls, $^{*}p < .1$.

Figure 6

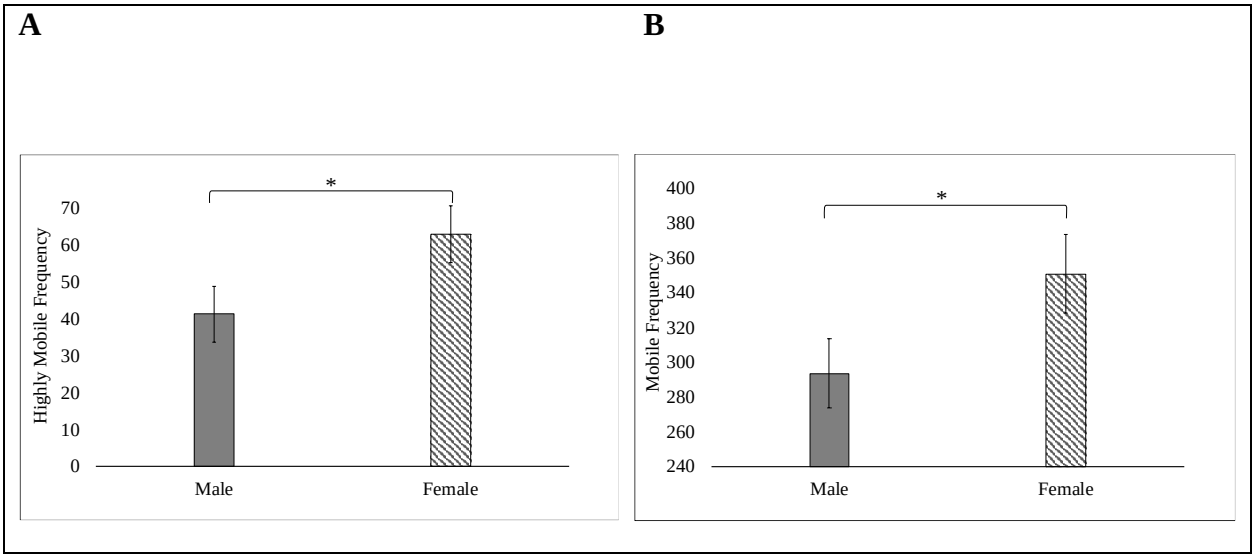
Highly mobile and immobile durations for the forced swim test as a function of sex.



Note. Data shown as mean ± standard error of the mean (SEM) of highly mobile (**A**) and immobile duration (**B**) for the forced swim test. **A.** Females had significantly higher mobile durations than males, $^{**}p < .05$. **B.** Males had significantly higher immobile durations than females, $^{**}p < .05$.

Figure 7

Highly mobile and mobile frequency for the forced swim test as a function of sex.

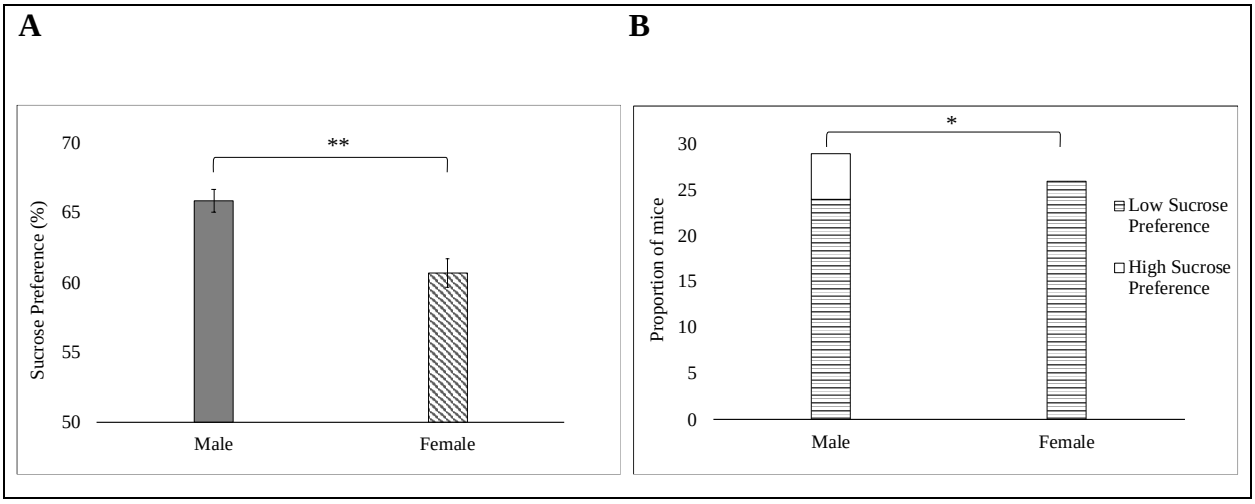


Note. Data shown as mean ± standard error of the mean (SEM) of highly mobile (**A**) and mobile frequency (**B**) for the forced swim test. **A.** Females had marginally higher highly mobile frequencies compared to males, $*p < .1$. **B.** Females had marginally higher mobile frequencies compared to males, $*p < .1$.

Appendix D

Figure 1

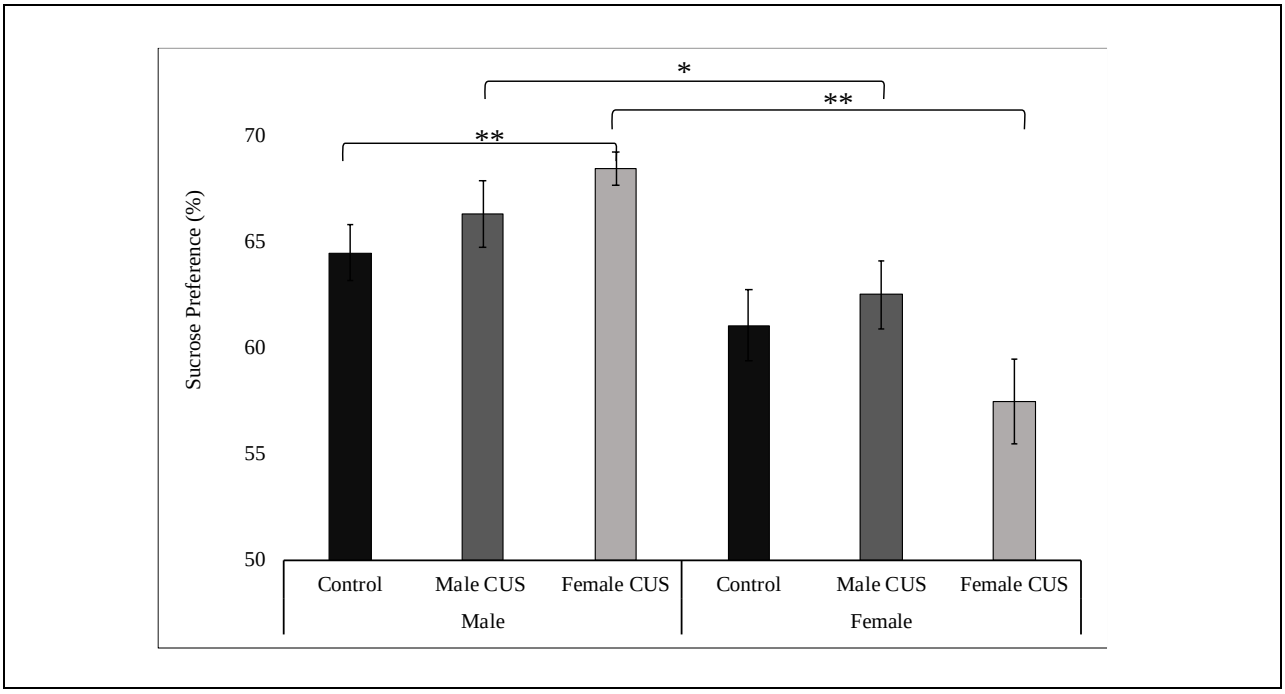
Sucrose preference scores for the sucrose preference test as a function of sex.



Note. Data shown as mean ± standard error of the mean (SEM) of raw sucrose preference scores (**A**) and proportion of males and females with high/low scores (**B**) for the sucrose preference test. **A.** Female mice had significantly lower raw sucrose preference scores than males, $^{**}p < .05$. **B.** Males had a marginally higher proportion of high sucrose preference scores compared to females, $^{*}p < .1$.

Figure 2

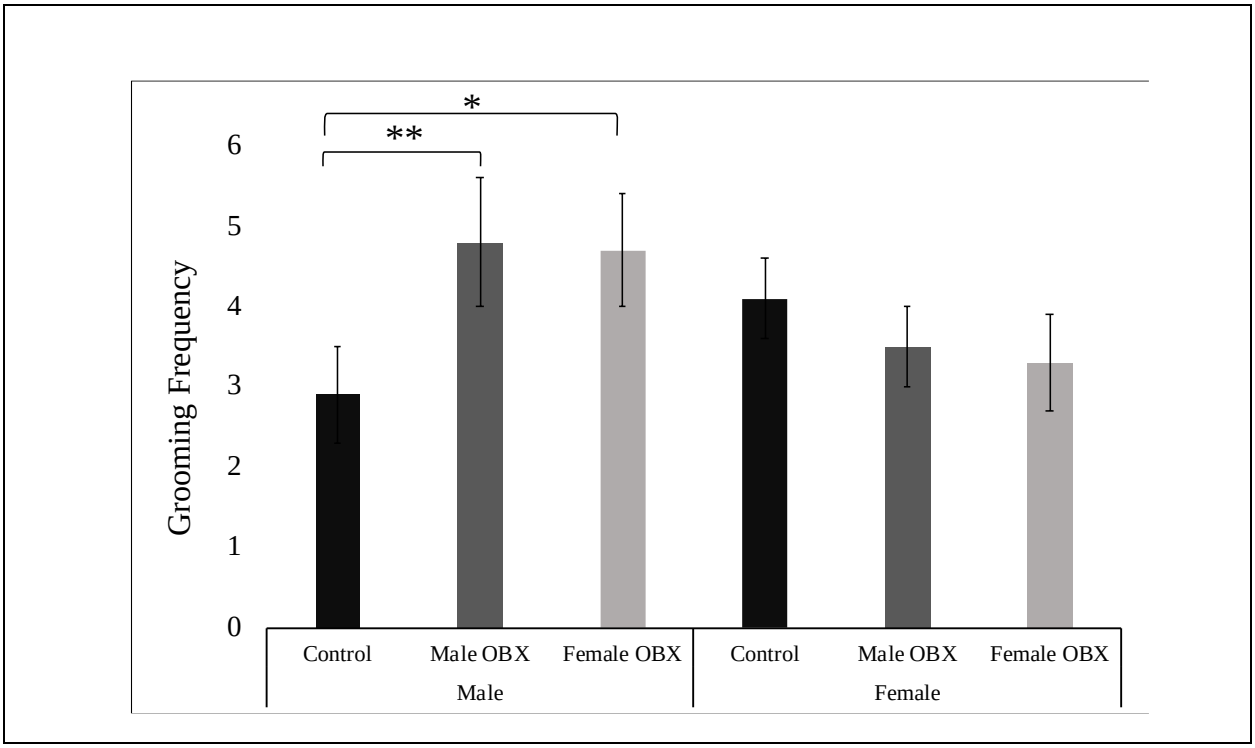
Sucrose preference scores for the sucrose preference test as a function of sex by treatment condition.



Note. Data shown as mean ± standard error of the mean (SEM) of raw sucrose preference scores for the sucrose preference test. Overall males had higher sucrose preference scores than females. Female CUS cecum increased sucrose preference scores in males compared to male controls, while it decreased sucrose preference scores in females, $^{**}p < .05$. Males treated with male CUS cecum had marginally higher scores than females treated with male CUS cecum, $^{*}p < .1$.

Figure 3

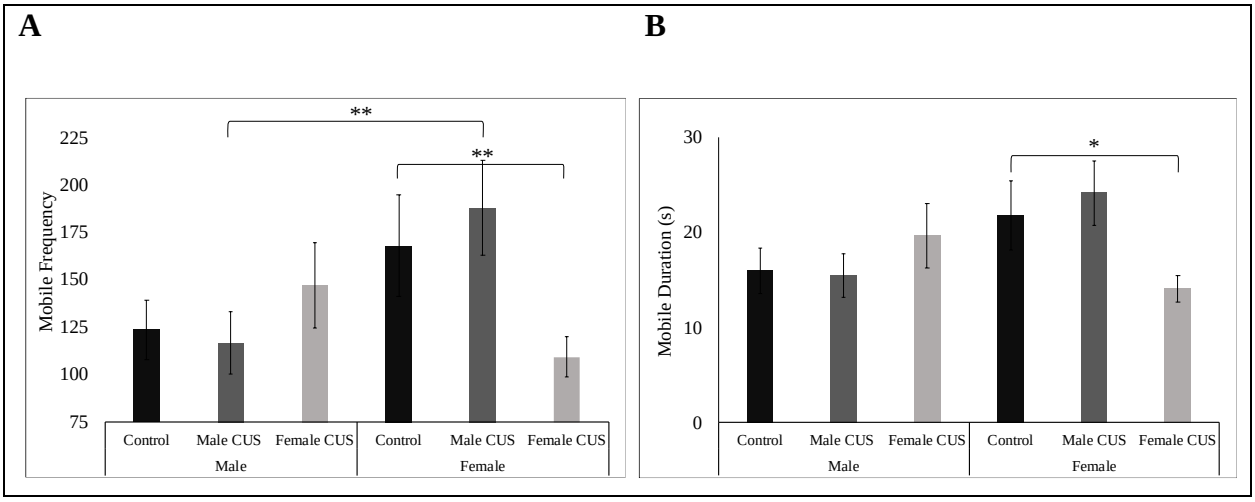
Grooming frequency for the splash test as a function of sex by treatment condition,



Note. Data shown as mean ± standard error of the mean (SEM) of grooming frequency for the splash test. Male CUS cecum significantly decreased grooming frequencies in males compared to male controls, $^{**}p < .05$. Female OBX cecum marginally decreased grooming frequencies in males compared to male controls, $^{*}p < .1$.

Figure 4

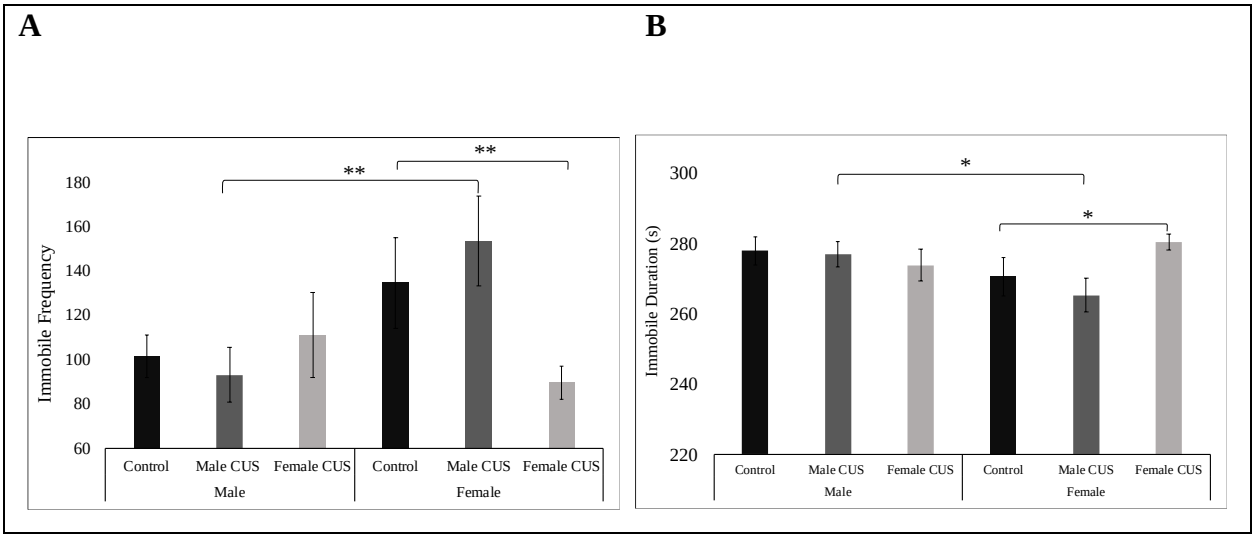
Mobile frequency and duration for the forced swim test as a function of sex by treatment condition.



Note. Data shows mean ± standard error of the mean (SEM) of immobile frequency (**A**) and duration (**B**) for the forced swim test. **A.** Female CUS cecum significantly decreased mobile frequencies in females compared to controls, $^{**}p < .05$. Male CUS cecum decreased mobile frequencies in females, increased it in males, $^{**}p < .05$. **B.** Female CUS cecum marginally decreased mobile durations in females compared to controls, $^{*}p < .1$.

Figure 5

Immobile frequency and duration for the forced swim test as a function of sex by treatment condition.

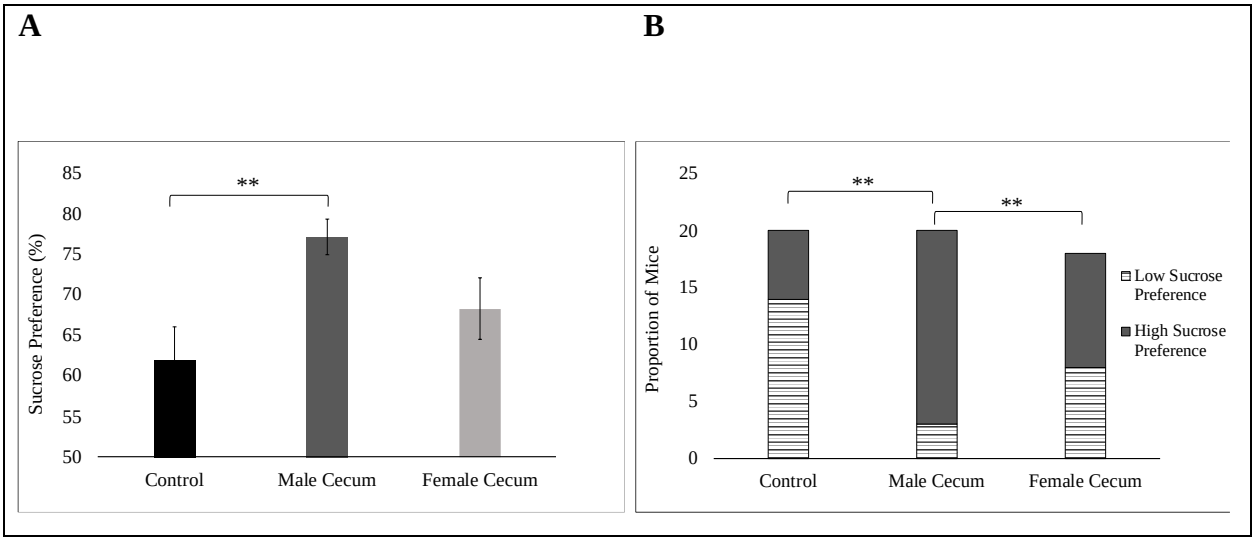


Note. Data shown as mean ± standard error of the mean (SEM) of immobile frequency (**A**) and duration (**B**) for the forced swim test. **A.** Female CUS cecum significantly decreased immobile frequencies in females compared to female controls, $^{**}p < .05$. Male CUS cecum increased immobile frequencies in females, decreased it in males, $^{**}p < .05$. **B.** Female CUS cecum marginally increased immobile durations in females compared to controls, $^{*}p < .1$. Male CUS cecum marginally increased immobile durations in males, decreased it in females, $^{*}p < .1$.

Appendix E

Figure 1

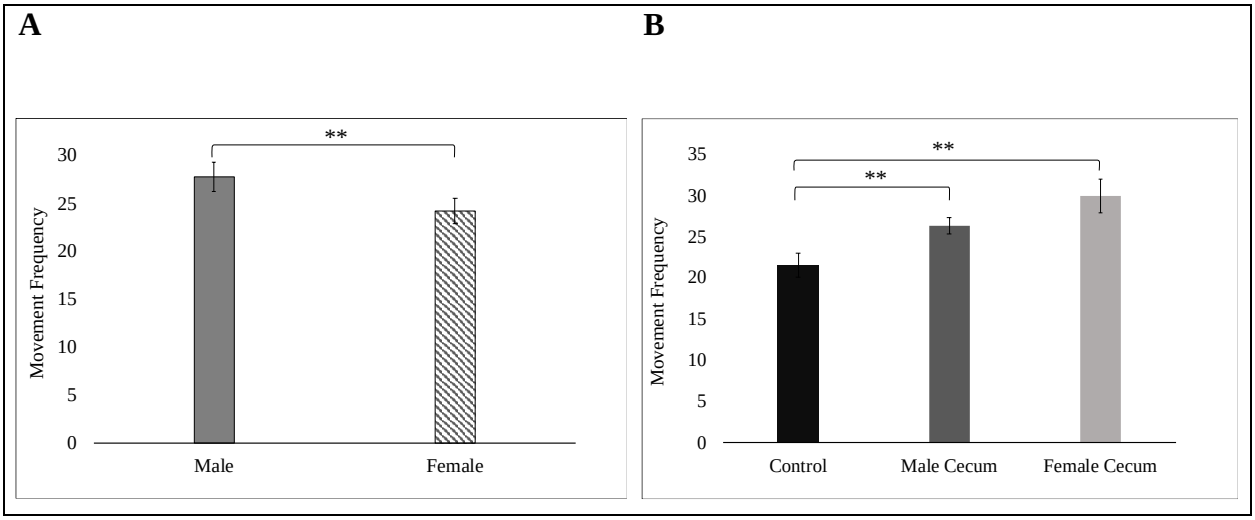
Sucrose preference scores for the sucrose preference test as a function of treatment.



Note. Data shown as mean ± standard error of the mean (SEM) of raw sucrose preference scores (**A**) and proportion of males and females with high/low scores (**B**) for the sucrose preference test. **A.** Mice treated with male cecum had significantly higher sucrose preference scores than controls, $^{**}p < .05$. **B.** Mice treated with male cecum had a significantly higher proportion of high sucrose preference scores compared to controls and mice treated with female cecum, $^{**}p < .05$.

Figure 2

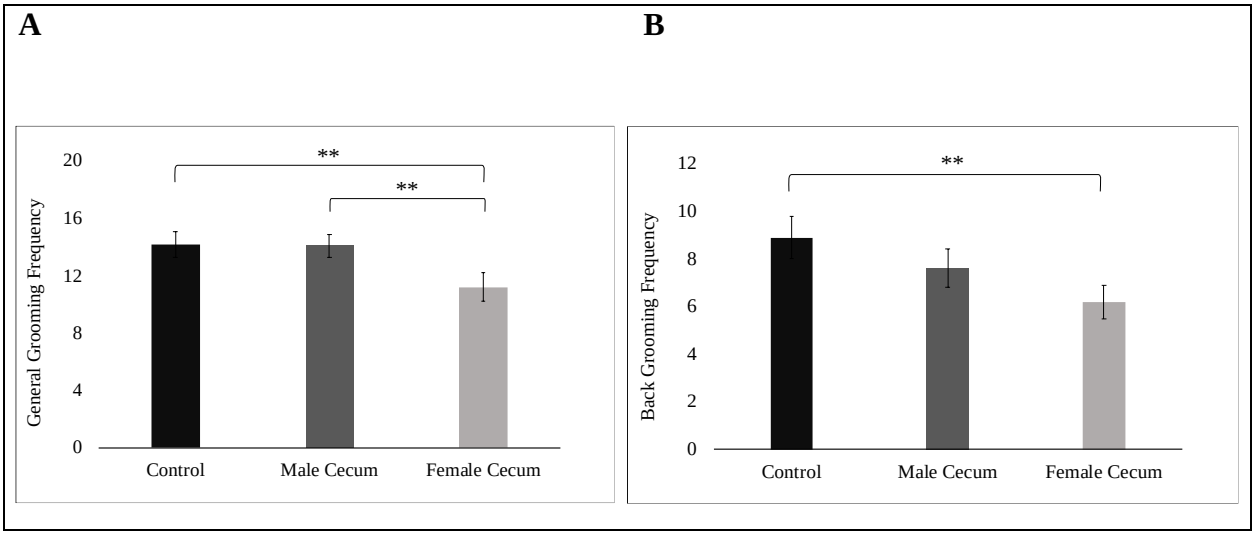
Movement frequency for the splash test as a function of sex and treatment condition.



Note. Data shown as mean ± standard error of the mean (SEM) of movement frequency for the splash test. **A.** Males had significantly higher movement frequencies compared to females, $^{**}p < .05$. **B.** Mice in the control group had significantly lower movement frequencies than mice treated with either male or female cecum, $^{**}p < .05$.

Figure 3

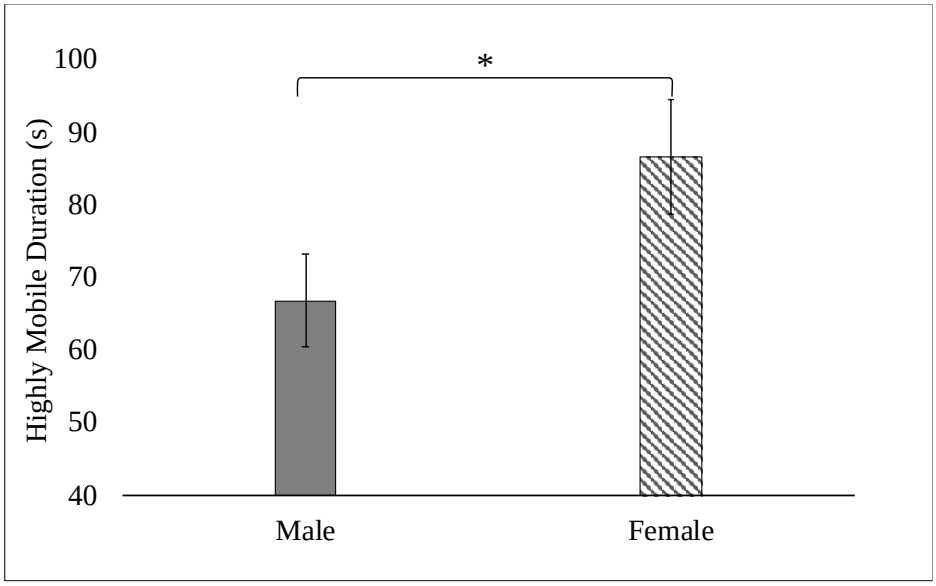
General and back grooming frequencies for the splash test as a function of treatment condition.



Note. Data shown as mean ± standard error of the mean (SEM) of general grooming frequency (**A**) and back grooming frequency (**B**) for the splash test. **A.** Mice treated with female cecum had significantly lower general grooming frequencies compared to controls and mice treated with male cecum, $^{**}p < .05$. **B.** Mice treated with female cecum had significantly lower back grooming frequencies compared to controls, $^{**}p < .05$.

Figure 4

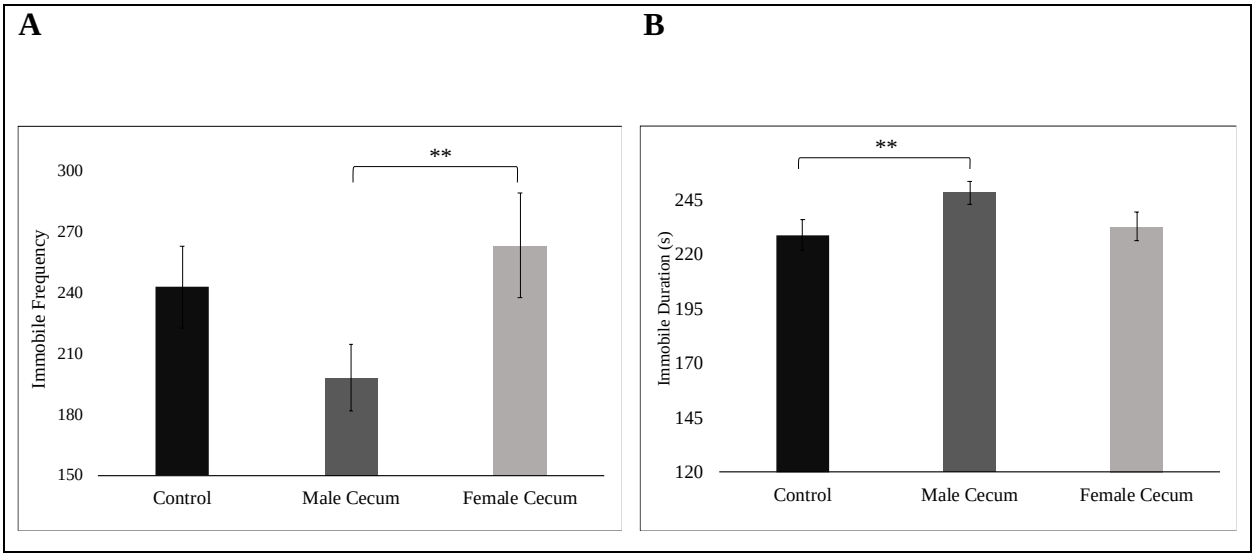
Highly mobile duration for the tail suspension test as a function of sex.



Note. Data shown as mean ± standard error of the mean (SEM) of highly mobile duration for the tail suspension test. Female mice had marginally higher highly mobile durations than males, *$p < .1$.

Figure 5

Immobile frequency and duration for the forced swim test as a function of treatment.



Note. Data shown as mean ± standard error of the mean (SEM) of immobile frequency (**A**) and immobile duration (**B**) for the forced swim test. **A.** Mice in the female cecum condition had significantly higher immobile frequencies than mice in the male cecum condition, $^{**}p < .05$. **B.** Mice in the male cecum condition had significantly higher immobile durations than mice in the control group, $^{**}p < .05$.