Get off to Sleep: Pubertal Depression Prevention by Metabolic Intervention

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Contents

Abstract

Puberty and adolescence are periods of brain-driven physiological development that display increased incidences of depression development. Adolescents display significant alterations to their stress response signaling, sleep patterns, and metabolism when compared to pre-pubescents. Increased exposure to stress, sleep disturbances, and impaired energy acquisition is typical during puberty and adolescence and similarly increases the likelihood of developing depression. A promising avenue of limiting the deleterious effects of stress and sleep disruption on pubertal and adolescent depressive behaviour is the use of treatments that blunt underlying metabolic impairments associated with depression. Treatments that directly or indirectly increase availability of the glucose metabolite L-lactate are associated with depression reduction. The investigations included in this dissertation evaluate the usability of L-lactate treatments in reducing depression development in pubertal CD-1 male and female mice. This work first examines a previously proposed oral lactate solution, its effect on energy substrate concentration and drowsiness, and its efficacy as a safe oral L-lactate treatment (Chapter 2). Subsequent research aimed to identify a pubertal model of depression that would allow future testing of L-lactate antidepressant treatment. Pubertal male and female mice exposed to chronic sleep disruption were evaluated for stress reactivity and depressive behavior and were identified as a model for antidepressant testing (Chapter 3). In the final study, we evaluated the effects of chronic sleep disruption on the expression of energy metabolites like L-lactate and glucose within the brain, its effect on neurotransmitters associated with depression, and changes to sleep architecture in relation to depression behaviour. Sleep disrupted and depressed animal models were administered L-lactate producing probiotics and were evaluated for improvements to energy substrate concentration, neurotransmitter expression, sleep recovery, and depression reduction (Chapter 4). The present thesis provides groundwork for the use of L-lactate therapies in depressed pubertal and adolescent groups and provides initial evaluations of probiotic intervention as a prevention strategy for juvenile depression.

Chapter 1

Problem Overview

Depression is a highly prevalent mood disorder responsible for quality-of-life reduction in nearly every country and culture sampled (James et al., 2018; Weissman et al., 1993; Weissman & Klerman, 1977). Depressive symptoms include general malaise and despair for an extended period, significant weight and appetite change, lethargy, inappropriate guilt, and/or suicidal thoughts and tendencies (Association, 2013). Approximately 280 million people worldwide suffered from major depression and depressive disorders in early 2020 (Global Health Data Exchange, http://ghdx.healthdata.org/) with women diagnosed twice as frequently as men (Hankin & Abramson, 1999; Hankin et al., 1998; Weissman et al., 1993; Weissman & Klerman, 1977). Preliminary data suggests that depression development has accelerated during the COVID-19 pandemic with adolescent samples especially effected (Cost et al., 2021). Additionally, Canadians have reported increased mental health deterioration associated with sleep pattern changes like delayed sleep onset during the pandemic (Robillard et al., 2021). The combined financial costs from depression-related treatment and absenteeism exceeds one trillion in US$ each year (Organization, 2008). The uniquely high prevalence of depression combined with its debilitating symptomology makes depression's worldwide financial burden the largest of any singular disease (Demyttenaere et al., 2004; Organization, 2008). Early prevention and treatment of depression has garnered much interest at the clinical, socioeconomic, and political levels.

Many life-long cases of depression first develop during puberty and adolescence (Hankin & Abramson, 1999; Hankin et al., 1998; Pine, Cohen, Cohen, & Brook, 1999; Weissman et al., 1993). Several physiological systems related to depression develop during puberty and adolescence (N. B. Allen, Barrett, Sheeber, & Davis, 2006; Blakemore, Burnett, & Dahl, 2010; Carskadon, 2011). Targeting signaling pathways that both mature during puberty and adolescence and influence depressive symptoms could provide effective treatment and prevention strategies. In the following sections, we have summarized the adolescent development of systems relevant to depression development.

Additionally, we provide evidence for a potential probiotic intervention that blocks several depression-related impairments. We hypothesize that depression development can be reduced with pubertal exposure to lactic acid probiotics.

1.0 Puberty and adolescence

Puberty is a period of development where brain-driven interactions between nervous and endocrine systems transform the physiology of an organism until it achieves reproductive maturity (Tanner, 1962). Pubertal onset follows increased hypothalamic-pituitary-gonadal (HPG) axis activity (Sisk & Foster, 2004). Adolescence follows puberty and includes maturation of cognitive ability and social behavior needed for healthy reproduction (Holder & Blaustein, 2014; Sisk & Foster, 2004). In the subsequent section, we discuss the signaling mechanisms responsible for initiating and facilitating reproductive maturity in rodent and human samples.

Gonadotropin releasing hormone (GnRH) secretion from the medial eminence of the basal hypothalamus drives reproductive maturation by signaling the anterior pituitary to stimulate egg, sperm, and gonadal hormone production within the ovaries and testis of humans and rodents (Sisk & Foster, 2004). Estrogens and progestins are major gonadal hormones produced in the ovaries where androgens are produced in the testis (Tanner, 1962). Gonadal hormones promote follicle maturation and spermatogenesis within the gonads of humans and rodents and produce secondary sex characteristics elsewhere (Marshall & Tanner, 1969, 1970; Tanner, 1962). Gonadal hormones feedback to the brain to regulate sexual behavior and GnRH release (Sisk & Foster, 2004). Continued pubertal development is activated and prolonged by transient GnRH secretion pulses controlled by gonadal negative feedback to the hypothalamus (Sisk & Foster, 2004). Puberty is initiated by a sudden increase in GnRH pulses however it is unclear what instigates this sudden acceleration (Sisk & Foster, 2004; J. T. Smith & Clarke, 2007). Therefore, the accelerated secretion of GnRH by unknown mechanisms both initiate and maintain reproductive maturation.

Few theories explain why GnRH pulses accelerate prior to pubertal onset (Jennes & Conn, 2002; Silverman, 1988). The Permissive Signal theory states that exposure to multiple internal and external clues initiate puberty (Sisk & Foster, 2004; M. E. Wilson,

1992). Permissive signals frequently relate to available energy and vary according to sex and species. Factors like available food, glucose concentration and insulin production can be used to measure if sufficient permissive conditions are met for pregnancy (Marshall & Tanner, 1969, 1970). Hindbrain and hypothalamic receptors monitor energy homeostasis and provide subsequent disinhibition of GnRH pulses when food is less available (Schneider, 2004; Schneider & Watts, 2002). The opposite is also observed: average pubertal onset occurs earlier in developed countries with easy food access than in less developed countries (Moodie et al., 2020). Female mammals are more likely than males to demonstrate delayed pubertal onset following food restriction due to the greater energy requirements of pregnancy (Marshall & Tanner, 1969, 1970; Tanner, 1962).

More recently, Kisspeptin peptides have been associated with the integration of permissive signaling initiating pubertal onset (d'Anglemont de Tassigny & Colledge, 2010; J.-H. Lee et al., 1996; J. T. Smith & Clarke, 2007; K. B. Smith et al., 2021). Kisspeptins are a group of amidated peptides encoded by the *Kiss1* gene (d'Anglemont de Tassigny & Colledge, 2010; J.-H. Lee et al., 1996). If the *Kiss1* gene or kisspeptin receptors are disrupted, pubertal onset fails to occur leading to impaired gametogenesis, underdeveloped gonads, and infertility (Funes et al., 2003). Kisspeptin neurons in the anteroventral paraventricular nucleus are expressed more in female than male rodents (Clarkson & Herbison, 2006; d'Anglemont de Tassigny et al., 2007). As kisspeptin stimulation disinhibits GnRH secreting cells, sexually dimorphic expression of kisspeptin neurons may contribute to earlier female pubertal onset (Clarkson & Herbison, 2006; d'Anglemont de Tassigny & Colledge, 2010; J. T. Smith & Clarke, 2007). A combination of external factors like food availability and internal factors like kisspeptin secretion likely initiate increased GnRH secretion and pubertal onset.

Pubertal onset and duration vary significantly between sexes and species (Marshall & Tanner, 1969, 1970; Sisk & Foster, 2004; Tanner, 1962). The average age for pubertal onset in girls and boys is 10-11 and 11-12 years old, respectively (Marshall & Tanner, 1969, 1970). Girls display pubertal development for 2-3 years longer than males if defining duration by first external sign of ovarian activity until first complete estrous cycle (Marshall & Tanner, 1969, 1970; Sisk & Foster, 2004). Rodent models of

female puberty follow similar definition of pubertal development duration (Ismail & Blaustein, 2013; Ojeda, Urbanski, Knobil, & Neill, 1994). First pubertal onset of rats and mice align with first ovulation and simultaneous vaginal opening at postnatal day 35 and 30, respectively (Holder & Blaustein, 2014; Ojeda et al., 1994). Puberty also ends in rodent models when the first complete estrous cycle occurs (Holder & Blaustein, 2014; Sisk & Foster, 2004). Pubertal development of female rats ends within 7 days and sometimes occurs within 24 hours of vaginal opening (Lohmiller, Swing, & Hanson, 2020; Parker Jr & Mahesh, 1976). CD-1 female mice display pubertal development for several weeks past pubertal onset which is further extended by same sex housing and deprivation of male pheromones (Korenbrot, Huhtaniemi, & Weiner, 1977; Vandenbergh, 1967). Male pubertal development occurs around postnatal day 42 in rat and CD-1 mouse models marked by separation of the glans penis and the prepuce (Korenbrot et al., 1977). We employ the CD-1 pubertal animal model as the extended pubertal duration better replicates pubertal onset and termination separation observed in humans (Ismail & Blaustein, 2013; Ismail, Garas, & Blaustein, 2011; Ismail, Kumlin, & Blaustein, 2013; Vandenbergh, 1967). Earlier pubertal onset and extended pubertal development in females is believed to contribute to many sexually dimorphic sensitivities expressed during puberty (Kane & Ismail, 2017; Marshall & Tanner, 1969; Tanner, 1962).

To summarize, pubertal maturation is sexually dimorphic in both humans and rodents (Korenbrot et al., 1977; Marshall & Tanner, 1969, 1970; Tanner, 1962; Vandenbergh, 1967). Initiation, propagation, and cessation of puberty is similar enough in human and rodent samples so that both mice and rats are useful animal models of human sexual maturation (Ojeda et al., 1994; Vandenbergh, 1967). In the subsequent section, we review the similarities between the sex-dependent vulnerabilities of puberty and depression.

2.0 Depression and brain signaling pathways

Depression is at least partially maintained by the depletion and dysregulation of synaptic serotonin, dopamine, and/or norepinephrine within the brain (Carr & Lucki, 2011). Several generations of antidepressants increase the availability of monoamines

(Carr & Lucki, 2011). Monoamine oxidase (MAO) inhibitors increase serotonin and norepinephrine concentration by blocking the metabolization of monoamines (Sandler, Reveley, & Glover, 1981). Tricyclic antidepressants increase the interaction of monoamines with synaptic receptors by blocking monoamine reuptake at the presynapse. The success of selective serotonin reuptake inhibitors (SSRIs) has refocused mechanistic investigations of depression onto serotonin production and signaling. In this section, we will summarize the functionality of serotonergic signaling within depression models.

Serotonin or 5-hydroxytrytophan (5-HT) is a biogenic monoamine that has area-specific effects on mood and arousal (Mohammad-Zadeh, Moses, & Gwaltney-Brant, 2008). 5-HT is produced by the hydroxylation and decarboxylation of the essential amino acid tryptophan within the presynaptic terminal of serotonergic fibres, catecholaminergic neurons and within the pineal gland (Clark, Weissbach, & Udenfriend, 1954). 5-HT within the central nervous system is primarily stored and produced in nine hindbrain cell clusters called raphe nuclei (Fuxe, 1964). The majority of 5-HT used within the forebrain and midbrain is synthesized in the dorsal raphe nuclei (Fuxe, 1964). Upon activation of ascending serotonergic fibres, 5-HT is secreted into the synaptic cleft to either bind postsynaptic 5-HT receptors or presynaptic autoreceptors. Postsynaptic 5-HT receptors vary in function according to type and location but include influence over mood, feeding, and digestion (Mohammad-Zadeh et al., 2008). Binding of presynaptic 5-HT_{1A} autoreceptors hyperpolarizes the neurons currently releasing 5-HT to reduce monoamine secretion (De Montigny, Blier, & Chaput, 1984). This creates a negative feedback loop for 5-HT secretion. Serotonin transport protein reuptakes 5-HT into the presynaptic terminal. 5-HT is either re-packaged into vesicles or metabolized by MAO-A into 5-hydroxyindoleacetic acid and eliminated by urine excretion (McIsaac & Page, 1959). Available 5-HT is converted to melatonin in the pineal gland to mediate sleep cycling and circadian rhythm (Mohammad-Zadeh et al., 2008). Serotonergic signaling is crucial to regulating metabolic energy acquisition, conservation, and mood. Reduced 5-HT concentration is associated with development of mood disorders like depression (Asberg, Thoren, Traskman, Bertilsson, & Ringberger, 1976; Heninger, Delgado, & Charney, 1996).

Men and women diagnosed with depression display reduced midbrain and forebrain 5-HT signaling from raphe serotonergic tracts (Burnet, Michelson, Smith, Gold, & Sternberg, 1994; Heninger et al., 1996). Dysfunctional serotonin receptor expression and/or activity provide plausible explanations for reduced 5-HT activity. However, investigations provide conflicting evidence on the roles of serotonin receptors (Carr & Lucki, 2011). Increased expression and activation of presynaptic 5-HT_{1A} receptors on the dorsal raphe limits serotonin production and inhibits 5-HT transmission to the midbrain and forebrain (Burnet et al., 1994; De Montigny et al., 1984). In rodent models of depression, increased immobility in the forced swim and tail suspension tests is interpreted as greater despair and depression (Castagné, Moser, Roux, & Porsolt, 2010; Castagné, Porsolt, & Moser, 2009). Increased 5-HT_{1A} expression on presynaptic raphe terminals is associated with increased forced swim and tail suspension test immobility and an enhanced response to stress in adult mice (Richardson-Jones et al., 2010). Alternatively, increased sensitization of autoreceptors on the dorsal raphe nuclei of mice similarly inhibits 5-HT release (Piñeyro & Blier, 1999). Yet, studies assessing postmortem brains of individuals with major depression disorder do not consistently report changes to dorsal raphe 5-HT_{1A} expression (Burnet et al., 1994; Mohammad-Zadeh et al., 2008). PET scan studies even suggest the opposite, that is, ligand binding to raphe 5-HT_{1A} receptors decreases in depression (Drevets et al., 2007). The role of dorsal raphe 5-HT_{1A} activity within depression development is unclear despite initial evidence to the contrary. These inconsistencies have shifted focus to 5-HT projections to various structures on depression development (Burnet et al., 1994; Lopez, Chalmers, Little, & Watson, 1998).

5-HT projections areas like the hippocampus, hypothalamus, and medial prefrontal cortex modulate memory, lethargy and appetite symptomology associated with depressive disorders (Chiba et al., 2012; Gilboa, Sekeres, Moscovitch, & Winocur, 2014; Lopez et al., 1998; McEwen, 2005; Mizoguchi, Shoji, Ikeda, Tanaka, & Tabira, 2008; Sekeres et al., 2021). 5-HT_{1A} receptors on the postsynaptic terminals of 5-HT projection areas are downregulated in the hippocampus and cortex of depressed humans and rodent models of depression (Lopez et al., 1998; McAllister-Williams, Ferrier, & Young, 1998; Murack et al., 2021). In our CD-1 model of adolescent depression, exposure to a single

novel stressor following 8 days of chronic sleep disruption reduced 5-HT_{1A} expression in the hippocampus and medial prefrontal cortex of mice displaying longer immobility durations in a forced swim test (Murack et al., 2021). Activation of postsynaptic 5-HT_{1A} receptors in the midbrain and forebrain are associated with antidepressant effects in animal models (Szabo & Blier, 2001). Subcutaneous injections of 5-HT_{1A} agonists such as buspirone and 8-OH-DPAT decrease duration of immobility in adult rats exposed to a forced swim test (Detke, Wieland, & Lucki, 1995). An effective antidepressant intervention may ensure sufficient activation of postsynaptic 5-HT_{1A} in the hippocampus and medial prefrontal cortex and improve regulation of depressive symptomology. However, the causes of reduced 5-HT signaling and its roles in depression onset are unclear. In the next section, we discuss the mechanisms responsible for pubertal vulnerability to depression development.

2.1 Pubertal Depression

The etiology of adult depression development can often be traced back to childhood and adolescence (Kessler, Avenevoli, & Merikangas, 2001). Several investigations report high percentages of young individuals with clinically significant depression symptoms (Fleming, Offord, & Boyle, 1989; Rutter, Izard, & Read, 1986). Self-report surveys indicate approximately 10-20% of Canadian children and adolescents aged 11 to 18 years old have experienced a depressive episode that exceeds clinically accepted cutoff points for adult depression diagnoses (Offord et al., 1987; Reinherz et al., 1989). Moreover, 14% of adolescents eventually develop lifelong major depression (Smajkic, 2009). Recent surveys suggest that changes to sleep schedules and increased isolation following COVID-19 closures have also contributed to an increase of depressive symptoms in Canadian children and adolescents (Cost et al., 2021; Robillard et al., 2021). Despite various age and mental health history, 37-56 % of Canadian youth displayed development or deterioration of depressive symptoms since March of 2020 (Cost et al., 2021). To mediate this pubertal vulnerability, we must first identify the underlying mechanisms responsible for increased depression during adolescence.

The frontal and limbic systems responsible for negative emotional regulation and cognitive control show less efficient activation in adolescence than in adulthood

(Ladouceur, 2012). Adolescent males and females display greater amygdala activation in a functional magnetic resonance imaging (fMRI) scan during an emotion regulation task compared to adults (Hare et al., 2008). Increased fMRI activation typically denotes inefficient processing within the brain. Additionally, adolescents displayed increased amygdala activity and slower reaction when identifying negative affect indicating impaired negative emotion regulation (Hare et al., 2008). Adolescents diagnosed with depression display slower reaction times to negative emotional backgrounds on an emotional *n*-back working task (Ladouceur et al., 2005). The ability to process negative emotion appears less efficient or impaired in healthy adolescents and even more so in depressed adolescents. These results suggest that some of the cognitive abilities that help overcome depression are still developing during adolescence.

Sexual dimorphism of depression begins to develop during puberty (Kessler et al., 2001; Marshall & Tanner, 1969, 1970; Tanner, 1962). Increased female susceptibility to depression is not observed until shortly after pubertal onset (Kessler et al., 2001). In preadolescent samples aged 11-13 years old, only 1.79-2.09% of boys and 0.31-2.19% of girls display serious depressive episodes. Following pubertal onset, 13 to 15-year-old girls begin to display at least twice the prevalence of depressive cases than males (Hankin & Abramson, 1999; Hankin et al., 1998). This sexually dimorphic trend continues into adulthood (Hankin & Abramson, 1999; Hankin et al., 1998; Kessler et al., 2001). As increased female vulnerability to depression occurs only after puberty, pubertal onset may instigate a sex-specific biological vulnerability to depression.

To summarize, lifelong depression often develops in early adolescence (Smajkic, 2009). Sexual dimorphism in reproductive development and depression prevalence occurs almost simultaneously (Kessler et al., 2001; Marshall & Tanner, 1969, 1970). A vulnerability to depression may arise during puberty, especially in pubertal females. Investigations into the mechanisms responsible for this pubertal vulnerability would provide prevention strategies for adolescent and lifelong depression. Due to the invasive investigations of these underlying pathways, many of the subsequent studies are limited to animal models.

3.0 Stress response and signaling pathways

A stressor is any external or internal stimulus that presents a potential challenge to an organism's well-being (Herman et al., 2011). This challenge provokes automatic physiological responses aimed to prepare the organism for "fight or flight" to remove or escape the stressor. Stressors vary in their intensity and duration. A single instance of mild-to moderate stress instigates a healthy stress response. However, single extreme stressors or repeat mild/moderate stress significantly alter future stress signaling (Albeck et al., 1997; Blackburn-Munro & Blackburn-Munro, 2001; Chiba et al., 2012; Dalla et al., 2005; Fairchild, Leitch, & Ingram, 2003; Izawa et al., 2007; Shapero, Curley, Black, & Alloy, 2019). Alterations to an organism's stress response can increase susceptibility to depression development (Blackburn-Munro & Blackburn-Munro, 2001; Checkley, 1996; Chiba et al., 2012; Mineur, Belzung, & Crusio, 2006). In the subsequent sections, we outline stress signaling pathways and how stressors can physically modify stress signaling.

The amygdala signals the hypothalamus to activate the "fight or flight" response when stressors are detected by sensory receptors (Spiess, Rivier, Rivier, & Vale, 1981). The hypothalamus controls many automatic processes necessary for survival like breathing, heartbeat, and blood pressure (DiMicco, Abshire, Hankins, Sample, & Wible Jr, 1986; Pho et al., 2021; Spiess et al., 1981). The hypothalamus will immediately activate a "short term" and "long term" response following stress detection (Herman et al., 2011; Joëls & Baram, 2009). However, the "short term" response is designed for immediate action and is unsustainable for extended stress exposure. The second "long term" stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Herman et al., 2011). The paraventricular hypothalamic nucleus (PVN) signals the anterior pituitary gland with corticotropin-releasing hormone (CRH) to release adrenocorticotropic hormone (ACTH) that releases glucocorticoid release from the adrenal cortex (Herman et al., 2011; Makino, Smith, & Gold, 1995; McGill et al., 2006; Vahl et al., 2005). Cortisol is a glucocorticoid that ensures continued activation of the "long term" stress response and supresses digestion and immune activity (Danilenko, Plisov, Hebert, Krauchi, & Wirz-Justice, 2008; Izawa et al., 2007). Corticosterone

replaces cortisol as the primary "long term" stress hormone in most rodents (Ismail et al., 2013; Murack et al., 2021; K. B. Smith et al., 2021). The "long term" stress response is more impacted by chronic stress in humans and rodents and is often more relevant to mood disorder research (Chiba et al., 2012; Leistner & Menke, 2018; Lopez et al., 1998).

Cortisol and corticosterone regulate their own secretion by a negative feedback loop (Gjerstad, Lightman, & Spiga, 2018). Detection of glucocorticoids in the hypothalamus and pituitary gland reduces CRH and ACTH secretion and subsequent cortisol and corticosterone release from the adrenal glands (Dallmant & Yates, 1969; Herman et al., 2011). Rodents under baseline and stressful conditions display increased CRH expression in the PVN following removal of adrenal glands indicating an extended response to stress (Aguilera, Kiss, Liu, & Kamitakahara, 2007; Harbuz & Lightman, 1989). Reduced glucocorticoid receptor expression in the PVN also extends the stress response (Laryea, Schütz, & Muglia, 2013). Glucocorticoid receptors are expressed in almost every tissue as the primary regulator of stress detection (Gjerstad et al., 2018; Herman et al., 2011). Glucocorticoid receptors also regulate CRH release from the PVN by inhibiting signaling from areas like the hippocampus (McEwen, 2005) and the prefrontal cortex (Chiba et al., 2012). Assessment of blood glucocorticoid concentration, glucocorticoid receptor expression and activity are effective methods of evaluating the health and sensitivity of the HPA axis.

The mechanisms underlying HPA axis hyperactivity following chronic stress are roughly separated in two formats. Firstly, chronic stress can increase glucocorticoid release by desensitizing the glucocorticoid negative feedback loop (Armario, Hidalgo, & Giralt, 1988; Marquez, Nadal, & Armario, 2004; Natelson et al., 1988). Adult male Wistar rats exposed to chronic restraint stress or partial water submersion show resistance to corticosterone negative feedback following a dexamethasone suppression test (Mizoguchi et al., 2001). Chronic stress inhibits glucocorticoid negative feedback by either decreasing glucocorticoid receptor expression or sensitivity (Mizoguchi et al., 2001). Alternatively, chronic stress can increase the sensitivity of stress detection resulting in frequent HPA activations. Male rats exposed to chronic variable stress display increased excitation of CRH secreting neurons in the PVN, increased CRH

sensitization in the pituitary, and more frequent activation of the HPA axis (Franco et al., 2016). It is unclear why different chronic stressors elicit glucocorticoid receptor inhibition or stress sensitization. However, the result in both cases is increased total glucocorticoid exposure (Gallucci et al., 1993; Murack et al., 2021).

In summary, the "long term" stress response of the HPA axis is susceptible to enduring impairments following prolonged HPA activation (Albeck et al., 1997; Chiba et al., 2012). Acute social stressors and chronic stress decrease expression and impair sensitization of glucocorticoid receptors (Belda, Fuentes, Daviu, Nadal, & Armario, 2015; Franco et al., 2016). Impaired feedback loops and sensitized stress detection prolong glucocorticoid release (Gjerstad et al., 2018). Prolonged HPA axis activation is frequently observed during puberty and adolescent development (Blakemore et al., 2010; Holder & Blaustein, 2014; Kane & Ismail, 2017). As previously stated, puberty is driven by increased gonadal hormone secretion (Sisk & Foster, 2004; Tanner, 1962). In the next section, we will discuss HPA axis maturation and the implications of increased pubertal sensitivity to stress on gonadal hormone signaling.

3.1 Pubertal stress and critical periods of HPA axis development

HPA axis maturation begins prior to pubertal onset in a process call adrenarche (Holder & Blaustein, 2014). Prior to adrenarche, the HPA axes of humans, rats and mice is hyporesponsive displaying lower basal glucocorticoid and ACTH release to mild stressors (Holder & Blaustein, 2014; Murack et al., 2021; Pignatelli, Xiao, Gouveia, Ferreira, & Vinson, 2006; Spinedi, Chisari, Pralong, & Gaillard, 1997; Walker, Walder, & Reynolds, 2001). Humans at 6 - 8 years old, rats near postnatal day 14, and mice near postnatal day 12 begin to display progressive increases in basal and stress stimulated glucocorticoid release (Holder & Blaustein, 2014; Pignatelli et al., 2006; Spinedi et al., 1997; Walker et al., 2001). Glucocorticoid release increases naturally throughout puberty and early adolescence until it reaches levels typical of adulthood. Additionally, females display larger increases in glucocorticoid concentration following pubertal onset in mouse models (Schmidt et al., 2003). Increased HPA axis activity during puberty has enduring effects on gonadal signaling associated with pubertal, adolescent, and mood disorder development in female samples (Laroche, Gasbarro, Herman, & Blaustein,

2009a, 2009b). In the following section, we discuss the dynamic relationship between HPA and HPG axis development and how it promotes pubertal stress sensitivity to mood dysfunction.

Exposure to severe stress during puberty impairs the ability of brain cells to recognize gonadal hormones (Laroche et al., 2009a, 2009b). Following ovariectomy, injection of estrogens is necessary to recover sexual receptivity in female mice (Blaustein, 2008). Shipping stress at postnatal day 28, 35, and 42 was sufficient to block progesterone and estradiol – mediated recovery of female sexual receptivity in pubertal C57Bl/6 mice (Laroche et al., 2009a, 2009b). Ovarian hormone resistance was not observed before or after puberty in mice shipped at postnatal day 21 and 49, respectively. Moreover, mice shipped at postnatal day 42 displayed the greatest disturbances to sexual behavior (Laroche et al., 2009a, 2009b). It was concluded that pubertal female mice exposed to stress experience gonadal hormone resistance in the brain. This resistance implies that stress can directly inhibit gonadal maturation of sexual behavior during adolescence.

Pubertal male rodents also show reduced sexual behavior following shipping stress (Laroche et al., 2009b). Male mice shipped at postnatal day 42 display greater intromission latencies, fewer mounting attempts, and reduced response to testosterone in adulthood than males shipped after postnatal day 50 (Laroche et al., 2009b). Pubertal male rats exposed to social instability display decreased copulatory efficiency and reduced number of ejaculations in later adulthood than male mice shipped during adulthood (McCormick, Mongillo, & Simone, 2013). Pubertal exposure to stress therefore increases sex hormone resilience in both female and male mice associated with diminished sexually receptivity.

Increased HPA axis sensitivity and its obstruction to gonadal hormone mediation of sexual development seems limited to puberty and adolescence (Holder & Blaustein, 2014; Murack et al., 2021; Pignatelli et al., 2006; Spinedi et al., 1997; Walker et al., 2001). Early pubertal rodents display a more extended glucocorticoid and ACTH release than late adolescents and adults following electric shock or restraint stress (Goldman, Winget, Hollingshead, & Levine, 1973; Romeo, Lee, Chhua, McPherson, & McEwen,

2004; D. M. Vázquez & Akil, 1993). Increased PVN activity and CRH secretion is observed in adolescent rats and likely accounts for extended glucocorticoid secretion (Romeo et al., 2006). Early adolescent rats display less glucocorticoid feedback than adults when administered dexamethasone (Goldman et al., 1973). Increased and extended glucocorticoid exposure during a period of gonadal-driven brain maturation may constitute the mechanisms behind increased pubertal susceptibility to stress and pubertal depression development. In the following section, we further disseminate the relationship between stress signaling and depression development.

3.2 Stress and depression

Social stress and recent trauma correlates positively with depression development (Hammen, Davila, Brown, Ellicott, & Gitlin, 1992; Kessler et al., 1994; Monroe, Slavich, & Gotlib, 2014). The likelihood of adult depression development increases with greater episodic and chronic stress exposure during adolescence (Hammen et al., 1992; Monroe et al., 2014). Chronic stress exposure displays a greater correlation with depression than episodic stress (Hammen et al., 1992). Additionally, chronic stress and not episodic stress significantly correlates with earlier age of depression onset (Hammen et al., 1992). Investigations into the relationship between chronic stress and depression could elicit greater understanding of depression development and lead to alternate depression prevention or treatment methods.

The effect of chronic stress on depression development is also sexually dimorphic. Females are more susceptible to depression development following chronic stress than males (Gallucci et al., 1993; McCormick, Smythe, Sharma, & Meaney, 1995; Romeo, 2010). Adult female Wistar rats display greater blood corticosterone and less sucrose intake, open field movement, and hippocampal serotonergic signaling than males following chronic exposure to various mild stressors (Dalla et al., 2005; Wulsin, Wick-Carlson, Packard, Morano, & Herman, 2016). Sleep-disrupted adolescent female mice express greater serum corticosterone, hippocampal glucocorticoid receptor expression, and forced swim test immobility than adolescent males (Murack et al., 2021). Female vulnerability to chronic stress likely arises from increased access to estrogens (Green & McCormick, 2016). Estrogens typically increase the HPA axis response to stress (Goel,

Workman, Lee, Innala, & Viau, 2011). Gonadectomized female rats display a decreased release of corticosterone and ACTH and show normalization of HPA axis responses following estradiol treatment (Burgess & Handa, 1992; Kalil, Leite, Carvalho-Lima, & Anselmo-Franci, 2013; Weiser & Handa, 2009). Females may experience a greater predisposition to depression development partly due to increased estrogen mediated HPA activity. In the following section, we discuss how increased glucocorticoid exposure promotes signaling phenotypes associated with depression.

Depressive symptoms may arise from changes to the activation and structure of serotonergic neurons downstream from the dorsal raphe nucleus (Chiba et al., 2012; Lopez et al., 1998; McEwen, 2005; Mizoguchi et al., 2008). Areas like the hippocampus and medial prefrontal cortex receive downstream serotonergic projection (Leistner & Menke, 2018; Lopez et al., 1998; Mohammad-Zadeh et al., 2008). Additionally, the activity and structure of hippocampal and prefrontal neurons can be impaired by continued glucocorticoid stimulation (Drouin et al., 1993; Leistner & Menke, 2018; Lopez et al., 1998; Madalena & Lerch, 2017; McEwen, 2005). One week of daily immobilization can produce a continuous glucocorticoid elevation and dendritic atrophy in hippocampal CA3 pyramidal neurons (Watanabe, Gould, & McEwen, 1992). Chronic restraint stress reduces the expression of pyramidal and granule CA1 neurons associated with learning and memory (Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). Chronic restraint also reduces dendritic length and density in the medial prefrontal cortex (Cook & Wellman, 2004). Dysfunctional serotonergic signaling within the frontal and limbic systems may arise from chronic stress exposure

The function of 5-HT_{1A} receptors implicated in depression development is modified by glucocorticoid modulation (Bosker, Klompmakers, & Westenberg, 1997; Fairchild et al., 2003; Joels, 2001; Meijer, Williamson, Dallman, & Pearce, 2000; Mohammad-Zadeh et al., 2008; Richardson-Jones et al., 2010). Acute corticosterone injection or exposure to acute stress decreases CA1 pyramidal cell sensitivity to 5-HT as demonstrated by greater hyperpolarization following 5-HT_{1A} activation (Hesen & Joels, 1996). Adrenalectomized rats with diminished corticosterone or after a glucocorticoid antagonist also express 5-HT insensitivity (Hesen & Joels, 1996). Low corticosterone

concentrations known to activate mineralocorticoid receptors in the hippocampus suppress these changes (Hesen & Joels, 1996). Selective activation and knockout studies of glucocorticoid and mineralocorticoid receptors implicate that activation of both receptor types seem necessary to reduce 5-HT_{1A} expression (Hesen & Joels, 1996; Ou, Storring, Kushwaha, & Albert, 2001). Ou, Storring, Kushwaha, and Albert discovered a glucocorticoid response element (GRE) on the 5-HT_{1A} promotor gene sequence in human, mouse, and rat samples (Ou et al., 2001). Heterodimerization of glucocorticoid and mineralocorticoid receptors following corticosterone exposure actively blocks this 5-HT_{1A} promotor sequence and reduces 5-HT_{1A} expression. Direct glucocorticoid suppression of postsynaptic 5-HT_{1A} expression in the hippocampus and medial prefrontal cortex may initiate development of depression symptoms. Moderation of glucocorticoid receptor expression and activation may therefore directly contribute to inhibition of genes needed for healthy serotonergic function.

An overwhelming number of investigations suggest hypothalamic and pituitary glucocorticoid receptors that regulate glucocorticoid negative feedback are desensitized in major depression disorder (Leistner & Menke, 2018). Glucocorticoid feedback desensitization in individuals with major depression was determined by reduced suppression of cortisol following dexamethasone testing (Calfa et al., 2003; Lowy, Reder, Gormley, & Meltzer, 1988; J. C. Nelson & Davis, 1997), reduced ACTH and unaffected cortisol after CRH testing (Holsboer, Von Bardeleben, Gerken, Stalla, & Müller, 1984), and elevated ACTH and cortisol levels following combination dexamethasone-CRH testing (Kaestner et al., 2005). However, individuals with major depression occasionally display increased glucocorticoid receptor sensitization similar to our findings (Levitan, Vaccarino, Brown, & Kennedy, 2002; Miller, Rohleder, Stetler, & Kirschbaum, 2005). Furthermore, work-related exhaustion studies tenuously associate sleep disorders and depressive symptoms with glucocorticoid receptor sensitization (Menke et al., 2014; Pruessner, Hellhammer, & Kirschbaum, 1999; Sonnenschein et al., 2007). Different sources of depression may alter HPA activity in unique ways.

To summarize, dysregulation of glucocorticoid signaling is observed together with several types of depression disorders (Leistner & Menke, 2018). Binding of

neuronal glucocorticoid receptors may directly inhibit gene promotor sequences controlling expression of hippocampal and prefrontal 5-HT receptors (Ou et al., 2001). Studies of exhaustion and depression indicate fatigue may promote depression development by impairing HPA axis activity (Menke et al., 2014; Pruessner et al., 1999; Sonnenschein et al., 2007). In the following section, we review some aspects of sleep and discuss how depression and adolescent maturation affect sleep habits.

4.0 Sleep and brain signaling pathways

Sleep is a reversable and repeating biobehavioral state defined by altered consciousness, reduced mobility, and limited perception of surrounding environment (Roebuck et al., 2013; Tubbs, Dollish, Fernandez, & Grandner, 2019). To fulfil the drive for sleep, most healthy adults require 7 to 8 hours of rest (Hirshkowitz et al., 2015). Sleep is considered a healthy behavior as its deprivation impairs many physiologically necessary systems like metabolism, stress regulation and emotional regulation (Borbély & Wirz-Justice, 1982; da Silva Rocha-Lopes, Machado, & Suchecki, 2018; Murack et al., 2021; Novati, Hulshof, Koolhaas, Lucassen, & Meerlo, 2011; Y. Tang, Preuss, Turek, Jakate, & Keshavarzian, 2009; Vetrivelan, Fuller, Yokota, Lu, & Saper, 2012). Sleep deficits occur when opportunities and/or ability to sleep are impaired (Tubbs et al., 2019). Modification of sleep processes can be objectively recorded by assessing changes to central neuron activation patterns (Roebuck et al., 2013). In the following section, the progression of sleep is presented, and healthy sleep architecture is discussed.

Sleep is organized into stages based on standardized scoring of neuronal electrical activity using electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) recordings (Roebuck et al., 2013). Electrodes are placed near areas of bioelectric fluctuations caused by firing neurons or muscular activity and record changes in their electromagnetic fields (Benbadis, 2015). Wakefulness is partially defined by brain activity characterized by disorganized high amplitude and high frequency EEG recordings (Wright Jr, Badia, & Wauquier, 1995). Sleep is staged by changes in neuronal activity as recorded by EEG (Berry et al., 2017; Berry et al., 2012).

Non-rapid eye movement (NREM) sleep encompasses the first three stages of the sleep cycle following sleep onset as defined by the American Academy of Sleep

Medicine (Berry et al., 2017; Berry et al., 2012). EEG recordings display increased wave amplitude and decreased wave frequency as sleep stages progress (Berry et al., 2012). Stage 1 sleep displays alpha activity or 8-12 Hz brain waves. Theta waves emerge as 4-7 Hz neural activity. Slight eye rolling and reduced muscle activity are also observed in stage 1 (Berry et al., 2012; Picchioni et al., 2008). Stage 2 sleep displays k-complex and spindles. K-complexes show transient large amplitude burst of activity (Bankman, Sigillito, Wise, & Smith, 1992). Spindles refer to 12-15 HZ activity that lasts 0.5-2 seconds in duration. Muscle activity continues to decrease, and eye movements are absent. Stage 3 or slow wave sleep shows delta waves at 1 Hz with high amplitude (Berry et al., 2012; Bes, Jobert, Muller, & Schulz, 1996; Dijk, Groeger, Stanley, & Deacon, 2010). Slow wave sleep relieves theoretical "sleep pressure" that accumulates during the day and coincides also with maintenance and repair of the body (Jenni, Achermann, & Carskadon, 2005). Rapid eye movement (REM) sleep is a period of rest that displays sudden increases in neural activity and an absence of motor activity excluding eye and diaphragm muscles (Berry et al., 2012). 5-HT inhibits REM activity and slowly decreases as sleep progresses (Armitage, 1996; Imeri, Mancia, Bianchi, & Opp, 1999). Sleep processes interchange between NREM and REM stages with decreasing durations of slow wave sleep and increasing durations of REM as sleep progresses (Roebuck et al., 2013; Tubbs et al., 2019). In short, we can categorize sleep stage by grouping neuronal and muscular activity. However, the initiation and termination of sleep is controlled by a variety of hypothesized pressures.

The Two Process Model of Sleep Regulation states that sleep propensity facilitates sleep cycle scheduling (Borbély, 1982). Sleep propensity is a combination of the inherent/homeostatic drive to sleep (S process) and the internal biological clock (C process). The "S process" reflects the gradual accumulation of chemical substances that promote a drive for sleep (Bourdon et al., 2018). Adenosine concentration increases as time awake is prolonged and positively correlates with increased sleep desire (Bourdon et al., 2018; Radulovacki, Virus, Djuricic-Nedelson, & Green, 1984; Silva et al., 2004). Alternatively, melatonin begins to increase as light exposure lessens near nightfall and begins to decrease in the latter half of the sleep phase (De Crescenzo et al., 2017; Robillard, Naismith, Rogers, Scott, et al., 2013; Shanahan & Czeisler, 1991). Slow wave

sleep relieves sleep pressure accumulated through the S process; however, the underlying mechanisms of sleep substance dissipation are not well understood (Berry et al., 2012; Bes et al., 1996; Dijk et al., 2010).

Circadian rhythm or the "C process" refers to the cyclic order of biochemical and behavioral changes like immune activity or feeding schedules that many organisms display in reference to a ~24-hour internal clock (Carskadon & Acebo, 2005; Crowley, Acebo, & Carskadon, 2007; Moore, 1983). Circadian rhythm is partially controlled by the suprachiasmatic nucleus of the hypothalamus (SCN) that signals individual cells with proteins like KaiA, KaiB, and KaiC (Benedetti, Fresi, Maccioni, & Smeraldi, 2008; Wright, Hughes, Kronauer, Dijk, & Czeisler, 2001). The SCN is reset daily by melatonin secretion following changes to light or dark exposure. Circadian drift gradually occurs without melatonin exposure (Benedetti et al., 2008; Wright et al., 2001). Sleep onset occurs when the increasing homeostatic drive for sleep exceeds the decreasing circadian drive for wakefulness (Borbély, 1982). As sleep progresses, substances believed responsible for sleep drive like adenosine and melatonin gradually decrease as sleep progresses (Borbély, 1982). The drive for circadian wakefulness slowly increases during sleep and eventually exceeds the declining drive for sleep leading to sleep termination. Delayed sleep onset, shallow sleep, and early sleep termination may arise from impaired sleep drive or circadian wakefulness (Borbély, Tobler, & Wirz-Justice, 1981; Borbély & Wirz-Justice, 1982). Diminished sleep drive or increased circadian drive for wakefulness during periods that organisms normally demonstrate greater sleep drive than circadian wakefulness could result in delayed sleep onset, shallow sleep, or early sleep termination. The pressures for sleep and wakefulness are often calculated according to the interactions between sleep drive and circadian rhythm driving wakefulness, sleep onset, sleep stage, and sleep termination. It is further hypothesized that psychological disorders involving improper sleep may result from dysregulation of the drive for sleep and circadian rhythm mechanisms.

In summary, sleep is a process characterized by variations in central neuronal and motor activity and controlled by the interaction between a drive for sleep and pre-existing circadian rhythms (Borbély, 1982; Borbély & Achermann, 1999). Sleep is essential to

many physiological processes, and its disruption leads to mood impairments (Borbély & Wirz-Justice, 1982; Gillin, Duncan, Pettigrew, Frankel, & Snyder, 1979). In the following section, we examine the relationship between sleep and depression.

4.1 Sleep and depression

People with depression often display a delayed sleep onset, early termination of sleep, and sleep fragmentation following repeated awakenings (Borbély & Wirz-Justice, 1982; Gillin et al., 1979). Stage 3 or slow wave sleep duration is reduced in depression (Borbély, 1982; Borbély & Wirz-Justice, 1982; Gillin et al., 1979; Gillin et al., 1981; Hauri, Chernik, Hawkins, & Mendels, 1974). Total REM duration is typically longer in people with depression (Borbély & Wirz-Justice, 1982; Gillin et al., 1979; Gillin et al., 1981). However, increased REM duration is often limited to the first REM period (DJ Kupfer, 1976; D Kupfer, Harrow, & Detre, 1969). Furthermore, latency to the first REM period is often shortened in people with depression. In this section, we summarize different theories explaining why sleep processes change during depression and whether a causal mechanism exists between depression and poor sleep.

Impaired sleep need or the drive for sleep (process S) may disrupt sleep during depression (Borbély, 1982; Borbély & Wirz-Justice, 1982; Bourdon et al., 2018). According to the Two Process model of sleep, sleep onset occurs when increasing sleep drive exceeds the decreasing circadian drive for wakefulness (Borbély, 1982). Consequently, sleep termination occurs when the circadian drive for wakefulness is greater than the drive for sleep. Theoretically, impairments causing sleep drive reduction or increased circadian wakefulness during the early rest phase would promote delayed sleep onset, shallow sleep or even early sleep termination. (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981; Borbély, Tobler, et al., 1981). Delayed sleep onset, shallow sleep, and earlier sleep termination are typical symptoms of depression disorders and may result from impaired sleep drive and circadian rhythms (Borbély, Baumann, et al., 1981; Borbély, Tobler, et al., 1981). People with depression demonstrate shortened slow wave duration associated with reduced sleep drive (Borbély, Baumann, et al., 1981; Borbély, Tobler, et al., 1981). Furthermore, young adults with depression display earlier and reduced peaked melatonin levels than healthy controls which may impair circadian

rhythm timing (Beck-Friis et al., 1985; Robillard, Naismith, Rogers, Scott, et al., 2013; Rubin, Heist, McGeoy, Hanada, & Lesser, 1992). However, conflicting publications have not reached a consensus (De Crescenzo et al., 2017; Robillard, Naismith, Rogers, Scott, et al., 2013). For example, Robillard and colleagues reported one third of their depressed young adult sample did not display early endogenous dim light melatonin onset as hypothesized (Robillard, Naismith, Rogers, Scott, et al., 2013). Regardless, depression is well correlated with sleep disturbances.

The anti-depressant effect of acute sleep deprivation further complicates the relationship between depression and sleep. As sleep deprivation increase exhaustion and stress, sleep loss should logically promote the development of depression-like symptoms (Borbély, Baumann, et al., 1981; Borbély, Tobler, et al., 1981; Borbély & Wirz-Justice, 1982). However, acute sleep disruption reliably creates a temporary antidepressant effect in ~60% of people with depression (Giedke & Schwärzler, 2002; Rudolf & Tölle, 1978). Total sleep deprivation must exceed 19-20 hours before an antidepressant effect can be observed (Knowles et al., 1979). Additionally, the antidepressant effect of total sleep deprivation is transient and typically disappears by the next sleep cycle. Acute sleep deprivation was therefore insufficient for investigating the depressive effects of sleep loss.

In summary, the relationship between sleep architecture and depression is dynamic. Regulation of REM sleep influences depressive symptoms (Borbély, 1982; Borbély & Wirz-Justice, 1982; Gillin et al., 1979; Gillin et al., 1981; Hauri et al., 1974). However, alternate factors may be responsible for the depression/sleep relationship. Signaling pathways for sleep, depression and stress all change throughout puberty and adolescence (Crowley et al., 2007; Crowley, Wolfson, Tarokh, & Carskadon, 2018; Green & McCormick, 2016; Hankin et al., 1998). Pubertal and adolescent sleep development may increase susceptibility to depression development.

4.2 Adolescent sleep

Average sleep habits and schedules begin to mature just prior to puberty and shift as adolescence progresses (Carpenter, Robillard, & Hickie, 2015; Carskadon, 2011; Crowley et al., 2018). Pubertal boys and girls display average sleep onsets between 9-

930 PM (Carskadon, 1990, 2011; Crowley et al., 2018; Pearson, Johnson, & Nahin, 2006). Timing of sleep onset increases throughout mid- and late adolescence until sleep patterns resemble adult sleep schedules (Carskadon, 1990; Robillard et al., 2014; Wolfson et al., 2003). The maturation of sleep patterns during adolescence is termed "juvenile phase delay" (Carskadon, 2011). Adolescents exposed to an increased sleep onset latency on non-work evenings display increased HPA activity and glucocorticoid release (Wittmann, Dinich, Merrow, & Roenneberg, 2006). Sleep onset is further delayed in adolescents and young adults with unipolar depression (Robillard et al., 2015; Robillard, Naismith, Rogers, Ip, et al., 2013; Robillard et al., 2014). In this section, we review the driving forces behind juvenile phase delay and how natural sleep phase shifting may increase adolescent susceptibility to depression development.

Maturation of sleep drive and circadian rhythm likely precipitate maturation of adolescent sleep architecture (Carskadon, 2011; Crowley et al., 2018). As previously mentioned, sleep onset and rise times are potentially governed by the interaction between an increasing and decreasing sleep drive and circadian rhythm (Borbély, 1982; Borbély, Baumann, et al., 1981; Borbély, Tobler, et al., 1981; Borbély & Wirz-Justice, 1982). Evidence suggests that circadian rhythms may temporarily lengthen during adolescence (Carskadon, 2011; Crowley et al., 2018). Adolescents aged 9-15 years old typically display a complete circadian rhythm cycle every 24.27 -24.35 hours (Carskadon & Acebo, 2005). Adult circadian cycles are shorter with average cycling occurring every 23.88 - 24.12h (Wright et al., 2001). Peaks in melatonin concentration become delayed during human adolescence and correlate with lengthening circadian rhythm durations (Carskadon, Acebo, Richardson, Tate, & Seifer, 1997). A longer circadian phase prolongs the need for wakefulness which potentially delays sleep onset. Circadian phase shifts may occur in adolescence as an effect of light entrainment from increased exposure to evening activities or even LED screens on phones, computers, and TVs (Baehr, Revelle, & Eastman, 2000; Shanahan & Czeisler, 1991).

The accumulation of the drive for sleep or "S process" also matures in adolescence (McGinnis, Lumia, Tetel, Molenda-Figueira, & Possidente, 2007). Pre-pubertal children and early adolescents display smaller increases to compensatory slow

wave activity following 24 h sleep deprivation than older adolescents (Jenni et al., 2005; Jenni & Carskadon, 2004; Tarokh, Carskadon, & Achermann, 2012). Slow wave activity is associated with an increased drive for sleep and increases following sleep deprivation (Bes et al., 1996). Younger adolescents may experience lower increases in sleep drive following sleep deprivation than older adolescents. Reduced compensatory increases to sleep drive in conjunction with longer circadian rhythms may allow younger adolescents to delay their sleep onset permanently without subsequent sleep rebound. Further research is required to elucidate the maturation of adolescent sleep drive and circadian processes.

Rodent models can be used to investigate sleep onset delay during adolescence (Hagenauer, Perryman, Lee, & Carskadon, 2009). Male and female rats show between 1 - 4 hours of sleep delay when entering adolescence (Hagenauer et al., 2009; McGinnis et al., 2007). Like humans, both male and female rats express an extended circadian rhythm duration in adolescence compared to adulthood (McGinnis et al., 2007). Mice also display sleep onset delay upon entering adolescence (Weinert, Eimert, Erkert, & Schneyer, 1994). Early adolescent mice even model the human tendency towards impaired sleep compensation following deprivation (A. B. Nelson, Faraguna, Zoltan, Tononi, & Cirelli, 2013). Early adolescent mice display less compensatory slow wave activity following 4-hours of sleep deprivation compared to late adolescent mice (A. B. Nelson et al., 2013). Therefore, rodent models can allow more invasive avenues of research into sleep onset delay observed during adolescence.

Post-mortem animal studies show impaired HPA axis signaling following chronic sleep disruption during puberty and adolescence when compared to non-sleep disrupted controls (Azogu et al., 2015; da Silva Rocha-Lopes et al., 2018; Murack et al., 2021). Adolescent male rats exposed to 21 days of REM sleep restriction display greater basal corticosterone concentrations, glucocorticoid receptor expression and increased anxiety-like behavior than rested counterparts (da Silva Rocha-Lopes et al., 2018). Chronic, but not acute, 4- hour sleep disruption in adolescent male rats increased glucocorticoid expression compared to non-sleep disrupted samples (Azogu et al., 2015). Early adolescent (postnatal day 42) and adult female (postnatal day 70) mice exposed to eight

days of 4-hour sleep disruption showed increased blood corticosterone and hippocampal glucocorticoid receptor expression than adults after exposure to a 30-minute novel restraint stressor (Murack et al., 2021). However, 3 hours of chronic sleep disruption was not sufficient to increase glucocorticoid release in adolescent male mice (Saré et al., 2019; Weibel, Follenius, Spiegel, Ehrhart, & Brandenberger, 1995). It is likely that mice require a longer duration of sleep restriction for HPA axis reactivity to be affected. Alternatively, low duration of sleep restriction may be sufficient in increasing HPA reactivity to a novel stressor only (Murack et al., 2021). While extended sleep disruption may predispose adolescent HPA axes to greater activity, it is unclear how this effect occurs.

The mechanisms underlying sleep and stress interactions are influenced by circadian rhythm (Van Reeth et al., 2000). Specific circadian phases therefore correlate with regular changes to HPA axis activity. For example, humans experience inhibited ACTH secretion during early sleep (Weibel et al., 1995; Weitzman, Zimmerman, Czeisler, & Ronda, 1983). ACTH secretion signals the release of cortisol. Delayed sleep onset or awakenings mid-sleep subsequently increases ATCH and cortisol secretion (Van Cauter, Van Coevorden, & Blackman, 1990). Young adult males display increased rates of plasma cortisol secretion during complete evening sleep deprivation when compared to normal night sleep (Weibel et al., 1995). Additionally, daytime sleep following evening sleep deprivation does not significantly inhibit plasma cortisol. Sleep deprivation may increase total daily cortisol secretion regardless of compensatory sleep. Cortisol secretion also follows a circadian rhythm as it is significantly suppressed by sleep that occurs only during night rest.

Spontaneous awakenings and partial sleep deprivation also influence cortisol secretion (Leproult, Copinschi, Buxton, & Van Cauter, 1997; Weibel et al., 1995). Plasma cortisol increases in young adult males 10 to 20 minutes following provoked or spontaneous awakenings during evening or morning compensatory sleep (Weibel et al., 1995). Furthermore, previous partial and complete sleep deprivation effects future circadian-controlled cortisol release (Leproult et al., 1997). Both partial 4-hour and complete sleep deprivation in young adult males increases plasma cortisol the following

day up to 6 hours prior to sleep onset (Leproult et al., 1997; Spiegel, Leproult, & Van Cauter, 1999). Early morning increases to cortisol expected after normal sleep and awakening is not curtailed by attempts to make up for sleep loss by late morning sleep following partial or complete sleep disruption. Additionally, partial and complete sleep disruption delayed cortisol inhibition by one hour during the following night of normal sleep (Leproult et al., 1997). Both partial and complete sleep deprivation clearly increases cortisol in both the short and prolonged term. Repeat delays to adolescent sleep onset may impair HPA axis inhibition by repeatedly increasing cortisol excretion.

It is unclear if adolescent delayed sleep onset resulting from maturing sleep drive and circadian rhythms behave similarly to sleep deprivation and increase release of cortisol. Finding comparative controls for a natural process like juvenile phase delay would be difficult in human samples. However, comparing cortisol of animal models that experience juvenile phase delay to those that do not may elucidate the effect of sleep maturation on cortisol. Regardless, periods of partial sleep deprivation and subsequent increases to cortisol release increase in prevalence during adolescence due to increased social engagements and weekend peer events (Wittmann et al., 2006). Repeated adolescent sleep deprivations may prolong HPA axis activation and increase the risk of depression development (Gjerstad et al., 2018). Identifying methods to limit significant sleep disruption could limit extended glucocorticoid release. One of the many roles for sleep is the conservation of cellular energy expenditure. In the next section, we review how brain activity is fueled and whether shifts in metabolism can be exploited to promote regular sleep patterns during adolescence.

5.0 Metabolism: Glucose and lactate

Mammals rely on the metabolism of glucose or its derivatives to provide the necessary energy to fuel biological function (Aronoff, Berkowitz, Shreiner, & Want, 2004; Nakrani, Wineland, & Anjum, 2020). Neurons in human brains consume approximately 25% of circulating glucose under healthy resting conditions but account for only 2% of total body weight (Berg, Tymoczko, & Stryer, 2002; L Pellerin, 2010). The metabolism of glucose and its related biomolecules fuel the mechanisms underlying sleep, sexual maturation, and depression (S. Arslanian & Suprasongsin, 1997; Baxter et

al., 1989; Poggiogalle, Jamshed, & Peterson, 2018). In this section, we will discuss how the metabolism of glucose and its byproducts fuel cellular activity, how metabolic pathways mature during puberty and adolescence, and how metabolism may be influenced to improve sleep and depressive symptoms.

Glucose is a 6-carbon monosaccharide central to energy consumption in plants, animals, and bacteria (Aronoff et al., 2004; Nakrani et al., 2020). Glucose can be synthesized from major biomolecules like proteins, carbohydrates, and lipids to be used for cellular energy production or a variety of other tasks (Bagherzadeh-Yazdi et al., 2021; Nyholm & Pascher, 1993; Okita, Rodriguez, & Preiss, 1981). Some of the macronutrients are broken down into glucose within the gastrointestinal tract and are released in the circulatory system (Goto et al., 2012). Glucose is absorbed into cells and used to create adenosine triphosphate (ATP) (Collins-Nakai, Noseworthy, & Lopaschuk, 1994). ATP is the energy currency of the cell fueling many major cellular functions within animals. Maximal conversion of glucose to ATP is a multi-step process (Boiteux & Hess, 1974; Chaudhry & Varacallo, 2018; Frey & Arabshahi, 1995). Glucose is first converted to pyruvate within the cytosol of a cell in a process called glycolysis (Chaudhry & Varacallo, 2018). Pyruvate either enters the mitochondria to create ATP through aerobic respiration or is used to synthesize lactate after exposure to lactic dehydrogenase (Boiteux & Hess, 1974; Chaudhry & Varacallo, 2018; Frey & Arabshahi, 1995).

Lactate is the base conjugate form of lactic acid and is observed in high concentrations following ischemia (Combs, Dempsey, Maley, Donaldson, & Smith, 1990) and lactic acid fermentation (Bangsbo, Johansen, Graham, & Saltin, 1993). Lactate has both an L- and D- enantiomer configuration. Most mammals express a lactic hydrogenase enzyme that readily metabolizes L-lactate (Holbrook, Liljas, Steindel, & Rossmann, 1975). However, enzymes that readily metabolize D-lactate are in low amounts in mammals. As a result, L-lactate is more easily metabolized (Freminet & Poyart, 1975; Garcia, Goldstein, Pathak, Anderson, & Brown, 1994). Evidence suggests that L-lactate is also produced within the central nervous system to provide for the brain’s heightened energy requirements (Magistretti & Allaman, 2018).

5.1 Astrocyte Neuron Lactate Shuttle Hypothesis

The energy demand of the brain arises from the firing of neurons needed to regulate behavior, cognition, and basic life function (Berg et al., 2002). Periods of increased activity or trauma increase neuronal demand for glucose (Cohen, Ugurbil, & Kim, 2002; Osei). The human brain compensates for this demand by increasing localized cerebral blood flow to active brain neurons (Cohen et al., 2002; Osei). However, the neuronal uptake of L-lactate in both basal and high activation conditions indicate a need for sources of energy in addition to glucose (Berthet et al., 2009; Combs et al., 1990; Larrabee, 1995, 1996; D. Smith et al., 2003). If true, active human neurons may produce cellular energy from alternate sources like L-lactate (Magistretti & Pellerin, 1996).

Astrocytes provide both structural and metabolic support to surrounding neurons (Hertz, Dringen, Schousboe, & Robinson, 1999; Magistretti & Pellerin, 1996). The Astrocyte Neuron Lactate Shuttle (ANLS) hypothesis states that astrocytes increase glucose absorption, rate of glycolysis, and extracellular lactate secretion to provide additional energy to brain neurons (Magistretti & Pellerin, 1996). The extensive history of the ANLS hypothesis including its many challenges have been reviewed in greater detail elsewhere (Magistretti & Allaman, 2018). However, several subsequent investigations support increased glucose uptake and preferential production of lactate within astrocytes (Allaman, Fiumelli, Magistretti, & Martin, 2011; Hamberger & Hydén, 1963; Hydén & Lange, 1962; Janzer & Raff, 1987; Magistretti & Pellerin, 1999; Nehlig, Wittendorp-Rechenmann, & Dao Lam, 2004; Luc Pellerin & Magistretti, 1994).

Astrocytes may control a larger portion of cerebral glucose flow then previously expected. The blood brain barrier is partly composed of astrocyte end-feet that cover capillaries (Janzer & Raff, 1987). As a result, astrocyte uptake of blood glucose occurs at a near equal rate to neurons under basal conditions (Allaman et al., 2011; Janzer & Raff, 1987; Magistretti & Pellerin, 1999; Nehlig et al., 2004; Luc Pellerin & Magistretti, 1994). Studies even suggest that much of the increased glucose uptake following functional activation occurs at astrocytes (Chuquet, Quilichini, Nimchinsky, & Buzsáki, 2010; Magistretti, 2009; Voutsinos-Porche et al., 2003). However, astrocytes only account for approximately 15% total brain glucose metabolism (Attwell & Laughlin, 2001; Rothman,

Behar, Hyder, & Shulman, 2003). Therefore, excess astrocytic glucose taken from cerebral blood is most likely released into extracellular space or repackaged for neuronal uptake (Janzer & Raff, 1987).

Glucose repackaging within astrocytes may take the form of glucose conversion to lactate via glycolysis (Hertz et al., 1999; Magistretti & Pellerin, 1996). As previously mentioned, glycolysis metabolizes glucose into pyruvate which is either processed in the mitochondria to create ATP or synthesized to make lactate and fewer ATP under hypoxic or anoxic conditions (Magistretti & Allaman, 2018). Astrocytes have limited access to enzymes specific to pyruvate metabolism and can more easily perform reductive conversions that thermodynamically favour pyruvate conversion to lactate (Hamberger & Hydén, 1963; Hydén & Lange, 1962; Magistretti & Allaman, 2015). Enzyme limitations and increased reductive activity results in astrocytes displaying a lactate:pyruvate ratio of 10:1, comparable to skeletal muscles during exercise (Brooks, 2020; Thevenet et al., 2016; Yellen, 2018). These combined characteristics support the possibility of lactate production in astrocytes, but do not provide evidence of an energy transport system to surrounding neurons.

Evidence of a lactate gradient has been observed between neurons and astrocytes (Demyttenaere et al., 2004; Mächler et al., 2016). Multiple *in vitro* studies support an astrocyte-neuron lactate shuttle (Chuquet et al., 2010; Luc Pellerin & Magistretti, 1994; Sada, Lee, Katsu, Otsuki, & Inoue, 2015; Weber & Barros, 2015; Zimmer et al., 2017). Double patch clamping of mouse pyramidal CA1 neurons and adjacent astrocytes showed that lactate is synthesized and released by astrocytes when exposed to a glucose-rich incubation medium (Sada et al., 2015). Blocking the lactate formation of astrocytes within a glucose-rich incubation medium hyperpolarized nearby pyramidal CA1 neurons (Sada et al., 2015). Pyramidal hyperpolarization suggests lactate formation within adjacent astrocytes was necessary for optimal pyramidal CA1 activation. Replacing a glucose-rich incubation medium with a lactate-rich one restored the pyramidal CA1 neuron's ability to produce action potentials. *In vivo* observations of the ANLS are more limited (Mächler et al., 2016; Magistretti & Allaman, 2018). Within-cell lactate concentrations may be observed by imaging nanosensors with a photon microscope

(Mächler et al., 2016). *In vivo* stimulation of lactate transport protein monocarboxylate transport protein 1 (MCT1) within the astrocyte and neurons of live mice revealed a decrease in astrocytic lactate and a delayed increase in neuronal lactate (Mächler et al., 2016). Lactate released from astrocytes into extracellular space seemed to be absorbed by surrounding neurons. However, these findings are relatively recent and require additional *in vivo* research before the ANLS hypothesis is universally accepted.

5.2 Neuron Astrocyte Lactate Shuttle Hypotheses

The Neuron Astrocyte Lactate Shuttle (NALS) hypothesis proposes an opposite flux of lactate within the brain presented by ANLS theories (Mangia, Simpson, Vannucci, & Carruthers, 2009). Neurons may metabolize glucose into pyruvate for synthesis of ATP or lactate. The NALS hypothesis implies that lactate is shuttled from neurons to adjacent astrocytes for disposal through the blood brain barrier or nearby ventricles (Dienel, 2012; Mangia et al., 2009). Evidence of the NALS hypothesis consists mostly of equations modelling cytosol kinetics, thermodynamics, and mitochondrial redox reactions that favour neuron to astrocyte transport (Mangia et al., 2009). However, it should be noted that the ANLS hypothesis has received similar mathematical modelling support in more recent years (Aubert & Costalat, 2005).

Lactate synthesis and transport within the brain may represent a necessary component for satisfying the brain's high energy demand (Cohen et al., 2002; Magistretti & Pellerin, 1996; Osei). Our current understanding of metabolism, absorption kinetics, and neuronal activity indicate glucose cannot be the only source of energy for the brain when highly active or damaged (Chuquet et al., 2010; Magistretti, 2009; Patricia, Raymond, Milot, Merali, & Plamondon, 2014; Voutsinos-Porche et al., 2003). Alternate sources of energy like lactate likely help meet this energy demand. Energy demand typically increases during periods of growth like puberty and adolescence (Joslowski et al., 2012). In the next section, we review how glucose metabolism is disrupted by adolescent maturation.

5.3 Adolescent metabolism

The absorption of cellular metabolic fuels is altered by puberty and adolescent maturation (Caprio et al., 1994; Joslowski et al., 2012). Cellular uptake of biomolecules like glucose, the amino acid leucine, and free chain fatty acids require exposure to the pancreatic hormone insulin (Caprio et al., 1994; Joslowski et al., 2012). Human cells display partial resistance to insulin and reduced glucose uptake during adolescence (Amiel et al., 1991; Caprio et al., 1994). Adolescent human males and females display as high as 40% reduction in the rate of insulin-mediated glucose uptake compared to adult male and females (Caprio et al., 1994). Cellular L-lactate absorption by MCT1 and MCT4 receptors does not require insulin (Park et al., 2018). However, lactate metabolism does mature throughout the human lifetime (Beneke, Hutler, Jung, & Leithauser, 2005; Riddell, 2008). Prepubescent humans display a reduced L-lactate concentration within peripheral blood following exercise than both adolescents and adults (Beneke et al., 2005). Prepubescents continue to display reduced blood L-lactate when controlling for different exercises, test sites and assays (Beneke et al., 2005). It is unclear if L-lactate glycolysis matures during adolescence (Riddell, 2008). However, changes to hormone production during adolescence may account for metabolic impairments following puberty.

Hormones that display greater secretion during puberty and adolescence are also associated with increasing cellular resistance to insulin (S. Arslanian & Suprasongsin, 1997; S. A. Arslanian & Kalhan, 1994). Growth hormone signals lipolysis or metabolism of free fatty acids for energy use (S. Arslanian & Suprasongsin, 1997; S. A. Arslanian & Kalhan, 1994; Snyder, Clemmons, & Underwood, 1988). Lipolysis competes with glucose metabolism, decreasing glucose uptake and increasing insulin resistance (S. Arslanian & Suprasongsin, 1997; S. A. Arslanian & Kalhan, 1994; Snyder et al., 1988). Growth hormone secretion increases prior to pubertal onset and throughout adolescence (d'Anglemont de Tassigny & Colledge, 2010; J.-H. Lee et al., 1996; J. T. Smith & Clarke, 2007; K. B. Smith et al., 2021). Increased growth hormone release during puberty may be partly responsible for adolescent insulin resistance and glucose intolerance.

Alternatively, leptin also signals lipolysis and reduces glucose absorption during adolescence (Manfredi-Lozano, Roa, & Tena-Sempere, 2018; M. J. Vázquez, Romero-Ruiz, & Tena-Sempere, 2015). Leptin is labelled a "satiety" hormone necessary to regulate the feeling of hunger (Casanueva & Dieguez, 1999). However, leptin secretion is also integral for signaling pubertal onset (M. J. Vázquez et al., 2015). Leptin concentration must reach a threshold before pubertal onset is attained (M. J. Vázquez et al., 2015). Leptin ensures that sexually immature organisms can be alerted to increased sustenance requirements during pubertal development and pregnancy (Ruscica et al., 2016; M. J. Vázquez et al., 2015). Leptin deficiencies after malnutrition and anorexia are associated with delayed pubertal onset, especially in women (Ruscica et al., 2016). Therefore, hormonal mediation of metabolic maturation may be sexually dimorphic.

Mammalian males and females have evolved to adapt separate metabolic strategies that become more salient following puberty (Mauvais-Jarvis, 2015; M. J. Vázquez et al., 2015). Males display greater blood glucose concentration and reduced fat formation following consumption of sustenance (Anderwald et al., 2011; Murack & Messier, 2019; M. J. Vázquez et al., 2015). Traditionally, male metabolic strategies ensure that glucose is readily available to fuel cells immediately for combat, exercise, etc. Alternatively, females more readily convert glucose to fat which can accumulate to signal both early puberty and/or pregnancy (Mauvais-Jarvis, 2015). Females therefore express lower blood glucose concentrations following feedings (Anderwald et al., 2011). Evidence suggests adolescent insulin resistance affects human males and females equally (Caprio et al., 1994).

L-lactate concentration seems largely sex independent (Beneke et al., 2005; Chen et al., 2018; Zhang & Ji, 2016). Pubertal, adolescent, and young adult human females and males display similar blood lactate concentrations during basal conditions, during exercise, and while recuperating (Beneke et al., 2005; Chen et al., 2018; Zhang & Ji, 2016).

Metabolism of fuels for cellular function mature throughout mammalian development and can be impaired by increased expression of satiety hormones during puberty and adolescence (Beneke et al., 2005; Caprio et al., 1994; Chen et al., 2018;

Joslowski et al., 2012; Zhang & Ji, 2016). Reduced access to cellular energy substrates like glucose and lactate impede neuronal growth and activity in the brain (Ruscica et al., 2016; Sada et al., 2015). Therefore, associations between impaired glucose and lactate metabolism and mental health vulnerabilities are expected.

5.4 Glucose, lactate, and depression

Altered metabolic pathways are associated with depressive disorders (Carrard et al., 2018; X.-Y. Lu, 2007; Xuezhen Wang, Zhang, & Lu, 2015). Anhedonia or the loss of interest in previously pleasurable items is a typical depression symptom (Association, 2013). Anhedonia has a bimodal effect on food intake (Association, 2013). A loss of appetite promotes severe weight loss during depression (Ruscica et al., 2016). Alternatively, people with depression often express either progressive or binge eating of high fat/carbohydrates to compensate for sensations of anhedonia (Frisco, Houle, & Lippert, 2013). Weight gain also follows increased stress exposure and repeated sleep deprivation (Murack et al., 2021). Impaired metabolic processing may be responsible for changes to eating behavior (Frisco et al., 2013; X.-Y. Lu, 2007). Leptin regulates energy metabolism by signaling the hypothalamus to initiate the sensation of satiety (Casanueva & Dieguez, 1999). Depression is often associated with leptin resilience and low leptin expression leading to extended hunger durations and overeating (Eikelis et al., 2006; Kraus, Haack, Schuld, Hinze-Selch, & Pollmächer, 2001; J. Liu et al., 2010). Leptin administration has even improved depressive behavior in rodent models but with limited success in humans (Garza, Guo, Zhang, & Lu, 2012; J. Liu et al., 2010; X.-Y. Lu, Kim, Frazer, & Zhang, 2006; Yamada et al., 2011). However, leptin's direct involvement with depression development has since been questioned and its antidepressant effects attributed to increased hippocampal neuroplasticity (Carvalho et al., 2014; Duman, Aghajanian, Sanacora, & Krystal, 2016; Thon, Hosoi, Yoshii, & Ozawa, 2014). Interventions targeting metabolic mechanisms impaired during depression may improve existing antidepressant treatments. In this section, we discuss the efficacy of several metabolic mechanisms recently targeted for antidepressant treatment.

People with depression often display metabolic dysfunction related to glucose metabolism (De Groot, Anderson, Freedland, Clouse, & Lustman, 2001; Goldney,

Phillips, Fisher, & Wilson, 2004). Men and women diagnosed with Type 2 diabetes display a greater comorbidity with depressive disorders compared to metabolically healthy adults (De Groot et al., 2001; Goldney et al., 2004; Nouwen et al., 2011). In some investigations, men and women with partial glucose metabolism impairments display greater depressive behavior (Adriaanse et al., 2008; Demakakos, Zaninotto, & Nouwen, 2014; Koponen, Kautiainen, Leppänen, Mäntyselkä, & Vanhala, 2015). However, several meta-analyses contradict these findings because partial glucose metabolism impairment and even undiagnosed diabetes do not consistently correlate with depression (Nouwen et al., 2011). Factors like treatment, stress, or social stigma may be responsible for some of the association between depression and type 2 diabetes but the extent is unclear.

Depression is associated with insulin resistance (Baxter et al., 1989; Kennedy et al., 2001; Mueller, Heninger, & McDonald, 1969; Winokur, Maislin, Phillips, & Amsterdam, 1988). Individuals diagnosed with major depression and subsequently exposed to an oral glucose tolerance test display greater basal glucose, blood glucose concentration, and insulin release in the periphery than healthy adults (Winokur et al., 1988). It is less clear how central glucose metabolism is affected by depression. Individuals diagnosed with unipolar depression displayed a reduced rate of glucose metabolism in the dorsal anterolateral prefrontal cortex compared to healthy controls according to positron emission topography (PET) scans (Baxter et al., 1989). Similar reductions to glucose metabolism were observed between bipolar depression and manic patients as well as obsessive compulsive patients with and without depression (Baxter et al., 1989; Mueller et al., 1969). Pharmacological treatment of unipolar depression and bipolar depression increased glucose metabolism and neuronal activity in the dorsal anterolateral prefrontal cortex (Baxter et al., 1989; Kennedy et al., 2001; L. Wang, Hermens, Hickie, & Lagopoulos, 2012). Despite close resemblance to insulin resistance, reduced brain glucose metabolism during depression may instead be a symptom of reduced brain neuronal activity. Several fMRI studies report general reductions to neuronal activity in the prefrontal cortex and increased limbic system activity in individuals diagnosed with depression (L. Wang et al., 2012). Paroxetine antidepressant treatment of males diagnosed with major depression both increases prefrontal cortical glucose metabolism and decreases hippocampal glucose metabolism indicating

moderating neuronal activity may be balancing glucose metabolism (Kennedy et al., 2001). It is unclear if changes to glucose metabolism during depression moderate neuronal activity, if changed neuronal activity influences glucose metabolism, or that a bi-directional relationship exists. However, evidence suggests that increasing access to energy substrates like L-lactate may reduce symptom expression related to depression (Allaman et al., 2011; Carrard et al., 2018; Lowenbach, Greenhill, & Durham, 1947; Onozato et al., 2020).

Peripheral blood lactate concentration fluctuates according to depression diagnoses and severity (Allaman et al., 2011; Carrard et al., 2018; Lowenbach et al., 1947; Onozato et al., 2020). People with depressive symptoms have lower peripheral blood L-lactate concentrations than healthy controls (Allaman et al., 2011; Carrard et al., 2018; Lowenbach et al., 1947; Onozato et al., 2020). Decreased peripheral L-lactate predicts increased severity of negative mental symptoms associated with depression like social withdrawal, anhedonia, and lack of motivation in adult men and women (Onozato et al., 2020). To assess the correlation between depression and peripheral blood L-lactate, adult male mice were peripherally injected with L-lactate, the antidepressant desipramine, or a control vehicle (Carrard et al., 2018). A single L-lactate administration was sufficient to reduce depressive behavior measured by a forced swim test and was comparable to the antidepressant effects of desipramine (Carrard et al., 2018). Additionally, a single L-lactate administration reduced phosphorylation of hippocampal genes associated with depression development like glycogen synthase kinase- 3 (GSK3) alpha, GSK3 beta, and cyclic-AMP response element-binding protein (Carrard et al., 2018). However, this L-lactate mediation of genetic expression and behavior occurred in a non-depressed rodent model.

To assess the effect of L-lactate in a model of depression, adult male mice were administered corticosterone for 21 days to create neurobehavioral changes similar to depression (Carrard et al., 2018). Twenty-one days of L-lactate administration abolished depressive behavior induced by corticosterone treatment as measured by the forced swim, tail suspension, and saccharin preference tests (Carrard et al., 2018). Chronic L-lactate and desipramine administration also increased hippocampal astrocyte activity and

hippocampal L-lactate concentration in adult male mice (Carrard et al., 2018). Alternate antidepressant therapies influence hippocampal activity and L-lactate concentration (Allaman et al., 2011; Carrard et al., 2021; Carrard et al., 2018). Selective serotonin-reuptake inhibitors (SSRIs) stimulate increased extracellular lactate release from cortical astrocyte cultures extracted from male Swiss albino mice (Allaman et al., 2011). L-lactate administration may assist existing antidepressant therapies. However, successful investigations into the antidepressant effects of L-lactate are limited to animal and injection models due to difficulties with oral L-lactate treatment in human samples (Lowenbach et al., 1947)

The original investigation of lactate's effect on sleep and depression included chronic administration of a racemic L- and D- lactate milk solution to people with major depression and medical students as healthy controls (Lowenbach et al., 1947). The oral treatment was successful in reducing sleep disruptions and depression symptoms. However, the milk/lactate solution was unpalatable and led to extreme albeit temporary gastric distress in all participants. Adult male mice were orally administered a similar racemic D- and L-lactate solution and displayed increased sickness behavior as well (Murack & Messier, 2019). Lower dosages eliminated sickness behavior but no longer increased peripheral blood L-lactate (Murack & Messier, 2019). However, drowsiness measured by running wheel activity proportionally increased with greater sodium lactate dosages indicating a causal mechanism between sleep and lactate (Béland-Millar, Larcher, Courtemanche, Yuan, & Messier, 2017; Murack & Messier, 2019). An alternate oral lactate manipulation is necessary if treatment in humans can be contemplated.

To summarize, depression is associated with decreased glucose tolerance and L-lactate availability (Allaman et al., 2011; Baxter et al., 1989; Carrard et al., 2018; Lowenbach et al., 1947; Onozato et al., 2020). Successful antidepressant treatments typically improve glucose metabolism and L-lactate availability in the brain (Allaman et al., 2011; Carrard et al., 2018; Kennedy et al., 2001). Considering sleep is needed to limit both excessive energy usage during rest and depression development, manipulating underlying metabolic processes could be used to regulate sleep patterns and improve antidepressant effects of existing therapies.

5.5 Glucose, lactate, and sleep

The timing of sleep and metabolism cycling are both influenced by circadian rhythm (Borbély, 1982; Borbély, Tobler, et al., 1981; Poggiogalle et al., 2018; Shanahan & Czeisler, 1991). Circadian shifts and sleep disturbances in healthy adults create similar metabolic profiles displayed by people with depression (Ogilvie & Patel, 2017; Spiegel et al., 1999). For example, sleep disturbances increase both glucose intolerance and insulin resistance typically resulting in increased weight gain (Ogilvie & Patel, 2017). Young adult men exposed to 6 consecutive evenings of 4-hour sleep expressed near 30% reduction in the acute response of insulin release to glucose and a decreased insulin sensitivity compared to men who slept 12 hours (Spiegel et al., 1999). The glucose metabolism profile of sleep-deprived men was more like that of older males with impaired glucose tolerance than healthy male controls. Even shorter durations of sleep disruption still effect glucose metabolism (Mavanji, Teske, Billington, & Kotz, 2013; Stamatakis & Punjabi, 2010). Healthy adult men between 18- 29 years old were exposed to mechanical- and auditory-based sleep disturbances during stage 2 sleep for only 2 consecutive evenings (Stamatakis & Punjabi, 2010). Sleep disrupted participants expressed both increased insulin resistance and decreased glucose effectiveness compared to rested males. However, body weight did not significantly change likely due to the shorter duration of the study (Stamatakis & Punjabi, 2010).

Weight gain following sleep disruption is often associated with changes in homeostatic hormones controlling appetite (Spiegal, Tasali, Penev, & Van Cauter, 2004; Spiegel et al., 2004; Spiegel, Tasali, Leproult, & Van Cauter, 2009). Sleep limited to 4-hour durations in young men decreased blood leptin concentration by 18% after only two evenings. These sleep-deprived young men also reported increased desire to eat (Spiegal et al., 2004). These findings indicate only two days of sleep deprivation was sufficient to modify hormone profiles to increase appetite (Spiegal et al., 2004; Spiegel et al., 2009). In an alternate study, leptin levels were reduced according to a dose-response decline after 6 days of 12 hour, 8 hour, and 4 hour periods of sleep in healthy young men (Spiegel et al., 2004). Peak leptin concentration was reduced by 26% between the 12- and 4-hour conditions while controlling for weight and caloric intake. As mentioned

previously, weight gain following increased anhedonia is often observed in unipolar and bipolar depressive disorders (Anderson, Cohen, Naumova, & Must, 2006; Association, 2013; Frisco et al., 2013). Sleep disturbances are a common symptom of depression and may contribute to the development of weight gain typical of depression development (Association, 2013). While the effect of sleep disturbances on glucose metabolism is clear, sleep's effect on L-lactate is more complex (Bourdon et al., 2018; Dash, Tononi, & Cirelli, 2012; Naylor et al., 2012).

Fluctuations of extracellular L-lactate concentration in the cortex of mice and rats has been associated with different arousal states and sleep stages (Bourdon et al., 2018; Dash et al., 2012; Murack & Messier, 2019; Naylor et al., 2012). Adult male mice display a lower average cortical L-lactate concentration while asleep (Bourdon et al., 2018; Dash et al., 2012; Naylor et al., 2012). A persistent L-lactate decline is displayed when transitioning to both sleep onset and to slow wave sleep (Dash et al., 2012; Naylor et al., 2012). L-lactate concentration increases at a rate of 15 μM/min when entering REM sleep (Dash et al., 2012; Naylor et al., 2012). Mice express an elevated L-lactate concentration when prematurely wakened or when exposed to a 6-hour mid-sleep disruption (Naylor et al., 2012). Glucose and glutamate were measured together with L-lactate; however, alertness and sleep phase could not be predicted from glucose concentration. Any causal mechanism between arousal state and extracellular L-lactate remains unclear (Lundgaard et al., 2017). Increased L-lactate concentration could be a by-product of increased neuronal activity classical observed during REM sleep (Berry et al., 2012; Berthet et al., 2009; Combs et al., 1990; Larrabee, 1995, 1996; D. Smith et al., 2003). Previous evidence supports a relation between REM sleep and available L-lactate (Ma et al., 2019). Male rats exposed to 7 days of REM deprivation displayed decreased lactate concentration in urine extracts. Interestingly, REM deprivation also increased depressive behaviour measured by the forced swim and tail suspension tests and increased gut Proteobacteria concentration suggesting a connection between sleep, depression, and microbiome composition. It is unclear if the relationship between L-lactate and sleep is bidirectional. Injection of sodium lactate in adult male mice 2 hours before typical sleep onset may increase drowsiness in a dose dependent manner (Murack & Messier, 2019). However, further research into sleep-influencing metabolic mechanisms is required.

In summary, depression diagnosis often coincides with limited glucose tolerance and reduced access to L-lactate (Allaman et al., 2011; Baxter et al., 1989; Carrard et al., 2018; Lowenbach et al., 1947; Onozato et al., 2020). Glucose metabolism is impaired by increased exposure to hormones secreted during puberty and adolescence (X.-Y. Lu, 2007; X.-Y. Lu et al., 2006; Spiegel et al., 2004; M. J. Vázquez et al., 2015). Additionally, chronic sleep disturbances caused by delayed sleep onset observed during adolescence may further reduce glucose tolerance (Mavanji et al., 2013; Schmid, Hallschmid, Jauch-Chara, Born, & Schultes, 2008). These metabolic factors combined with natural sensitivity to stress make puberty and adolescence a particularly vulnerable period for depression development. Identifying an oral intervention that increases glucose tolerance and extracellular L-lactate may help reduce the likelihood of sleep-mediated metabolic impairments and depression during adolescence.

6.0 Lactic acid probiotics

Lactic acid bacteria are microorganism species that were first identified by their ability to excrete lactic acid or its base conjugate lactate following carbohydrate fermentation (Evivie, Huo, Igene, & Bian, 2017; Klaenhammer & De Vos, 2011). Lactic acid bacteria are used for industrial purposes to both ferment and preserve foods (Gezginc, Topcal, Comertpay, & Akyol, 2015; Klaenhammer & De Vos, 2011). Alternatively, many *Lactobacillus* and *Bifidobacterium* strains of lactic acid bacteria are marketed as “probiotics” that provide health benefits when consumed (Evivie et al., 2017). Lactic acid probiotics improve weight reduction and glucose management in both humans and rodent hosts (Aalling, Nedergaard, & DiNuzzo, 2018; Bagarolli et al., 2017; Evivie et al., 2017; Kadooka et al., 2010; Takemura, Okubo, & Sonoyama, 2010). Adult humans administered *lactobacillus gasseri* SBT2055 showed decreased BMI, weight, and hip circumference (Kadooka et al., 2010). Rats exposed to high fat diets and fed *lactobacillus paracasei* display reduced body fat, weight, and number and size of adipocytes (B.-H. Lee, Lo, & Pan, 2013). Lactic acid probiotics are an intriguing approach to reduce disease burden caused by underlying metabolic impairments. In this section, we discuss how probiotic interventions influence stress reduction, depression reduction, and sleep quality.

Several mechanisms have been suggested for probiotic's reduction of weight gain (Tsai, Cheng, & Pan, 2014). Lactic acid probiotics reduce hormones associated with hunger and increase hormones required to improve glucose metabolism (Hong, Chung, & Kim, 2015; Tsai et al., 2014). Diet-induced obese rats displayed reduced weight gain, body fat, and blood glucose after treated with *lactobacillus plantarum* DK211 (Hong et al., 2015). These obese rats also expressed reduced plasma satiety hormones like ghrelin following probiotic treatment suggesting that probiotics induced weight loss by reducing hunger (Hong et al., 2015). Moreover, insulin sensitivity was increased in the same obese rats increasing ease of glucose absorption and reducing formation of fat storage cells (Hong et al., 2015). The resulting effect of probiotic administration is often lower fasting blood glucose concentration (Al-Salami et al., 2008; Nikbakht et al., 2018). Meta analysis assessments agree that lactic acid probiotics consistently lower fasting blood glucose, and are more effective when administered in multispecies mixtures (Nikbakht et al., 2018). Administration of lactic acid bacteria as adjunct diabetic therapies are currently under investigation (Al-Salami et al., 2008; Bayat et al., 2016; Feizollahzadeh, Ghiasvand, Rezaei, Khanahmad, & Hariri, 2017; Tao, Gu, Mao, Zhang, & Pei, 2020). If impaired glucose metabolism from sleep loss and pubertal hormone secretion increases the risk of adolescent depression development, then early probiotic intervention may attenuate that risk.

6.1 Lactic acid probiotics, stress, and depression: metabolic mechanisms

Lactic acid probiotic consumption may reduce symptoms associated with stress (Diop, Guillou, & Durand, 2008; Gruenwald, Graubaum, & Harde, 2002; K. S. Smith, Greene, Babu, & Frugé, 2019). Men and women who scored high on stress assessments consumed probiotic mixture capsules containing *B Bifidobacterium longum* for 3-weeks (Diop et al., 2008). These stressed adults reported a significant reduction in stress induced nausea and stomach pain following probiotic treatment (Diop et al., 2008). However, probiotic mediation of stress may have been localized to alleviated stomach discomfort and not direct HPA axis interaction. A similar study requiring moderately stressed men and women with no stomach problems to consume a multi-probiotic capsule containing *B. longum* for 6 months (Gruenwald et al., 2002). Adults administered this probiotic

capsule displayed improved stress-coping abilities on the List of Adjectives test (Gruenwald et al., 2002). In our own lab, we have observed that chronic administration of a lactic acid bacteria mixture can attenuate the anxiolytic and depressive effects of lipopolysaccharide injections in adolescent mice (Murray et al., 2019; Murray et al., 2020). Extended probiotic use may reduce the long-term impact of adolescent stress on depression development.

Certain lactic acid probiotics that reduce stress and exhaustion also display antidepressant effects in adult samples (Pinto-Sanchez et al., 2017; K. S. Smith et al., 2019; Tamtaji et al., 2019). Mild and moderately depressed individuals with comorbid irritable bowel syndrome display decreased depressive severity on the Hamilton Depression rating scale following 6-weeks of *B. longum* treatment (Pinto-Sanchez et al., 2017). Once again, alleviation of irritable bowel symptoms may have improved depression behavior. Adult outpatients diagnosed with moderate depression without comorbid bowel issues were administered a probiotic mixture containing *B. longum* and *Lacticaseibacillus rhamnosus* for 6 weeks (Ghorbani et al., 2018). Outpatients scored lower on the Hamilton Depression scale after probiotic intervention. In a similar study, adults diagnosed with major depression and administered a lactic acid probiotic mixture containing *B. longum* for 8 weeks displayed reduced depressive scores on the self-report Beck Depression Inventory (Tamtaji et al., 2019). Moreover, this probiotic treatment reduced both insulin resistance and blood insulin levels in people with depression. Improved insulin sensitivity following lactic acid probiotic treatment may reduce adolescent risk for depression if depression development is mediated by poor glucose tolerance. The mechanisms behind the antidepressant effect of lactic acid probiotic remain unclear.

Several pathways explaining the antidepressant effect of probiotics have been proposed and are explained elsewhere in greater detail (Borovikova et al., 2000; Bravo et al., 2011; Gayathri & Rashmi, 2017). Many microorganisms living within the gastrointestinal tract influence afferent neurotransmission within the Vagus nerve (Avolio et al., 2019; Borovikova et al., 2000; Bravo et al., 2011; Dutta et al., 2019). Adult male mice chronically administered *L. rhamnosus* (JB-1) display lower stress-induced plasma

corticosterone, reduced anxiety-like behavior assessed within an elevated plus maze, and reduced depression behavior in a forced swim test (Bravo et al., 2011). Vagotomised mice administered *L. rhamnosus* (JB-1) did not display these antianxiety and antidepressant effects (Bravo et al., 2011). Alternatively, probiotics interact with the brain by influencing immune responses (Borovikova et al., 2000). Microbial-secreted acetylcholine within the gut propagates an anti-inflammatory effect within the HPA axis of mice and reduces depression symptoms (Borovikova et al., 2000; Köhler et al., 2014).

Gastrointestinal absorption of metabolites secreted from probiotics may also influence brain chemistry and behavior (Bourdon et al., 2018; Matsumoto et al., 2012; Schroeder & Backhed, 2016). In the colonic lumen of humans, microorganisms secrete tryptophan, L-lactate, and over 150 alternate metabolites with the capability to influence homeostasis and existing disease (Matsumoto et al., 2012). As mentioned previously, tryptophan is necessary for the synthesis of serotonin, a neurotransmitter often depleted in depressive disorders and targeted by several types of antidepressants (Hilakivi-Clarke, 1991; O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). Administration of *B. longum* and *L. helveticus* simultaneously reduced depressive scores and tryptophan breakdown in humans with mild and moderate depression symptoms (Kazemi, Noorbala, Azam, Eskandari, & Djafarian, 2019). Lactic acid probiotics may reduce depression by direct secretion of metabolites like tryptophan or serotonin associated with antidepressant effects that can absorbed into the circulatory system for distribution.

L-lactate produced from lactic acid probiotics and absorbed into the circulatory system may contribute to depression reduction. However, only limited evidence provided by short bowel patients and high lactic acid probiotic diets suggest that blood lactate concentration can be directly influenced by gut microbiota (Nakagawa et al., 2018; Uchida et al., 2004).

Lactic acid probiotic consumption improves glucose regulation in human and rodent models while simultaneously reducing depression scores in some samples (Tamtaji et al., 2019). It is unclear if lactic acid probiotics can improve L-lactate availability with a change in diet (Kazemi et al., 2019; Matsumoto et al., 2012; Uchida et al., 2004). It is equally unclear if lactic acid probiotics influence sleep regulation.

6.2 Lactic acid probiotics and sleep

Previous research indicates that sleep and microbiome composition may have a bidirectional relationship (Ogawa et al., 2020; Poroyko et al., 2016). Changes in sleep have been associated with modifications to microbiome composition (Poroyko et al., 2016). Adult male C57BL/6J mice exposed to tactile sleep disruption for four weeks displayed decreased flora concentration belonging to the Lactobateriaceae and Bifidobateriaceae families. Conversely, manipulation of the gut microbiome may influence sleep patterns (Ogawa et al., 2020). Adult male C57BL/6J mice treated with a broad-spectrum antibody profile displayed reduced NREM duration during the sleep phase, longer NREM and REM during the awake phase, reduced total wakefulness, and reduced consolidation of NREM, REM, and wakefulness. Changes in sleep architecture often predict modifications to microbiome composition and vice-versa. Sleep may be improved if the microbiome composition is appropriately modified.

Several human studies indicate improved sleep following lactic acid probiotic consumption (Irwin, McCartney, Desbrow, & Khalesi, 2020). However, reports of improved sleep are typically limited to subjective sleep scales (Dhiman et al., 2014; Kato-Kataoka et al., 2016; Kelly et al., 2017; Marotta et al., 2019; Nakagawa et al., 2018). Healthy adult men and women consuming *L. helveticus* MIKI-020 for 4 weeks displayed greater self-reported calmness, motivation, and decreased fatigue as measured by the Visual Analogue scale (Nakagawa et al., 2018). Multiple investigations report improved self-reported sleep quality according to the Pittsburgh Sleep Objective Index in adults after ~8 weeks of lactic acid probiotic treatment (Dhiman et al., 2014; Kato-Kataoka et al., 2016; Kelly et al., 2017; Marotta et al., 2019). Subjective reports of improved sleep quality were typically greater in healthy individuals than participants diagnosed with anxiety or depression (Irwin et al., 2020). Despite overwhelming subjective reports, it has proven difficulty to identify how sleep has been objectively improved by probiotic treatment (Nakagawa et al., 2018).

Multiple studies do not reveal significant effects of treatment on objective polysomnography or actigraphy measures of sleep (Monoi et al., 2016; Nakakita, Tsuchimoto, Takata, & Nakamura, 2016; Nishida et al., 2017). However, isolated studies

have shown differences in sleep efficiency. Adults treated with *L. helveticus* MIKI-020 for 4 weeks displayed improved sleep efficiency (total REM and NREM sleep/duration from sleep onset to rise time) using EEG recordings (Nakagawa et al., 2018). Given the recency of these investigations, the mechanisms underlying subjective and objective sleep improvements are unclear.

Depression is multifaceted disease with an immense impact on individual quality of life and societal expenses (Association, 2013; James et al., 2018; Weissman et al., 1993; Weissman & Klerman, 1977). Lifelong depression often develops during puberty and adolescent development (Pine et al., 1999). Adolescents experience natural stress susceptibility, sleep disturbances, and metabolic impairments that predispose young men and women to depression development (Caprio et al., 1994; Carskadon, 2011; Ge, Conger, & Elder Jr, 2001; Joslowski et al., 2012). Lactic acid probiotic administration may simultaneously improve pubertal and adolescent stress reactivity, sleep, and metabolism and potentially ameliorate early depression develop (Diop et al., 2008; Gruenwald et al., 2002; Nakagawa et al., 2018; K. S. Smith et al., 2019; Tamtaji et al., 2019).

References

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Chapter 2: The impact of lactic acid and medium chain triglyceride on blood glucose, lactate and diurnal motor activity: a re-examination of a treatment of major depression using lactic acid

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Abstract

While investigating the effect of alternative energy substrates on extracellular brain glucose or lactate, Béland-Millar (2017) noted a reduction of physical activity after intraperitoneal administration of lactate and ketone bodies. These observations were similar to an older study that examined the impact of drinking a sodium lactate/lactic acid solution before sleep in hospitalized patients with major depression. Patients and control participants self-reported drowsiness, early sleep onset and better overall sleep after consumption. Some patients showed improved mood after several days of treatment. We re-evaluated the effects of the solution used (0.59 g/kg) as well as several smaller doses (0.47, 0.35, 0.24 and 0.12 g/kg) on blood lactate and glucose in CD-1 mice and on sleep onset associated activity reduction. Because of adverse effects with the lactate/lactic acid solution, we also examined the effects of a medium chain triglyceride (MCT) solution (10, 5, 2.5, and 1 ml/kg) on blood lactate and glucose. Oral gavage administration of lactic acid/lactate produced adverse effects particularly for the largest doses. However consumption of 10 and 5 ml/kg volumes of MCT oils significantly increased blood lactate concentration to levels comparable to Lowenbach's solution without piloerection indicative of adverse effects. To evaluate pre-sleep activity reduction produced by lactate, mice were intraperitoneally administered diluted sodium lactate (2.0 g/kg, 1.0 g/kg, 0.5 g/kg, 0.25 g/kg, or saline) for 6 days, 120 minutes before their sleep period and their running activity was measured. Larger lactate doses reduced pre-sleep running each day up to 60 minutes post injection. Smaller doses reduced running after a single treatment only. These results suggest that the modulation of blood lactate levels may be useful in treating sleep onset problems associated with depression.

Keywords: glucose, lactate, monocarboxylate transporter, lactic acid, sleep, running, depression

1.0 Introduction

In 1947, Lowenbach (1947) used an unconventional treatment for major depression on a psychiatric ward. They administered a mixture of lactic acid and sodium lactate diluted in milk to their patients before their sleep onset. Lowenbach et al. hypothesized that electroconvulsive therapy (ECT), administered without curarization at the time, led to a large rise in blood lactate as a result of the muscle contractions produced by seizures. They reasoned that increases in blood lactate could be an important factor in the beneficial effect of ECT. The study was completed a few years before 1951 when the introduction of suxamethonium as a synthetic alternative to curare sharply reduced muscle contractions and led to the more widespread use of modern ECT (Dorkins, 1982). The large rise in blood lactate is mostly abolished by curarization without any decrease in ECT effectiveness in major depression (Posner, Plum, & Van Poznak, 1969), an observation that contradicted Lowenbach et al.'s hypothesis. This observation together with the discovery of the first anti-depressant iproniazid in 1951 led to the fall into obscurity of that early experiment. However ,there were a number of noteworthy observations in the Lowenbach study despite it being an uncontrolled, "natural" experiment.

First, researchers mention that the immediate effects of drinking the lactate/lactic acid mixture both in healthy volunteers (medical students) and patients alike were «sensations …described in such terms as "a gentle feeling of drowsiness," "general lassitude," "I feel relaxed", [and] "a tendency to want to sleep"»(Lowenbach et al., 1947). As sleep is a clinical issue in most severely depressed patients, the lactic acid solution was given to patients before going to bed. Lowenbach et al., after ensuring all barbiturate treatments were discontinued, observed that many patients had improved sleep either the first night or after a few nights using the lactate/lactic acid solution. The efficacy of the treatment on depressive symptoms was more variable. Out of 45 patients treated, some patients showed remission (16%), while a few more "improved markedly" (11%) or were "somewhat improved" (31%). Three out of the 26 patients that improved with the solution returned within a year with a recurrence of depression – the remainder (23) were depression-free for up to three years after which they were lost to follow-up. Many

patients (42%) had minimal or no response for depressive symptoms after a two-week lactic acid/lactate trial. Drinking the lactic acid/sodium lactate solution leads to severe initial side effects. Immediately post-administration, participants reported extreme cramps, nausea and poor palatability for 1-3 days of consecutive exposure. In conclusion, it appeared that the improvement on sleep of the lactic acid/lactate solution was more common than the effect on depressive symptoms. However participants reported initial aversion to prolonged solution consumption. An alternative solution could likely improve compliance if this treatment was to be used again in humans.

We became aware of the Lowenbach et al. study after we observed unusual and profound activity reduction in animals following an intraperitoneal 2 g/kg injection of either sodium lactate, beta-hydroxybutyrate (BHB) or acetate (Lowenbach et al., 1947). Further experimentation revealed that these substances sharply raised blood lactate while also raising brain extracellular glucose (Béland-Millar et al., 2017). These observations support an examination of the effect of raising blood lactate on spontaneous activity in a mouse model. Since the lactic acid/lactate solution used by Lowenbach was aversive (and in the absence of dose-response curves), we tested the effects of various doses, routes of administrations and alternate solutions on the reduction in running wheel activity that occurs towards sleep onset in mice. This behavioral paradigm was chosen because it lends itself to automatic quantification and follows a well-known circadian pattern. The compulsion observed in mice to use running wheels provides a useful measure of activity and alertness, particularly the absence of activity associated with sleep onset (Cosgrove, Hunter, & Carroll, 2002; Rhodes et al., 2001).

Multiple physiological and behavioral processes, including running wheel activity, are regulated by the suprachiasmatic nuclei of the hypothalamus (Cheng et al., 2002; Moore, 1983; Schaap & Meijer, 2001). The circadian rhythm of that structure approximates a 24-hour diurnal cycle. At the beginning of the rodent sleep period, locomotor activity is reduced (Oliverio & Malorni, 1979; Ono, Shimizu, & Yoshida, 1991). Blood and brain lactate concentration also reliably oscillates according to the wake and sleep cycles of mice including a rise during rapid eye movement (REM) sleep (Naylor et al., 2012). Astrocytes and neurons can produce lactate as a product of

glycolysis (Schurr, Payne, Miller, & Rigor, 1997). Lactate can also be transported across the blood-brain barrier based on concentration gradients (Pardridge, 2007) through bidirectional diffusion using the MCT1 monocarboxylate transporter (Hellmann, Vannucci, & Nardis, 1982; Knudsen, Paulson, & Hertz, 1991). At present, it is unknown if blood lactate levels modulate or result from sleep/wake cycles.

Because of the adverse effects and poor palatability of lactate solutions, we examined the effect of medium chain triglycerides on blood lactate. Medium chain triglyceride (MCT) oils are odorless, tasteless liquids that consist of fatty acid chains approximately 6-10 carbons long (Bach & Babayan, 1982; Hopman, Jansen, Rosenbusch, & Lamers, 1984; Marten, Pfeuffer, & Schrezenmeir, 2006) and are safe to consume (Jeukendrup & Aldred, 2004; Traul, Driedger, Ingle, & Nakhasi, 2000). Compared to long chain triglycerides, absorption of the medium chain fatty acids is more efficient (Bach & Babayan, 1982) and more quickly transported to the liver and across mitochondrial barriers (Ikeda, Okamura-Ikeda, & Tanaka, 1985). Rapid oxidization leads to a sudden increase of acetyl coenzyme A (Crozier, 1988; Metges & Wolfram, 1991). Excess acetyl coenzyme A is converted to ketone bodies. As the ketone body BHB is associated with increases in blood lactate, MCT oils may be a possible alternative to oral lactate solutions. Although omega-3 oils such as PUFA have been shown to have a small but reliable anti-depressive effect particularly with co-administration of anti-depressive drugs (Mocking et al., 2017), blood lactate concentration is not effected and so they are a poor emulation of Lowenbach's solution.

We evaluated the effect of Lowenbach's solution on blood lactate while attempting to reduce adverse effects by administering a sodium lactate/lactic acid solution at 100, 80, 60, 40, and 20% original dosage. Because of poor efficacy and high toxicity in mice of the original Lowenbach's solution, we used MCT oil to safely and efficiently raise blood lactate concentration. Solutions were administered near the end of the wake cycle. Finally, we examined the translation of the subjective observations of lactate-induced drowsiness published by Lowenbach (Lowenbach et al., 1947) and noted by Béland-Millar (Béland-Millar et al., 2017) to objective recordings with sodium lactate injections in a rodent model of late day diurnal motor activity. We hypothesized that

blood L-lactate concentration can be significantly influenced through oral treatments and that increased blood L-lactate would reduce late day activity in a dose-dependent manner.

2.0 Methods and Materials

2.1 Animals

Male CD-1 mice 6-8 weeks of age (Charles River Canada, St-Constant, Québec, Canada) were housed under standard laboratory conditions and reverse light cycle (07:00 to 19:00) with "Lights ON" starting the sleep period at 19:00. Light intensity reached maximal intensity levels of 200 lux measured at cage level during Lights ON and 23 lux (red light) during Lights OFF. Mice were individually housed in cages immediately upon arrival and for the remainder of the experimental procedure. Mice had *ad libitum* access to standard chow (Teklad Global 18% Protein 2018, Teklad Lab Animal Diets, Envigo, Mississauga, Canada) and water, unless otherwise specified. All experimental procedures were approved by the University of Ottawa Animal Care Committee and met the guidelines of the Canadian Council on Animal Care (CCAC).

2.2. Experiment 1

Lactic acid / sodium lactate groups (see Figure 1A): Thirty mice were randomly assigned to six different groups and individually housed. Animals had *ad libitum* access to food and water until 2 hours prior to testing where food was removed. Peripheral blood samples were collected by lightly piercing the dorsal tail vein with a 23-gauge needle (Becton, Dickson and Company, Franklin Lakes, NJ). First drops collected were not sampled to avoid pooled blood. Plasma lactate and glucose concentrations (mmol/L) were measured using a lactate meter (StatStrip Xpress, Nova Biomedical, Innovation House, Runcorn, Cheshire, UK) and a glucose meter (Accu Check, Aviva, Roche Diagnostics, Mannheim, Germany) with the appropriate test strips. The lactate meters are sensitive to 0.1mM increments for concentrations in the range of ~1.0-10 mM and <0.5mmol/L analytical error (Bonaventura et al., 2015). The glucose meters are sensitive to 0.1mM increments within ± 0.5mM analytical error for concentrations in the range of ~1.0-5.6 mM and ± 10% above ~5.6mM (Freckmann et al., 2015). Baseline lactate and glucose

plasma concentrations were measured after the fasting period. A 1:1 solution of lactic acid (USP) and sodium lactate 60% w/w (Alfa Aesar, Tewksbury, MA, USA) was diluted to obtain a 0.2 ml volume that was administered using a gavage needle so that mice received a lactate dose of 0.59 g/kg (n=5), 0.47 g/kg (n=5), 0.35 g/kg (n=5), 0.24 g/kg (n=5) or 0.12 g/kg (n=5). Control mice were administered sterile water (n=5). The 0.59 g/kg dose was determined to approximate the original dosage used in Lowenbach's (1947) study. The remaining groups approximate 80%, 60%, 40% and 20% of the original dosage. Lactate and glucose measurements were recorded every 30, 60, 90, 120, 150 and 180 minutes post-administration. Treatments were repeated for three consecutive days.

MCT groups (see Figure 1A): Twenty-five mice were randomly assigned to five different groups and exposed to housing, food and manipulation conditions described above. Baseline lactate and glucose concentration measurements were recorded after a 1-hour fasting period. Mice were administered by gavage 10 ml/kg (n=5), 5 ml/kg (n=5), 2.5 ml/kg (n=5) or 1 ml/kg (n=5) medium chain triglyceride oil (NOW Foods, Inc., Bloomingdale, Il., U.S.A.). Dosage concentrations were replicated according to previous experimentation (Kesl et al., 2016) and calculated to compare dose response to 50% incremental decreases. The manufacturer reports MCT oil containing approximately 35-40% capric acid and 60-65% caprylic acid with 1% undetermined fatty acid chain length. Sterile water was administered to control animals. Lactate and glucose concentrations, behavior and weight were recorded as described above.

2.2.1 Adverse effect assessment

Adverse effects of lactate and MCT gavage were assessed each day at 30, 60, and 180 mins post-administration (see Figure 1A) according to a modified version of the sickness behavior procedure (Gandhi, Hayley, Gibb, Merali, & Anisman, 2007; Ismail et al., 2011). Assessments occurred immediately prior to vein sampling. Mice were scored "1" if piloerection (curled body posture) was present. Piloerection is common in mice experiencing discomfort or pain (Jirkof, 2014; Renn et al., 2011; Van Loo et al., 1997). Animals that had piloerection at each of the three observations were scored 3 for that day. Mice scoring a daily total of 3 (i.e. 3h of adverse effects) were removed from further

treatment and allowed to recover (see Figure 3A and B). Mice were weighed before and after experimental manipulation to assess severe weight gain or loss indicative of sickness (± 10% per 3 days) following CCAC guidelines.

2.3 Experiment 2

2.3.1 Acclimatization and Running Wheel.

Fifty mice were randomly assigned to 5 separate dosage groups and individually housed in cages (25 cm x 16 cm x 44 cm) containing one ENV-044 Low Profile Wireless Running Wheel (MedAssociates.inc). Animals were individually housed so that each animal had only access to one wheel. All wheels had a wireless connection to a DIG-804 USB Interface Hub. Running wheel setup and protocol closely follow established guidelines (Goh & Ladiges, 2015). Mice were given 48 hours of unlimited running wheel access to acclimatize them to the use of the running wheels (see Figure 1B). Since the experiment examined the effect of treatments on running behavior, mice were removed from the sample if they recorded less than 275 m traveled in the final 24 hours of acclimatization as detailed below (Cobos et al., 2012). A single animal met this criterion.

2.3.2 Pre-treatment Activity Recording.

Mice were manipulated for approximately 30 seconds each evening (at 17:00) to minimize the effect of handling and gavage on subsequent running activity. In order to equate the running propensity of mice across groups before the rest period (Lights ON), voluntary running activity was recorded continuously for 5 consecutive days after acclimatization (see Figure 1B). Voluntary running activity was quantified as the number of rotations completed by mice on the running wheel. Pre-treatment voluntary running activity was partitioned into 30-min segments. Baseline reference values were averaged over 5 days for each individual mouse at each time segment before and after the onset of the rest period. The values obtained were used to assign animals to each group as described below.

2.3.3 Identification of Active runners.

Mice activity varies near the end of the wake period (Lightfoot, Turner, Daves, Vordermark, & Kleeberger, 2004; Swallow, Carter, & Garland, 1998; Turner, Kleeberger, & Lightfoot, 2005). To ensure that the modulation of activity by lactate could be observed, a novel calculation of late active running was created using average trends in mice running wheel activity (See Supplementary material 1). Mice were scored on their activity before and after Lights ON in order to equally represent the various running patterns in each injection group. Mice that did not run reliably before (e.g. "inactive runners," n=11) or that remained active after Lights ON (e.g. "overactive runners," n=8) were removed from analysis. Detecting changes in "inactive runner" activity during the observation period was difficult. Animals that continued running after Lights ON would increase a bias towards Type 1 error after the removal of inactive runners. To equate the groups on spontaneous running behavior, mice were assigned to groups according to their average individual voluntary running activity from 17:00 to 19:00 each day of the pre-treatment period (see Supplementary material 1). Running behavior was coded as "2" if a mouse demonstrated a progressive decrease in running approaching Lights ON with almost no running thereafter. Mice that decreased running activity approaching Lights ON but still continued running at a high rate after were coded "1". Mice demonstrating inconsistent or near zero running activity before Lights ON and high voluntary running activity after Lights ON or near absence of running before and after Lights ON were coded "0". Thus mice with the most consistent running behavior on each of the 5 days received a total of 10 points. Mice that received a score of 4 or less (n=19) were excluded from the analysis (see Supplementary material 2).

2.3.4 Treatment Activity Recording.

Four dilutions of sodium DL-lactate 60% w/w (Alfa Aesar) were prepared daily (1/5, 1/10, 1/15, and 1/20 dilutions). All lactate solutions were tested for acid-base neutrality (pH = 7.391 ± 0.012). Saline solution pH was 5.5. Mice were assigned to five different dosage groups according to scored running patterns to ensure an acceptable distribution of active runners. Approximately 120 minutes prior to Lights ON (17:00), mice received an intraperitoneal (i.p.) injection of either 2.0 g/kg (n=7), 1.0 g/kg (n=6),

0.5 g/kg (n=6) or 0.25 g/kg (n=6) dosages of 1/5, 1/10, 1/15, or 1/20, respectively, diluted sodium lactate or saline (0.9%) (n=6). I.p. injections replaced oral gavage as large volume consumption may impede running wheel activity. The effect of lactate injections on blood lactate concentration has been investigated previously (Béland-Millar et al., 2017). I.p. injection doses of lactate that produced similar blood lactate as MCT's were used in Experiment 2 (see Supplementary material 3). After the injection, mice were immediately returned to their cages and running recording began. Mice were injected once a day for 6 consecutive days following completion of pre-treatment recording. Treatment length was chosen to emulate maximum treatment duration reported previously(1947). The 120 minutes of running wheel activity between injection and Lights ON were divided according to the number of rotations achieved per 30-min segments: 0-30, 30-60, 60-90, and 90-120 mins post-injection.

2.4 Data analysis.

Due to expected high variation in baseline data (Lightfoot et al., 2004; Shivavedi, Chatterjee, & Kumar, 2014; Swallow et al., 1998; Turner et al., 2005), all measurements were transformed as values relative to baseline recording to analyze the individual intervention effects. This is common in experiments implementing peripheral blood lactate (Béland-Millar et al., 2017) and running wheel activity (Cobos et al., 2012) paradigms. In Experiment 1, blood lactate and glucose concentrations were analyzed as values relative to pre-administration baseline measurements. In Experiment 2, relative running wheel activity was calculated by dividing the number of rotations after treatment by the average baseline rotations recorded pre-treatment. Baseline was the averaged running wheel rotations completed during the same 30-min time segments across five days of pre-experimentation.

A series of split-plot ANOVAs were used to evaluate each experimental procedure. The effect of i.p. lactate on late-day activity and the effect of orally administered lactate and MCT oil on blood metabolites were analyzed separately. Changes in weight and resting blood lactate/glucose concentrations were analysed separately using one-way (Dosage) and two-way (Dosage by Day) ANOVAs, respectively, and pairwise comparisons to control group values. No outliers were

identified. Non-sphericity and heterogeneity were corrected according to the Greenhouse-Geisser method. *Post hoc* pairwise comparisons ($\alpha = 0.05$) were used to assess experimental and control (saline 0.9%) groups. Due to the novelty of the Active Running paradigm, pre-planned comparison independent-samples t-tests ($\alpha = 0.05$, one-sided) were performed in Experiment 2 to assess individual lactate dosage group running wheel activity in comparison to saline controls immediately after injection (Hilton & Armstrong, 2006). It was expected that activity would decrease with lactate injection. All statistical procedures were performed using SPSS v. 24 (IBM, Armonk, New York, USA).

A significant percentage of the lactate gavage groups did not complete the experimental procedure due to severe adverse effects. Linear mixed modeling (LMM) is a parametric analysis common with repeated measures and longitudinal studies where non-compliance of participants or death make standard analyses of variance unusable (Chakravarty, Hubert, Lingala, & Fries, 2008; Corey, Edwards, Levison, & Knowles, 1997; T. Murphy et al., 2010; Verbeke, 1997) while using descriptive statistics similar to ANOVA. LMMs allow for missing time points and sample specific covariance structures affording a more flexible and accurate approach. The Satterthwaite approximation was used to compute the denominator degrees of freedom. Statistical significance limits remain the same as with ANOVA.

3.0 Results

3.1 Experiment 1

3.1.1 Effects of lactate/lactic acid gavage on blood glucose and lactate

LMM analysis of blood lactate concentration after lactate gavage included fixed effects of dosage ($F(5, 22.9) = 4.11$, $p = 0.004$), treatment day ($F(2, 54.9) = 6.18$, $p = 0.008$), and time post gavage ($F(6, 73.8) = 33.43$, $p < 0.001$) and the interactions of dosage and treatment day ($F(10, 47.4) = 3.29$, $p = 0.003$) and dosage, treatment day and time ($F(102, 95.1) = 1.60$, $p = 0.011$). The Toeplitz covariance matrix was selected to represent the distribution of sample covariance.

Three-way pairwise comparisons revealed few significant differences between dosage groups on the first day of treatment (see Figure 2). After the second treatment day, mice that received 0.59 g/kg lactate solution had significant increases in relative blood lactate for 3 hours post-gavage compared to control. During the final treatment day, the same effect was observed in mice administered 0.47 g/kg lactate solution from 60 to 180 mins post gavage. Mice administered 0.12 g/kg had significant increases in relative blood lactate concentrations at 120- and 180-mins post gavage.

An additional LMM was performed on the relative blood glucose of the same mice (see Figure 2). The same linear model was used, and only the fixed effects of treatment day ($F(2, 48.1) = 6.87$, $p = 0.002$) and time post gavage ($F(6, 77.2) = 39.2$, $p < 0.001$) were significant. Decreases in blood glucose were greater overall on Day 2 and 3 compared to Day 1.

3.1.2 Lactate gavage piloerection, weight change and survival rate

Several mice administered Lowenbach's lactate solution were removed from the experiment due to prolonged piloerection (see Figure 3B). In the 0.59 g/kg group, mice were removed after Day 1 (n=3) and Day 2 (n=1). Mice in the 0.47 g/kg group were removed after Day 1 (n=1) and Day 2 (n=1). One mouse each was removed from the 0.24 g/kg and 0.12 g/kg dosage groups after Day 1 and Day 2, respectively. Of these mice, 5 recovered within seven days of removal. Three mice were euthanized due to prolonged piloerection. All euthanized mice were from the 0.59 g/kg group. All animals in the saline and 0.35 g/kg groups completed the experiment without adverse events.

Kruskal-Wallis tests showed significant differences in piloerection at all assessments on Day 1 ($p<0.05$), 30 min assessment only on Day 2 ($p< 0.05$), and Day 3 at 30 min and 60 min ($p<0.05$). Pairwise comparisons to the saline group (Figure 3A) indicated significant increases in piloerection for the 0.59 g/kg group at 30 and 60 min on Day 1, and for the 0.47 g/kg group at 30 min on Day 1 and 2 and at 60 min on Day 2 only. Weight did not differ significantly across groups (see Table 1). Pre-gavage blood lactate ($F(5, 16) = 1.668$, $p = 0.199$) or glucose values ($F(2, 32) = 0.137$, $p = 0.873$) were not significantly different across dosages and days.

3.2.1 Effects of MCT gavage on blood glucose, lactate, and weight

A split plot ANOVA (5x3x7) performed on the relative blood lactate concentrations of mice administered MCT oil (n=20) revealed main effects of dosage ($F(4, 20) = 6.77$, $p = 0.001$, $\eta^2 = 0.575$) and time after gavage ($F(3.0, 60.7) = 26.17$, $p < 0.001$, $\eta^2 = 0.567$; Greenhouse-Geisser corrected) but not treatment day. *Post-hoc* comparisons revealed significant increases in blood lactate in mice administered 10 ml/kg of MCTs compared to lower dosages and control ($p < 0.05$).

A significant three-way interaction (dose x time x day) ($F(48, 240) = 1.73$, $p < 0.01$, $\eta^2 = 0.257$) is presented in Figure 4. Mice administered 10 ml/kg MCT demonstrated significantly greater blood lactate levels compared to control at Day 1 and Day 2 across all time periods ($p<0.01$). Compared to control, the 5 ml/kg dose produced a moderate and significant blood lactate increase 30 mins after gavage on Day 1 ($p = 0.055$) and Day 2 ($p <0.01$), respectively.

A split plot ANOVA (5x3x7) evaluated relative blood glucose concentrations and indicated main effects of dose ($F(4, 20) = 6.00$, $p = 0.002$, $\eta^2 = 0.545$), treatment day ($F(2, 40) = 6.82$, $p = 0.003$, $\eta^2 = 0.254$) and time of measurement ($F(3.5, 69.8) = 18.89$, $p < 0.001$, $\eta^2 = 0.486$). Blood glucose significantly decreased after 10 ml/kg compared to 2.5 ml/kg, 1.0 ml/kg and water. Blood glucose levels significantly decreased between Day 1 and Day 2, Day 1 and Day 3 ($p < 0.01$), but not Day 2 to Day 3.

Analysis of a three-way interaction ($F(48, 240) = 2.01$, $p < 0.001$, $\eta^2 = 0.286$) revealed significant decreases in blood glucose after Day 1 for the 10 ml/kg group compared to water, for two consecutive hours (see Figure 4). Similar effects were observed for the first administration of 5 ml/kg and 2.5 ml/kg at 30 and 60 min, respectively. Upon second day administration of 10 ml/kg, blood glucose levels differed significantly from control at 90- and 120-mins post injection and moderately at 60 ($p= 0.093$), 150 ($p=0.054$) and 180 ($p=0.070$) minutes. Mice administered 5 ml/kg showed moderately lower blood glucose ($p= 0.063$) 30 mins post injection. After Day 3 treatment, mice administered 10 ml/kg MCT oil again demonstrated a significant decrease in blood glucose compared to control for 90 consecutive minutes post administration.

3.2.2 MCT aversive effects and weight loss

A single mouse administered 5 ml/kg was removed from procedure on Day 2 due to complications resulting from excessive gavage abrasion on the esophagus (verified upon necropsy). No piloerection was observed after MCT administration. Pairwise comparisons reveal significant weight loss for mice administered 10 ml/kg and 5 ml/kg ($p < 0.05$) and moderate weight loss with 2.5 ml/kg administration ($p = 0.058$) compared to control.

3.3 Experiment 2

3.3.1 Effects of lactate injection of running activity around the onset of rest period

The 5x6x3 ANOVA performed on the voluntary running activity of active runners (n=31) indicated a significant effect of dose ($F (4, 26) = 5.66$, $p = 0.002$, $\eta^2 = 0.465$), treatment day ($F (2.7, 69.5) = 3.48$, $p = 0.025$, $\eta^2 = 0.118$) and time period ($F (2, 52) = 24.71$, $p < 0.001$, $\eta^2 = 0.487$). Post hoc analysis determined that administration of lactate regardless of dilution significantly reduced voluntary running activity below saline levels. However, voluntary running activity did not differ significantly between dosage groups.

Planned comparisons show administration of 2.0 g/kg lactate significantly reduced immediate voluntary running activity on treatment Day 1 ($t (11) = 1.87$, $p = 0.044$), Day 2 ($t (11) = 2.43$, $p = 0.017$), Day 4 ($t (11) = 2.26$, $p = 0.023$), Day 5 ($t (11) = 2.42$, $p = 0.017$) and Day 6 ($t (11) = 2.45$, $p = 0.016$) and with moderate significance Day 3 ($t (11) = 1.75$, $p = 0.054$). Only mice administered 1.0 g/kg lactate significantly decreased voluntary running activity within 30 mins of i,p, injection consistently across repeat treatments (Day 1: $t (10) = 2.20$, $p = 0.026$; Day 2: $t (10) = 2.05$, $p = 0.034$; Day 3: $t (6.5) = 2.62$, $p = 0.018$; Day 4: $t (6.5) = 2.14$, $p = 0.036$; Day 5: $t (10) = 1.82$, $p = 0.049$; Day 6: $t (6.2) = 2.12$, $p = 0.038$). Lower dose sodium lactate injections 0.5 and 0.25 g/kg reduce voluntary running activity after Day 1 ($t (10) = 3.10$, $p = 0.006$), Day 2 ($t (7.1) = 1.91$, $p = 0.049$) and Day 1 ($t (10) = 2.39$, $p = 0.016$) treatment only, respectively.

3.3.2 Baseline running behavior and weight.

Baseline activity of mice injected 2.0 g/kg sodium lactate were significantly more active from 18:00 to 18:30 (90 min time segment) during their baseline recordings compared to other doses (p <0.05). There were no significant weight change between doses (F (4, 26) = 0.609, p = 0.662) (see Table 1).

4.0 Discussion

4.1 Methodological Considerations

Blood lactate and voluntary running activity briefly increased after gavage or injection procedures (see Figures 2, 4 and 5). Gavage needle insertion causes discomfort and stress in small animals (Alban et al., 2001; Roberts, Soames, James, Gill, & Wheeldon, 1995). However alternative administration procedures also have drawbacks: it is difficult to control dosage of a substance added to chow in an *ad libitum* context. Mixing solutions into the water source was not feasible in the present case: lactate has poor palatability and MCT oils cannot emulsify properly in water.

Individual housing of mice is associated with a variety of maladaptive risk factors including increased aggressiveness, defense escape activity (Kršiak, 1975) and heart rate (Späni, Arras, König, & Rülicke, 2003). However it was necessary to seclude mice to accurately record individual late day running wheel activity in Experiment 2. This housing procedure has been implemented elsewhere with minor confounds (Lightfoot et al., 2004; Swallow et al., 1998). Single housing in Experiment 1 was necessary to justify comparisons between metabolite concentration and activity procedures. As isolation is linked with increased anxiety and higher locomotive behavior, it is likely that blood lactate and general running wheel activity increased due to housing protocol. Day 1 baseline blood lactate concentrations are greater than average (Béland-Millar et al., 2017; Ferreira et al., 2007; Schurr et al., 1997), a possible response to isolation. Comparisons to control groups and analysis of data as a percentage relative to individual baselines in both experiments allow for direct analysis of the effect of lactate/MCT oil and sodium lactate on blood metabolite concentration and late day activity, respectively.

Different control solutions were administered between experiments. In Experiment 1, to ensure proper dilution while maintaining equal volume and consistency, water was required. Water as a control solution was important for blood metabolite comparisons to Lowenbach's lactate solution and to MCT oil consumption. There is little discrepancy between the effect of water gavage and i.p. saline injections on blood lactate when comparing Figures 2 and 4 to the published observations of Béland-Millar et al. (2017) suggesting that control conditions in the two experiments had similar effects on blood lactate (see Supplementary material 3).

Mice were administered lactate prior to sleep to model the experimental conditions in the Lowenbach (Lowenbach et al., 1947) study. The time of injection was changed from 30 minutes prior to the designated sleep period in Lowenbach's experiment to 120 minutes in the present study because CD-1 mice typically show activity until two hours prior to the Lights ON sleep period (Goh & Ladiges, 2015; Harri et al., 1999). Decreases in relative voluntary running activity resulting from our experimental manipulation were expected to be more easily assessed during that period.

Though strain dependent (Brant & Kavanau, 1964; Festing, 1977), typical running wheel behavior is categorized by a sharp activity increase up to 48 hours in duration (Goh & Ladiges, 2015) after first exposure to a running wheel. Voluntary running activity then decreases and stabilizes between Day 3 and Day 7 of an unlimited exposure. The averaged measurements across 5 days of the pre-experimental period should be an accurate estimate of the individual voluntary running activity before the sleep period. The number of lactate administrations required to influence running activity was determined according to two sources. Béland-Millar et al. (2017) reported a decrease in activity after 1 to 3 days of treatment in a mouse sample. Lowenbach et al. (1947) observed significant behavioral changes after 4 days of treatment.

4.2 Orally administered lactate solution modulates blood lactate but at doses that produce adverse effects in mice.

In Experiment 1, only Lowenbach's original solution and 80% dilution significantly increased peripheral blood lactate by Day 3 administrations. However samples administered the original dosage or 80% dilution demonstrated aversive effects

by Day 1 and Day 2/3, respectively. Lowenbach's solution successfully alters blood lactate but only at doses that produce adverse effects. Consumption of diluted lactate solution is not a viable method of addressing lactate moderated drowsiness.

The causes of the adverse effects of Lowenbach's solution in mice are unknown. All doses tested were less concentrated than the reported acute oral LD50 4.875 g/kg of lactic acid in mice (Clary, Feron, & Van Velthuijsen, 1998; Rowe, Sheskey, & Owen, 2006). However adverse effects were most prevalent in mice administered 0.59 g/kg and 0.47 g/kg. Post mortem autopsy revealed no physical damage to the esophagus, lung or stomach. We cannot discuss the possibility of acute ketoacidosis due to the absence of arterial blood gas analysis. Since the pH of all solutions were close to 7.0, it is unlikely that the solutions produced irritation of tissues. Lowenbach (1947) reports stomach discomfort, nausea and vomiting as a common symptom of initial lactate treatment. Rodents are unable to vomit (Horn et al., 2013) but the solution may have caused nausea until lactate was transported out of the intestinal lumen via the monocarboxylate transport protein slc5a8 (Iwanaga, Takebe, Kato, Karaki, & Kuwahara, 2006). Although the mechanism of discomfort remains unknown, a combination of limiting slc5a8 proteins and the inability to vomit may have created a toxic gastric environment from which lactic acid/lactate could not be removed.

Limited transport protein availability may also explain the discrepancy between immediate piloerection and delayed peripheral blood lactate modulation until Day 3. Multiple days of exposure are likely required for sufficient MCT transporter protein slc5a8 expression to adapt to the increased availability of oral lactate (J. Wang, Peng, Li, & Pan, 2013).

4.3 Orally administered MCT oils appear to be a safer and efficient alternative to raise blood lactate levels

MCT oils significantly and safely increased blood lactate in mice. Similar MCT dosages and their effects on blood metabolite concentrations have been previously investigated in a rodent model (Kesl et al., 2016). In our experiment, the 5 ml/kg dosage was the lowest volume that increased blood lactate concentration. Mice gavaged 5 ml/kg of MCT oil had both a 30-min peak blood lactate rise and also the 60-min return to

baseline observed by Béland-Millar et al. (2017). Acute and chronic administration of 10 ml/kg produced the greatest long-lasting increase of blood lactate. MCTs did not cause observable adverse effects and were effective after the first administration. These characteristics suggest that MCT oils are a possible alternative to Lowenbach's solution.

The exact mechanism by which MCT oils increase peripheral blood lactate is currently unknown. However limited availability of enzymes required for the tricarboxylic acid cycle in hepatocytes is responsible for the conversion of excess acetyl coenzyme A to the ketone bodies acetoacetate, BHB and acetone (Seaton, Welle, Warenko, & Campbell, 1986). When the production of ketone bodies is reduced, hepatocytes synthesize pyruvate and then lactate from acetyl coenzyme A. Alternatively, less prevalent acetone is converted from remaining acetoacetate (Guthrie & Jordan, 1972; Mitchell et al., 1995) or via acetoacetate decarboxylase (Tagaki & Westheimer, 1968). Acetone conversion to lactate occurs in the liver via the methylglyoxal pathway in low concentrations or by the propanediol pathway in other organs when blood concentrations are higher (Casazza, Felver, & Veech, 1984). Large volumes of MCT oil would be necessary to create the acetone concentrations necessary for the large peripheral blood lactate increase observed. This may explain why only large MCT doses produce significant blood lactate increases. Previous research demonstrates increased blood lactate after BHB injections (Béland-Millar et al., 2017) likely via conversion to acetoacetate during ketolysis (Chang, Olson, & Schwartz, 2013) and spontaneous creation of acetone. Large MCT oils doses likely increase blood lactate through an increase in ketone bodies.

MCT oil consumption also decreased blood glucose. The hypoglycemic effect of MCT oil consumption has been reported in previous rodent (Kashiwaya et al., 2010; Kesl et al., 2016) and human (Miles, Haymond, & Gerich, 1981; Schwartz, Boyes, & Aynsley-Green, 1989; Senior & Loridan, 1968) experiments. Kashiwaya et al. (Kashiwaya et al., 2010) demonstrated a decrease of blood glucose and insulin after 14 days of ketogenic supplementation in a rodent model suggesting reduced glucose production or increased insulin sensitivity. In humans, 3-hour ketone infusions that decreased glucose production and uptake are correlated with changes in B-cell pancreatic functionality (Miles et al.,

1981). Increased ketone concentrations provided by a large dose of MCT oil likely reduces hepatic glucose production by providing an alternate source of energy for cell function (Owen et al., 1967).

4.4 Peripheral lactate injections reduce diurnal motor activity prior to sleep.

Unpublished observations of Béland-Millar (2017) noting a correlation between activity reduction and peripheral blood lactate increase was successfully reproduced and quantified in the present experiment. This effect could be observed immediately after administrating dosages up to 25% of the original 2.0 g/kg dose. However, lower lactate doses were less effective over multiple days of treatment.

Due to the toxicity of Lowenbach's oral solution in a rodent model and the confounding effects of large gavage volume on running activity, lactate was administered via i.p. injections in Experiment 2 to simulate blood lactate levels produced by MCT oil in Experiment 1. By comparing Day 1 blood lactate concentration after 10 ml/kg MCT gavage to Béland-Millar's 2.0 g/kg sodium lactate i.p injection (2017), we observed similar blood lactate fluctuation up to 120 minutes post administration (see Supplementary material 3). Direct measures of MCT oil consumption on running wheel activity is yet required to support our claims. However, previous literature supports this hypothesis as long term ketogenic diets promote deeper and more efficient sleep by reducing inflammation and increasing adenosine activity (Barrea et al., 2022; Hallböök, Lundgren, & Rosén, 2007). Experiment 2 dosages were calculated as percentages of original dose (i.e. 2.0g/kg) in a similar stepwise manner.

Late day voluntary running in mice typically decreases with greater proximity to the completion of the wake cycle (Edgar, Kilduff, Martin, & Dement, 1991; Lightfoot et al., 2004; Valentinuzzi, Scarbrough, Takahashi, & Turek, 1997). We argue that rapid reduction of late day activity is a useful measure of the ability to facilitate sleep onset. In our experiment, the effect of blood lactate rise was strong enough to influence activity by inducing quiescence earlier before the Lights-ON sleep period. Despite being typical of drowsiness and sleep onset, these results will have to be replicated with more typical measures of sleep onset and quality using EEG.

The mechanism behind lactate's influence on activity reduction remains unknown. Lactic acid build-up is classically associated with prolonged muscle contractions in the absence of sufficient oxygen supply (Hill & Lupton, 1923; Margaria, Edwards, & Dill, 1933). A tempting explanation of the reduced activity in our experiment may be that systemic lactate injection induces muscular pain. However recent studies disprove lactic acid's direct influence on muscle fatigue, instead supporting ATP deficiency as a likely culprit (D. Allen & Westerblad, 2001; Schurr, 2014; Westerblad, Allen, & Lannergren, 2002).

Lowenbach et al. (1947), and more recently Carrard et al. (2018), have indicated an effect of increased peripheral blood lactate on depressive symptoms. Lowenbach's theory linking the anti-depressive effects of ECT to increased blood lactate is unlikely in the face of the effectiveness of ECT without large blood lactate changes with curarization. However our data supports Lowenbach's observations by showing decreased diurnal activity after a lactate injection.

Noradrenergic neurons in the locus coeruleus are inhibited during slow wave sleep and almost quiescent in REM sleep (Aston-Jones & Bloom, 1981; Gervasoni et al., 1998). However, extracellular lactate in the locus coeruleus is largely excitatory as demonstrated by *in vitro* lactate bath (F. Tang et al., 2014). It is unclear how locus coeruleus activation can be reconciled with the present results. Béland-Millar et al. (2017) recorded extracellular lactate concentrations in the primary motor and visual cortices only. It is possible that the locus coeruleus is protected from excitation resulting from changes to extracellular lactate concentration during sleep. Naylor et al. (2012) noted increases in extracellular lactate during subsequent REM stages in the primary motor cortex. These findings together with Tang et al. (2014) suggest that lactate fluctuations during sleep may be localized to specific areas.

More than a single mechanism is likely responsible for lactate's influence on behavior. Compared to baseline recordings, activity increased immediately following lactate injection. This is likely due to the stress associated with the injection procedure. A single administration of any lactate dose significantly reduced activity compared to a saline control for 30 mins post-injection on Day 1. Lactate injections may be moderating

the increased activity caused by handling stress at lower doses. Measures of anxiety reduction will be necessary to further address this observation. It is possible that the observed activity reduction represents a combined effect of lactate on stress-related responses, and then on late-day diurnal activity.

4.5 Decreased Body Weight in MCT oil consumption only

Higher doses of MCT oil consumption produced significant weight loss. MCT oils are increasingly used in ketogenic diets to promote the metabolism of fats over carbohydrates. Large MCT oil consumptions are associated with “loose stool” in human participants. Henderson et al. (2009) reported a 20% incidence of similar symptoms after consumption of a ketogenic agent. Kesl et al. (2016) also reported similar circumstances in a rat sample. They were successful in reducing symptoms and maintaining ketone concentration by oral administration of a BHB salt. Though no gastrointestinal side effects were observed, analysis of weight reduction and stool collection after BHB salt consumption in comparison to MCT oils would be necessary.

5.0 Conclusions

Increasing blood lactate levels appear to facilitate activity reduction towards the transition to sleep in the mouse. This study suggests that Lowenbach’s lactate solution has a number of practical drawbacks as a potential facilitator of sleep and eventual adjunct therapy for depression. MCT oils are proposed as an alternative. The present results are consistent with previous studies that have found evidence that lactate (Carrard et al., 2018) and ketogenic diets can produce anti-depressive-like effects in rodents (Brietzke et al., 2018; P. Murphy, Likhodii, Nylen, & Burnham, 2004).

Conflicts of Interest

None.

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Acknowledgements

We would like to thank our technicians Jacky Liang and Josh Davis for lab and animal housing assistance.

References

See end of document.

Tables, Figures, and Captions

See below.

Table 1

Experiment 1: Oral Administration							
Dosage	*Mean Baseline*						*Weight change (g)*
	Lactate			*Glucose*			
	Day 1	*Day 2*	*Day 3*	*Day 1*	*Day 2*	*Day 3*	
Lactate solution							
0.59 g/kg (5)	2.30 ± 0.15	1.90 ± 0.10	1.60	8.92 ± 0.49	8.45 ± 1.25	9.20	0.93 ± 0.50
0.47 g/kg (5)	2.90 ± 0.37	2.20 ± 0.19	2.17 ± 0.24	8.94 ± 0.60	9.95 ± 1.00	9.53 ± 0.43	1.74 ± 1.37
0.35 g/kg (5)	2.88 ± 0.11	2.15 ± 0.21	2.83 ± 0.77	9.75 ± 0.33	8.9 ± 0.65	9.35 ± 0.93	-1.33 ± 0.50
0.24 g/kg (5)	3.35 ± 0.51	2.68 ± 0.49	3.52 ± 0.54	7.18 ± 0.67	6.81 ± 1.24	7.62 ± 1.08	0.03 ± 1.44
0.12 g/kg (5)	2.14 ± 0.12	2.55 ± 0.25	2.58 ± 0.92	9.18 ± 0.76	8.07 ± 0.98	8.55 ± 0.45	-1.94 ± 1.19
Water (5)	2.94 ± 0.23	3.22 ±0.88	2.96 ± 0.61	8.36 ±0.61	7.52 ± 1.19	6.30 ± 1.55	-0.96 ± 0.32
MCT oils							
10 ml/kg (5)	2.76 ± 0.40	2.48 ± 0.25	2.63 ± 0.18	8.64 ± 0.41^{+}	9.98 ± 0.72	9.83 ± 1.16	-2.38 ± 0.49 ^{x}
5 ml/kg (5)	3.64 ± 0.25	2.38 ± 0.26	2.50 ± 0.40	10.30 ± 0.91	10.63 ± 0.6	9.73 ± 0.77	-2.50 ± 0.19 ^{x}
2.5 ml/kg (5)	3.18 ± 0.25	2.2 ± 0.29	2.42 ± 0.41	8.22 ± 0.74^{+}	9.28 ± 0.48	10.30 ± 0.23	-2.14 ± 0.34
1 ml/kg (5)	3.08 ±0.27	2.00 ± 0.28	3.06 ± 1.05	9.58 ± 0.25	9.76 ± 0.70	9.72 ± 1.11	-1.90 ± 0.34
Water (5)	2.78 ± 0.21	3.01 ± 0.54	2.70 ± 0.70	8.45 ± 0.66^{+}	7.77 ± 1.01	7.45 ± 1.35	-1.08 ± 0.34

Experiment 2: I.P Injection of Sodium Lactate - Average Baseline Running Wheel Rotations Post Administration					
Dosage	*0-30 mins*	*30-60mins*	*60-90 mins*	*90-180 mins*	*Weight*
2.0 g/kg (7)	1197.45 ± 171.45	861.77 ± 147.38	594.63 ± 110.73	187.35 ± 125.25	0.58 ± 0.28
1.0 g/kg (6)	1210.14 ± 190.76	1008.72 ±181.33	256.25 ±57.93*****	98.32 ±65.23	0.12 ± 0.55
0.5 g/kg (6)	910.53 ±132.89	625.25 ± 115.49	272.80 ±61.57*****	87.23 ± 45.52	0.55 ±0.16
0.25 g/kg (6)	882.37 ± 149.19	685.06 ± 118.82	274.94 ± 65.19*****	97.88 ± 58.32	0.98 ± 0.39
Saline (6)	817.25 ± 146.84	819.20 ±124.13	360.83 ± 80.86*****	95.72 ± 71.25	0.83 ± 0.65

Table 1 Pre-treatment characteristics of mice gavaged with a lactate solution (n=30; n_f=22) or MCT (n=25; n_f=24) oils and mice that received an i.p injection of lactate (n=31; n_f=31). Metabolite blood concentration is measured in millimoles per liter; variance is measured as the standard error of the mean (mmol/L ± SEM). Blood lactate and glucose baseline was recorded approximately thirty minutes prior to solution administration. Running wheel baseline activity was computed as the average number of rotations completed in a 30-min segment observed on each of five days of unlimited wheel exposure. Animals were weighed 12 hours before first administration, and 12 hours prior to final administration. Negative weight change values denote weight loss. Group sizes are in parentheses.

+ = significant difference compared to 5 ml/kg MCT oral administration within same day measurements ($p < 0.05$).

x = significant difference in weight change from water (control) values ($p < 0.05$).

***** = significant difference compared to 2.0 g/kg i.p. injection within same time period ($p < 0.05$).

n_f = final sample size

Figure 1.

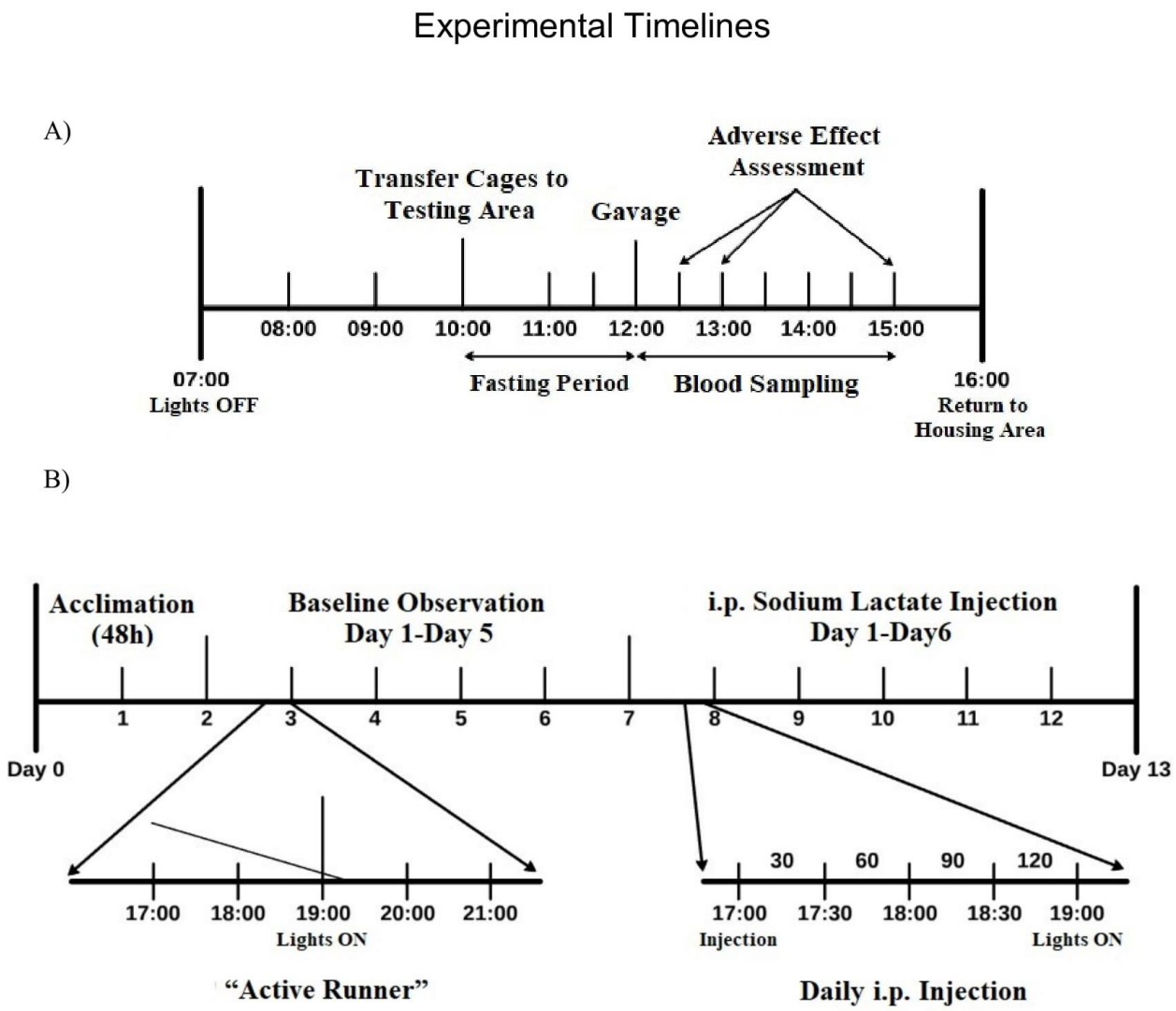

Experimental Timelines
A)
Adverse Effect Assessment
Transfer Cages to Testing Area
Gavage
08:00
09:00
10:00
11:00
12:00
13:00
14:00
15:00
07:00 Lights OFF
Fasting Period
Blood Sampling
16:00 Return to Housing Area
B)
Acclimation (48h)
Baseline Observation Day 1-Day 5
i.p. Sodium Lactate Injection Day 1-Day6
1
2
3
4
5
6
7
8
9
10
11
12
Day 0
Day 13
17:00
18:00
19:00
20:00
21:00
Lights ON
30
60
90
120
17:00
17:30
18:00
18:30
19:00
Injection
Lights ON
"Active Runner"
Daily i.p. Injection

Figure 1. Methodological design of Experiment 1 (A) and Experiment 2 (B). Blood sampling occurred at administration and every subsequent 30 minutes for an elapsed 180 minutes. Experiment 2 depicts expected “Active Runner” baseline activity decline (approximately 50% reduction every 60 minutes) and the daily injection schedule of sodium lactate treatment.

Figure 2.

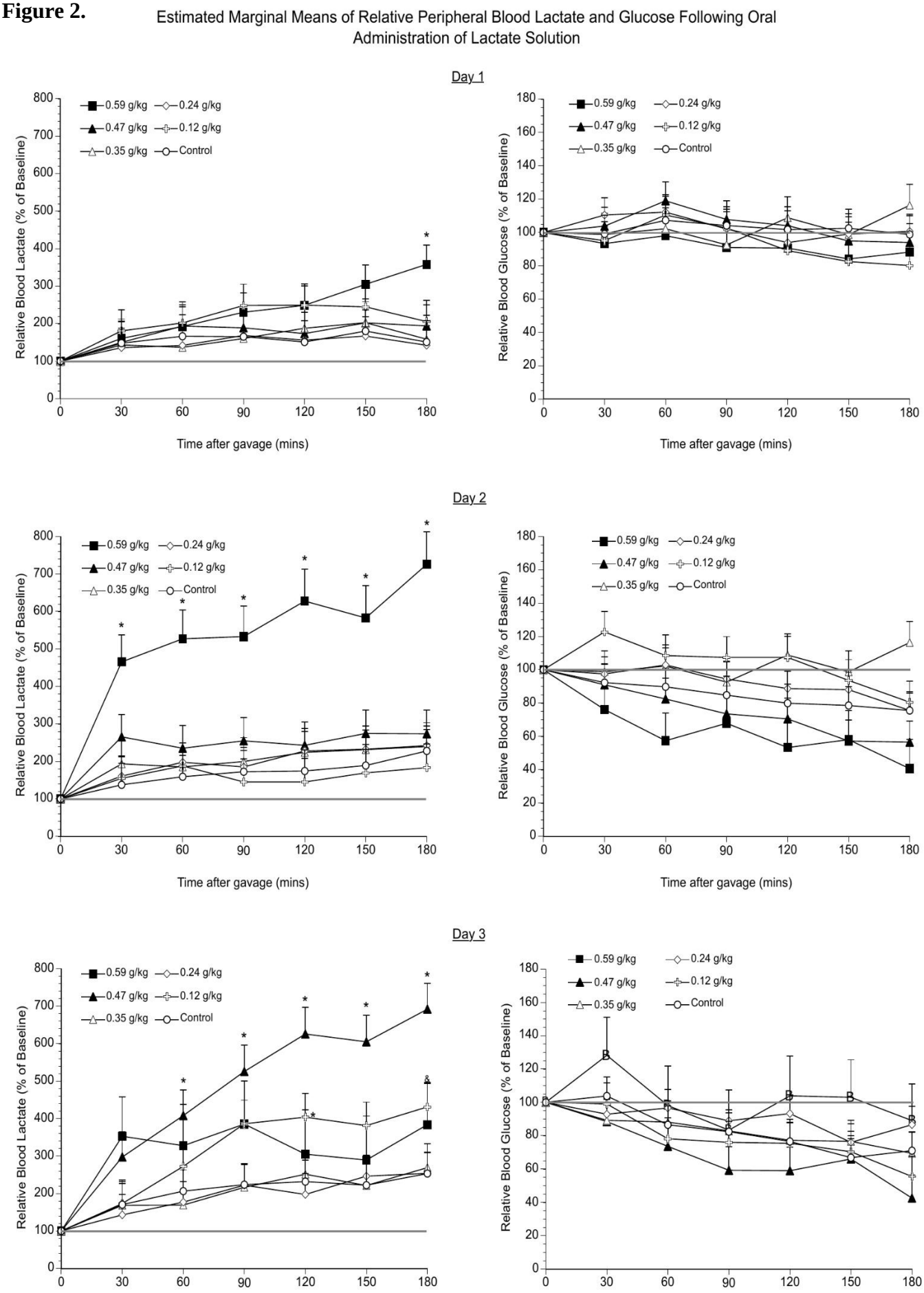

Estimated Marginal Means of Relative Peripheral Blood Lactate and Glucose Following Oral Administration of Lactate Solution
Day 1
Day 2
Day 3
0.59 g/kg
0.24 g/kg
0.47 g/kg
0.12 g/kg
0.35 g/kg
Control
Relative Blood Lactate (% of Baseline)
Relative Blood Glucose (% of Baseline)
Time after gavage (mins)

Figure 2. Peripheral blood lactate and glucose concentrations relative to baseline measurements after one to three days of 0.59 g/kg (n=5; n_f=1), 0.47 g/kg (n=5; n_f=3), 0.35 g/kg (n=5; n_f=5), 0.24 g/kg (n=5; n_f=4) or 0.12 g/kg (n=5; n_f=4) diluted 1:1 lactic acid (USP) and sodium DL-lactate 60% w/w solution or water control (n=5; n_f=5). Blood levels were measured every 30 minutes over 180 minutes after oral administration.

Values are expressed as a percent of their baseline measured 30 minutes prior to administration and error bars represent the standard error of the mean.

***** = significant difference from water ($p < 0.05$)

n_f = final groups sample size

Figure 3.

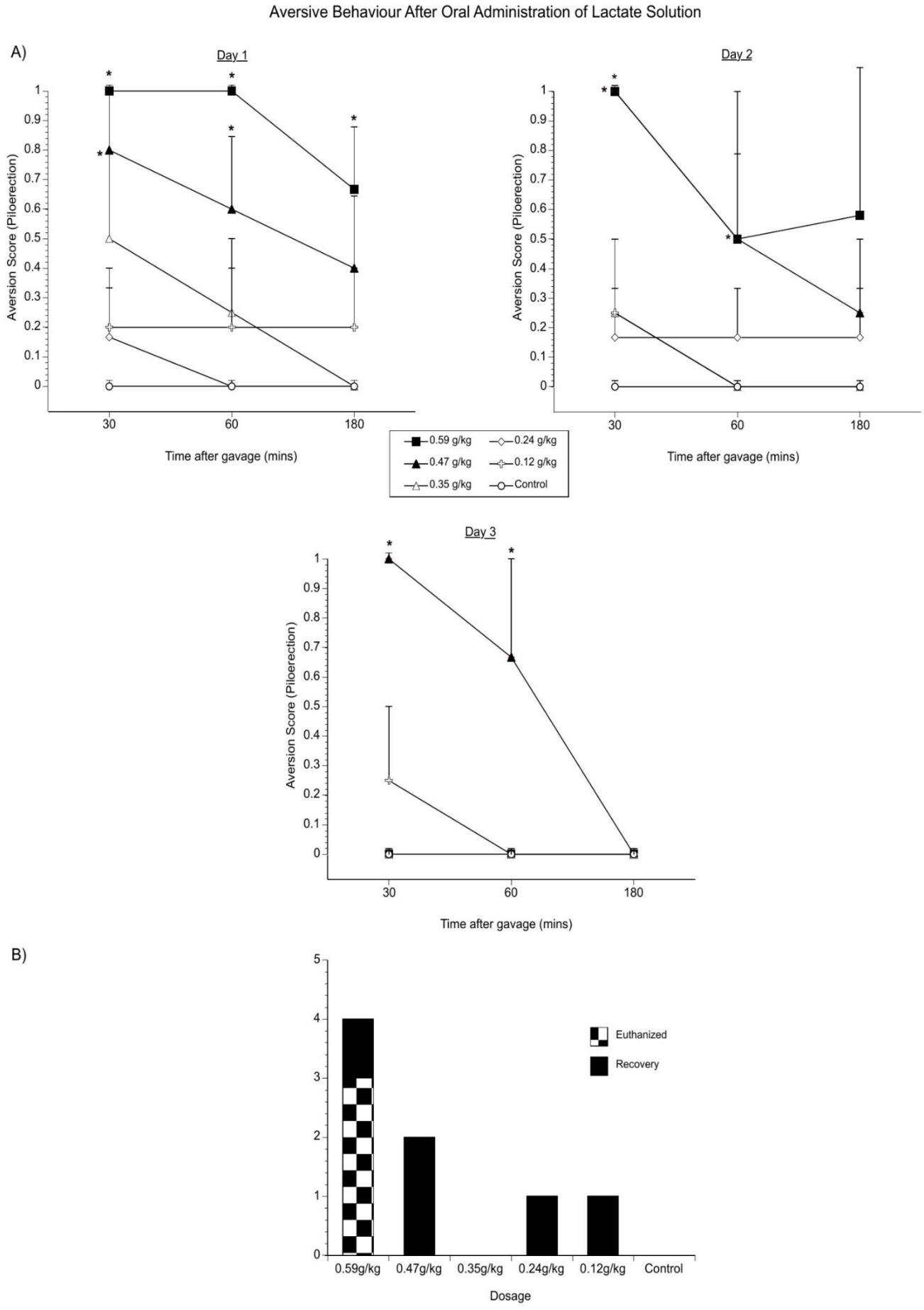

Aversive Behaviour After Oral Administration of Lactate Solution
A)
Day 1
Day 2
Day 3
Aversion Score (Piloerection)
Time after gavage (mins)
0.59 g/kg
0.47 g/kg
0.35 g/kg
0.24 g/kg
0.12 g/kg
Control
B)
Euthanized
Recovery
0.59g/kg
0.47g/kg
0.35g/kg
0.24g/kg
0.12g/kg
Control
Dosage

Figure 3. Aversive behavior incidence (A) and recovery (B) of mice administered 0.59 g/kg (n=5; n_f=1), 0.47 g/kg (n=5; n_f=3), 0.35 g/kg (n=5; n_f=5), 0.24 g/kg (n=5; n_f=4) or 0.12 g/kg (n=5; n_f=4) diluted 1:1 lactic acid (USP) and sodium DL-lactate 60% w/w solution or water control (n=5; n_f=5).

***** = significant difference from water (p= 0.05)

n_f = final groups sample size

Figure 4. Relative Peripheral Blood Lactate and Glucose Following Oral Administration of MCT Oils

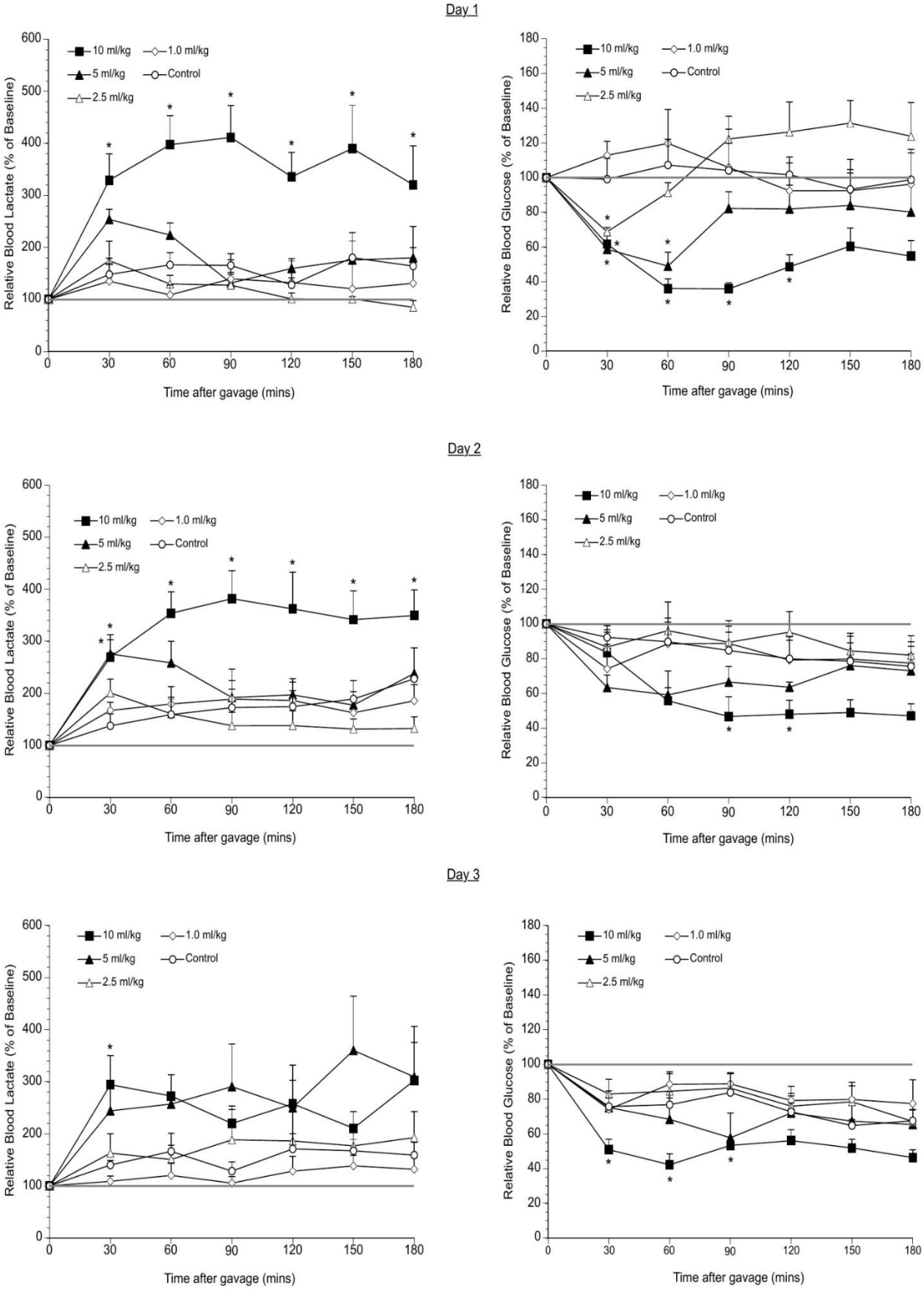


Figure 4. Peripheral blood lactate and glucose concentrations relative to baseline measurements after one to three days of 10 ml/kg (n=5; n_f=5), 5 ml/kg (n=5; n_f=4), 2.5 ml/kg (n=5; n_f=5) or 1.0 ml/kg (n=5; n_f=5) of medium chain triglyceride oils (~40% capric acid, ~60% caprylic acid) or water control (n=5; n_f=5). Blood levels were measured every 30 minutes elapsed over 180 minutes after oral administration.

Values are expressed as a percent of their baseline measured 30 minutes prior to administration and error bars represent the standard error of the mean.

***** = significant difference from water ($p < 0.05$)

n_f = final groups sample size

Figure 5.

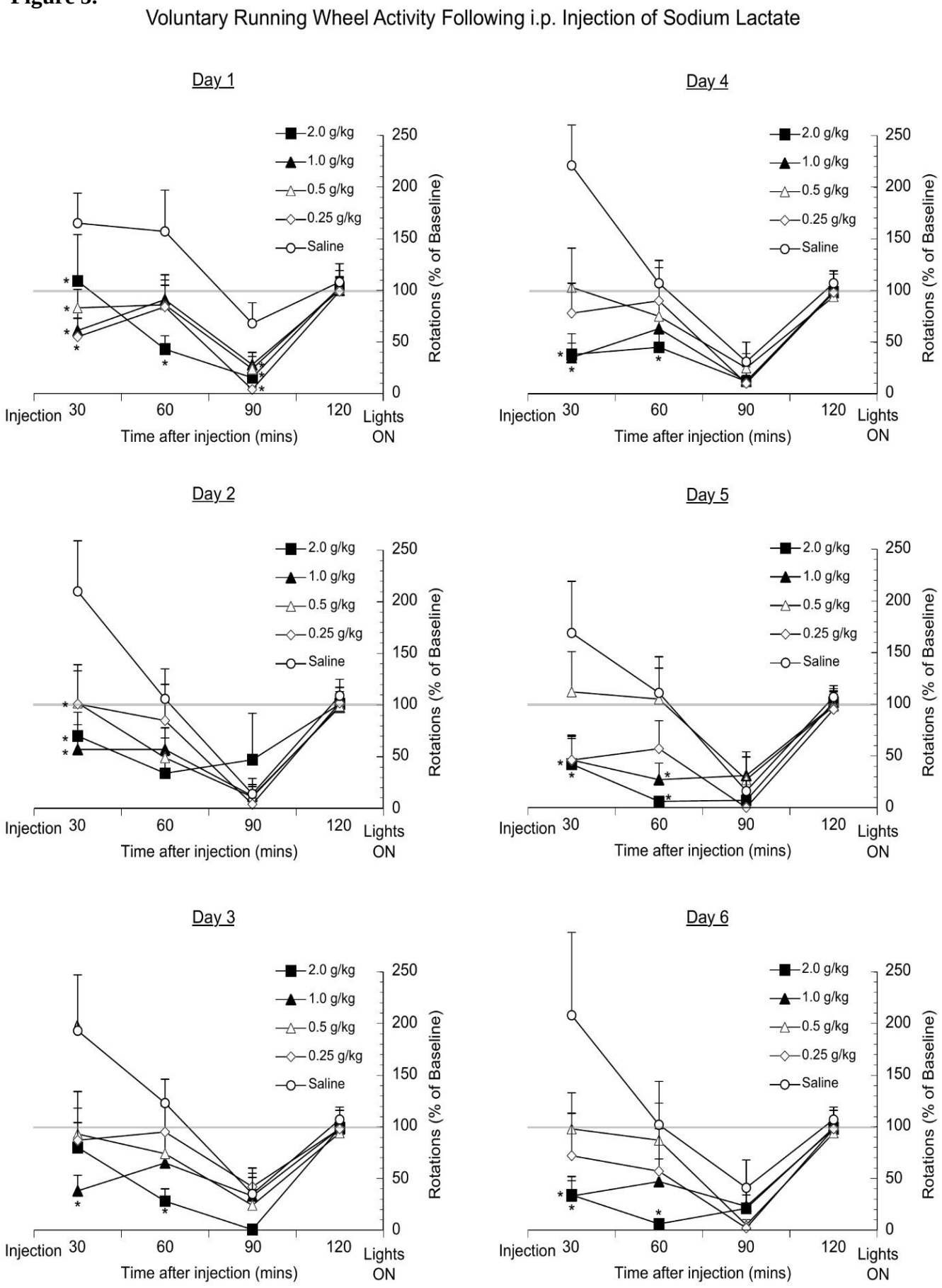

Voluntary Running Wheel Activity Following i.p. Injection of Sodium Lactate
Day 1
Day 4
Day 2
Day 5
Day 3
Day 6
2.0 g/kg
1.0 g/kg
0.5 g/kg
0.25 g/kg
Saline
Rotations (% of Baseline)
Injection
30
60
90
120
Lights ON
Time after injection (mins)

Figure 5. Relative running activity after one to six days of 2.0 g/kg (n=7; n_f=7), 1.0 g/kg (n=6; n_f=6), 0.5 g/kg (n=6; n_f=6), 0.25 g/kg (n=6; n_f=6) i.p. injections of diluted sodium lactate or 0.9% saline (n=6; n_f=6). Values are expressed as a percent of their baseline value (averaged across five days of pre-treatment) and error bars represent the standard error of the mean.

***** = significant difference from saline ($p < 0.05$)

n_f = final groups sample size

Supplementary material 1: Active Runner Categorization Protocol

Each day of pre-treatment observation, mice were scored according to late day and early evening running wheel activity (17:00 to 19:00 and 19:00 to 21:00, respectively). Late day activity scoring is calculated in reference to the daily average running wheel rotations, per 30 minutes segment, during peak activity. Peak running wheel activity plateaus daily between 09:00-12:00 and begins to decline between 17:00-18:00 (Harri et al., 1999). Active running behavior is scored as "2"; it is characterized by consistent relative decline of activity before Lights ON (17:00-19:00) and low or absent activity after Lights ON (19:00-21:00). Over-active running behavior is scored as "1"; it is characterized by consistent relative decline of activity before Lights ON but high running wheel activity thereafter. Inactive running behavior is scored as "0"; it is characterized by an absence of pre-Lights ON running wheel activity (regardless of absence or presence of activity post-Lights ON). Each mouse was evaluated at four time segments 30 minutes in duration pre- and post- Lights ON. Each time segment was evaluated as pass/fail. Mice that achieve three or four passes for either pre- or post-Lights ON activity are awarded 1 point for a possible score total of 2. Mice that receive 0 for pre-Lights ON activity will automatically receive 0 total score. As we will be prioritizing wake cycle activity analysis, pre-Lights ON activity absence is penalized.

"Active runner" Categorization

(Pre-Lights ON: decreasing but present activity; Post-Lights ON: low or absent activity)

1. Calculate average rotation per 30 minute segment during peak performance (09:00 to 12:00) (Harri et al., 1999).

Example:

Day 1	09:00-09:30	09:30-10:00	10:00-10:30	10:30-11:00	11:00-11:30	11:30-12:00	**Average**
ID 9	1642	976	0	0	1104	1804	**921**

2. Evaluate activity 120 minutes before designated sleep period (Lights ON).
    a. To pass: rotations made between 17:00 and 17:30 should be similar to the peak average. Allow for +/-10% error.
    b. Repeat (a) for rotations made between 17:30 and 18:00.
    c. To pass: rotations made between 18:00 and 18:30 should be at least 50% of rotations completed between 17:30 and 18:00.
    d. To pass: rotations made between 18:30 and 19:00 should be at least 50% of the rotations completed between 18:00 and 18:30.

Example:

Day 1	17:00-17:30	17:30-18:00	18:00-18:30	18:30-19:00
ID 9	1389	831	224	0
Ideal Range	1013-829	1013-829	<416	<112
Pass (y/n)	n	y	y	y

Day 1 ID 9 → 3 Passes = 1 point awarded.

3. Evaluate activity 120 minutes after the designated sleep period (Lights ON).

    a. To pass: rotations made between 19:00 and 19:30 should be similar to the peak average. Allow for +/-10% error.

    b. Repeat (a) for rotations made between 19:30 and 20:00.

    c. Repeat (a) for rotations made between 20:00 and 20:30.

    d. Repeat (a) for rotations made between 20:30 and 21:00.

Example:

Day 1 ID 9 → 4 Passes = 1 point

Conclusion: total 2 points → "Active runner"

"Over-active Runner" Categorization

(Pre-Lights ON: decreasing but present activity; Post-Lights ON: high activity)

1. Calculate average rotation per 30 minute segment during peak performance (09:00 to 12:00) (Harri et al., 1999).

Example:

Day 1	18:30-19:00	19:00-19:30	19:30-20:00	20:00-20:30	20:30-21:00
ID 9	0	0	0	0	0
Ideal Range	-	~0	~0	~0	~0
Pass (y/n)	-	y	y	y	y

Day 1	09:00-09:30	09:30-10:00	10:00-10:30	10:30-11:00	11:00-11:30	11:30-12:00	**Average**

ID 23	1232	845	1193	511	0	638	**737**

2. Evaluate activity 120 minutes before designated sleep period (Lights ON).
    a. To pass: rotations made between 17:00 and 17:30 should be similar to the peak average. Allow for +/-10% error.
    b. Repeat (a) for rotations made between 17:30 and 18:00.
    c. To pass: rotations made between 18:00 and 18:30 should be at least 50% of rotations completed between 17:30 and 18:00.
    d. To pass: rotations made between 18:30 and 19:00 should be at least 50% of the rotations completed between 18:00 and 18:30.

Example:

Day 1	17:00-17:30	17:30-18:00	18:00-18:30	18:30-19:00
ID 23	632	741	411	195
Ideal Range	811-663	811-663	<371	<206
Pass (y/n)	y	y	n	y

Day 1 ID 23 →4 Passes = 1 point

3. Evaluate activity 120 mins after the designated sleep period (Lights ON).
    a. Fail: Rotations made between 19:00 and 19:30 should be greater than 50% of the rotations completed between 18:30 and 19:00.
    b. Repeat (a) for rotations made between 19:30 and 20:00.

c. Repeat (a) for rotations made between 20:00 and 20:30.

d. Repeat (a) for rotations made between 20:30 and 21:00.

Example:

Day 1 ID 23 → 0 Passes = no points awarded

Conclusion: total 1 point → "Over active runner"

"Inactive Runner" Categorization

(Pre-Lights ON: low or absent activity; Post-Lights ON: absent, low or high activity)

1. Calculate average rotation per 30 minute segment during peak performance (09:00 to 12:00) (Harri et al., 1999).

Example:

Day 1	09:00-09:30	09:30-10:00	10:00-10:30	10:30-11:00	11:00-11:30	11:30-12:00	**Average**
ID 16	416	0	774	1932	1760	2059	**1157**
ID 27	1545	2274	1786	1850	2190	0	**1608**

2. Evaluate activity 120 minutes before designated sleep period (Lights ON).

Day 1	18:30-19:00	19:00-19:30	19:30-20:00	20:00-20:30	20:30-21:00
ID 23	195	615	711	307	244
Ideal Range	-	<98	<98	<98	<98
Pass (y/n)	-	n	n	n	n

a. Fail: rotations made between 17:00 and 17:30 should be dissimilar to the peak average. Allow for +/- 10% error.

b. Repeat (a) for rotations made between 17:30 and 18:00.

c. Fail: rotations made between 18:00 and 18:30 should be greater than a 50% reduction of rotations completed between 17:30 and 18:00.

d. Fail: rotations made between 18:30 and 19:00 should be at greater than a 50% reduction of rotations completed between 18:00 and 18:30.

Example:

Day 1	17:00-17:30	17:30-18:00	18:00-18:30	18:30-19:00
ID 16	0	0	786	584
Ideal Range	1273-1041	1273-1041	~0	~0
Pass (y/n)	n	n	n	n
ID 27	0	0	463	736
Ideal Range	1769-1447	1769-1447	~0	~0
Pass (y/n)	n	n	n	n

Day 1

Day 1 ID 16 → 0 Passes = no points awarded

Day 1 ID 27 → 0 Passes = no points awarded

3. Evaluate activity 120 mins after the designated sleep period (Lights ON).

a. Fail: Rotations made between 1900 and 1930, should be greater than 50% of the rotations completed between 1830 and 1900.

b. Repeat (a) for rotations made between 1930 and 2000.

c. Repeat (a) for rotations made between 2000 and 2030.

d. Repeat (a) for rotations made between 2030 and 2100.

Example:

Day 1	18:30-19:00	19:00-19:30	19:30-20:00	20:00-20:30	20:30-21:00
ID 16	584	818	880	936	1169
Ideal Range	-	~0	~0	~0	~0
Pass (y/n)	-	n	n	n	n

Day 1	18:30-19:00	19:00-19:30	19:30-20:00	20:00-20:30	20:30-21:00
ID 27	736	227	0	0	0
Ideal Range	-	<368	<368	<368	<368
Pass (y/n)	-	y	y	y	y

Day 1 ID 16 →

0 passes = no points awarded

Day 1 ID 27 → 4 passes = no points awarded* (See first paragraph)

Conclusion: Day 1 ID 16 → 0 points = "Inactive runner"

Day 1 ID 27 → 0 points = "Inactive runner"

To summarize…

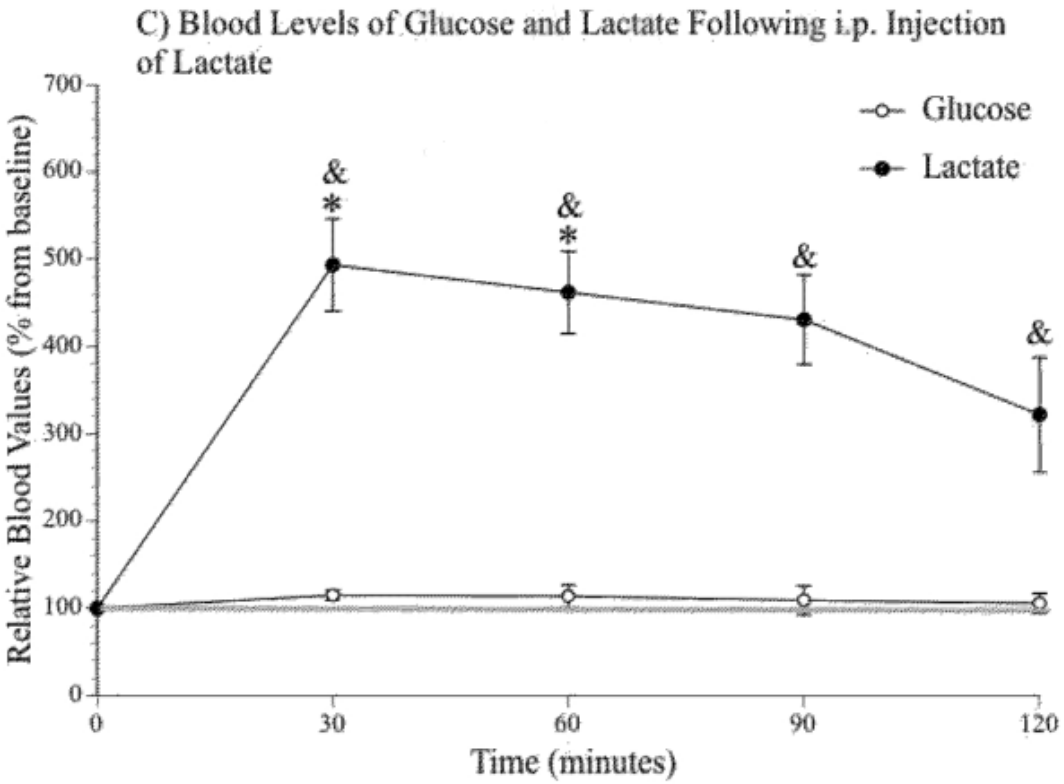
Behavior	Pre-Lights ON (17:00-19:00)	Post-Lights ON: (19:00-21:00)
Active Runner	Consistent decreasing activity	Absent or low activity
Over-active Runner	Consistent decreasing activity	High activity
Inactive Runner	Inconsistent activity	Absent or low activity
	Inconsistent activity	High activity

Supplementary material 2: Scored Baseline Running Wheel Activity

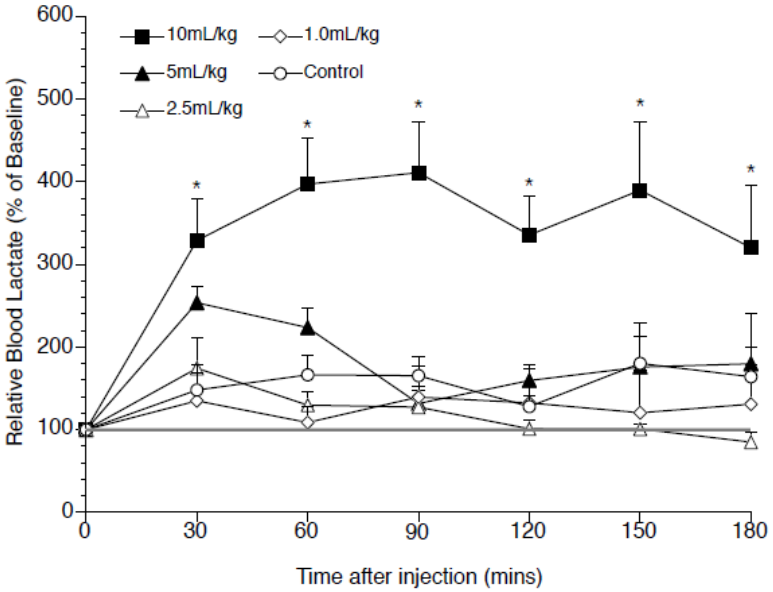
Round 1									Round 2								
Dosage (g/kg)	ID	Day					Tally	Total (%)	Dosage (g/kg)	ID	Day					Tally	Total (%)
		1	2	3	4	5					1	2	3	4	5		
Saline	1	2	2	2	2	2	10/10	60	Saline	47	2	2	2	0	2	8/10	52
	17	2	2	2	2	2	10/10			40	0	1	2	2	1	6/10	
	6	1	1	2	2	0	6/10			31	1	0	1	2	2	6/10	
	7	1	2	0	0	0	3/10			39	2	0	0	2	0	4/10	
	16	0	0	0	0	1	1/10			27	0	0	0	0	2	2/10	
0.25	2	2	2	2	2	2	10/10	76	0.25	38	2	2	0	2	2	8/10	56
	9	2	2	2	2	2	10/10			56	1	2	2	2	0	7/10	
	19	1	2	2	2	2	9/10			44	2	2	0	0	2	6/10	
	8	1	2	2	0	0	5/10			35	0	2	0	0	2	4/10	
	18	1	2	0	1	1	5/10			57	1	0	2	0	0	3/10	
0.5	3	2	2	2	2	2	10/10	82	0.5	52	2	1	2	0	2	7/10	54
	10	2	2	2	2	2	10/10			43	2	2	0	0	2	6/10	
	20	1	2	2	2	2	9/10			37	1	1	1	0	2	5/10	
	22	1	1	2	2	2	8/10			55	1	0	2	2	0	5/10	
	11	0	0	2	1	1	4/10			32	0	2	0	0	2	4/10	
1	21	2	2	2	2	2	10/10	76	1	34	1	1	1	2	1	6/10	40
	13	1	2	2	2	2	9/10			42	0	2	0	2	2	6/10	
	4	2	2	2	0	2	8/10			49	1	1	0	0	2	4/10	
	23	1	2	1	0	2	6/10			29	0	0	0	0	2	2/10	
	12	1	0	2	2	0	5/10			54	0	0	0	2	0	2/10	
2	5	0	2	2	2	2	8/10	72	2	48	0	2	2	2	2	8/10	42
	14	1	2	1	2	2	8/10			41	1	1	0	2	2	6/10	
	24	1	2	2	1	1	7/10			30	2	1	0	0	2	5/10	
	15	0	2	2	2	0	6/10			33	1	1	0	0	2	4/10	
-	-	-	-	-	-	-	-	-		45	0	0	0	0	2	2/10	
-	-	-	-	-	-	-	-	-		46	0	0	0	0	0	0/10	
-	-	-	-	-	-	-	-	-	Running Wheel Broke	36	0	0	0	0	0	0/10	0
-	-	-	-	-	-	-	-	-		53	0	0	0	0	0	0/10	

Supplementary material 2: Baseline running wheel activity of sample mice scored according to the novel identification of active runners. The table includes daily and culminative scoring of active running wheel activity during baseline recording. Scoring follows the protocol outline in Supplementary material 1.

Supplementary material 3: Previous Sodium Lactate and Current MCT Lactate Comparison



Béland-Millar, A., Larcher, J., Courtemanche, J., Yuan, T., & Messier, C. (2017). Effects of Systemic Metabolic Fuels on Glucose and Lactate Levels in the Brain Extracellular Compartment of the Mouse. *Frontiers in neuroscience*, *11*.



Supplementary material 3: Graphical comparisons of previous blood lactate concentration after i.p. injection of sodium lactate to blood lactate current oral gavage of medium chain triglycerides.

Chapter 3: Chronic sleep disruption induces depression-like behavior in adolescent male and female mice and sensitization of the hypothalamic-pituitary-adrenal axis in adolescent female mice

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Abstract

Depression is a prevalent mood disorder responsible for reduced quality of life for over 280 million people. Depression commonly develops during adolescence and becomes twice as prevalent in females than in males. However, the mechanisms underlying adolescent depression onset and sex differences in the prevalence rate remain unclear. Adolescent exposure to stress and subsequent sensitization of the hypothalamic-pituitary-adrenal (HPA) axis contributes to mood disorder development, and females are particularly vulnerable to HPA sensitization. Repeated exposure to stressors common to adolescent development, like sleep disruption, could partially be responsible for adolescent female susceptibility to depression. To address this possibility, 80 adolescent and adult CD-1 mice (Male, n= 40; Female, n= 40) were manually sleep disrupted for the first four hours of each rest cycle or allowed normal rest for eight consecutive days. Depression-like behavior was assessed with the forced swim test. 5-HT_{1A} and glucocorticoid receptor expression and concurrent cellular activation via glucocorticoid receptor/c-Fos colocalization were examined in various brain regions to assess cellular correlates of depression and HPA-axis activation. Both adolescent male and female mice displayed significantly greater depression-like behavior and prelimbic c-Fos expression after chronic sleep disruption than non-sleep disrupted adolescent and sleep disrupted adult counterparts. However, sleep disrupted adolescent females demonstrated greater dorsal raphe 5-HT_{1A} expression than sleep disrupted adolescent males. Adolescent females and males had decreased medial prefrontal 5-HT_{1A} expression after chronic sleep disruption, but only adolescent females expressed decreased hippocampal 5-HT_{1A} expression compared to controls. Chronic sleep disruption significantly increased corticosterone release, glucocorticoid expression in the CA1, and activation of glucocorticoid immunoreactive cells in the prelimbic cortex of adolescent females but not in adolescent males. These findings suggest that chronic sleep disruption during adolescence could give rise to depressive symptoms in male and female adolescents through differing signaling mechanisms.

Keywords: Adolescence; Depression; Chronic sleep disruption; Sex differences; Forced swim test; Immunohistochemistry

1.0 Introduction

Over 280 million people worldwide are affected by depression (James et al., 2018). Depression often begins during adolescence, a period of development following the onset of puberty which finalizes an organism's transition into adulthood (Tanner, 1962). Women are twice as likely to develop depression than men during adolescence (Hankin et al., 1998). This diagnostic disparity persists into adulthood and is observed across multiple countries (Weissman et al., 1993). Early-life stress is a predictor of the onset of depression in adolescents (Ge et al., 2001) and produces long-lasting changes to neuroendocrine signaling pathways (Romeo, 2010; Romeo & McEwen, 2006). Adolescents, especially females, are typically more susceptible to sensitization of the stress response after repeated stress challenges (Gallucci et al., 1993; McCormick et al., 1995; Romeo, 2010). Reducing exposure to chronic stress during adolescence before it engenders depression is a cost effective and efficient method of reducing depression development (James et al., 2018). However, exposure to certain chronic stressors may be inherent to normal adolescent development and therefore difficult to avoid entirely.

Adolescent development often includes a delay in sleep/wake schedule. Early adolescent sleep includes an early sleep onset similar to childhood sleep schedules. Mid- and late adolescent sleep patterns resemble adult sleep schedules as sleep onset becomes more delayed (Crowley et al., 2007). Delayed adolescent sleep onset is driven by a temporary lengthening of the intrinsic circadian cycle or "internal day" (Carskadon & Acebo, 2005) combined with a delayed accumulation of drowsiness measured by Slow Wave Activity (Jenni et al., 2005) resulting in an approximate +4-hour shift in average sleep onset by young adulthood (Chaput, Dutil, & Sampasa-Kanyinga, 2018; Crowley et al., 2007). Adolescent sleep delay is further exacerbated by "social jet lag" or the discrepancy between biological rhythm and reduced social constraints to conform to a regular sleep pattern on non-workdays (Roenneberg, Wirz-Justice, & Merrow, 2003; Wittmann et al., 2006). Chronic sleep onset delay acts as a chronic stressor (Puttonen, Härmä, & Hublin, 2010; Wittmann et al., 2006). Therefore, adolescent sleep delay is a stressor common to human development (Minkel et al., 2014). Adolescent sleep disruption has become more common in recent years alongside increased access to LED screens (Van den Bulck, 2004; van der Schuur, Baumgartner, Sumter, & Valkenburg,

2018) especially in adolescent females (van der Schuur et al., 2018). Increased chronic sleep disruption during adolescence may exacerbate modern development of depression.

Disruptions of sleep duration, onset and efficiency correlate highly with a variety of depressive states yet the causative direction of the relationship remains disputed (Lustberg & Reynolds III, 2000). According to the monoamine hypothesis, depression is caused by a dysregulation of neurotransmitter functionality in the central nervous system (Heninger et al., 1996). Brief decreases in serotonergic production and signaling are associated with depressive symptoms (Heninger et al., 1996). Many pharmacological treatments for depression are designed to increase the availability of serotonin (5-HT) in the synapse (Leonard, 1996). The majority of 5-HT used for signaling in most mammalian mid- and forebrain regions is produced in the dorsal raphe nucleus, a hindbrain region located near the brainstem (Michelsen, Schmitz, & Steinbusch, 2007). The G-protein coupled somatodendritic autoreceptor 5-HT_{1A} regulates dorsal raphe nuclei activity via a negative feedback loop (De Montigny et al., 1984). Increased 5-HT_{1A} receptor expression in the dorsal raphe nuclei inhibits 5-HT production resulting in decreased signaling to mid- and forebrain areas (Burnet et al., 1994). This decrease is characteristic of depressive disorders (Asberg et al., 1976). 5-HT_{1A} also functions as a postsynaptic receptor in projection areas innervated by 5-HT (De Montigny et al., 1984) and expression is typically downregulated in cortical and hippocampal tissue of depressed humans and rodent models (Lopez et al., 1998). Additionally, exposure to chronic sleep disruption decreases neuronal volume (Novati et al., 2011) and alters monoamine signaling (da Silva Rocha-Lopes et al., 2018) within rat and human hippocampi. Novati et al. (2011) observed reduced hippocampal volume in adolescent male rats after one month of daily 20-hour sleep disruption sessions. Adolescent male rats also express less dorsal hippocampal 5-HT_{1A} after 21 days of 18-hour sleep disruption sessions (da Silva Rocha-Lopes et al., 2018). Cellular activity in cortical and hippocampal tissue is heavily influenced by the hypothalamic-pituitary-adrenal (HPA) axis (Romeo et al., 2006). HPA-axis activity may influence mechanisms responsible for initiating 5-HT_{1A} expression typical of a depressive phenotype (Chiba et al., 2012; Ge et al., 2001; Guidotti et al., 2013).

The HPA-axis is a stress-responsive neuroendocrine system that helps an organism adapt to increasing demands from the environment and re-establish homeostasis after a challenge (Kudielka & Kirschbaum, 2005). Upon stress exposure, the paraventricular nucleus of the hypothalamus secretes corticotrophin releasing hormone which signals the pituitary gland to release adrenocorticotropic hormone and, in turn, causes the adrenal glands to secrete glucocorticoids. Chronic stress exposure sensitizes the HPA-axis and results in increased production and transport of glucocorticoids to the central nervous system (Gallucci et al., 1993). Corticosterone is the most prevalent glucocorticoid in mice and binds to glucocorticoid receptors in areas implicated in stress reactivity such as the hippocampus (McEwen, 2005) and medial prefrontal cortex (Chiba et al., 2012). Adolescent females are more vulnerable than males to stress-induced sensitization of HPA-axis (Girard-Joyal et al., 2015; McCormick & Mathews, 2007). Activation of the HPA axis may be inferred by observing increased co-expression or "colocalization" of the c-Fos protein, an immediate early gene, in cells immunoreactive to glucocorticoid receptor antibodies, and increased serum corticosterone after an acute novel stressor (Girard-Joyal et al., 2015; Lehner et al., 2009; Ostrander, Richtand, & Herman, 2003). Chronic sleep disruption selectively moderates HPA-axis functionality in adult male rats (Koban, Le, & Hoffman, 2006; Martins, Marques, Tufik, & D'Almeida, 2010; Meerlo, Koehl, Van der Borght, & Turek, 2002). Chronic paradoxical sleep disruption over 5 consecutive days increases corticotropin releasing hormone in the paraventricular nucleus of adult male rats (Koban et al., 2006). Adult male rats also experience increased serum corticosterone after 21 consecutive days of 18-hour chronic sleep disruption (Martins et al., 2010). Interestingly, 8 consecutive sessions of 18-hour chronic sleep disruption have been shown to sensitize the HPA-axes of adult male rats to novel stressors (Meerlo et al., 2002). Adult female rats show increased corticosterone release after 4 days of paradoxical sleep deprivation (Antunes, Andersen, Baracat, & Tufik, 2006). Weight gain also follows sleep deprivation in human and animal models displaying heightened HPA activity (da Silva Rocha-Lopes et al., 2018; Koban et al., 2006; Spiegel et al., 2004; Spiegel et al., 1999; Spiegel et al., 2009; Vetrivelan et al., 2012). However, it is unclear if adult females experience similar HPA-axis sensitization to novel stress following chronic sleep disruption (Girard-Joyal et al., 2015; Lehner et al.,

2009; Ostrander et al., 2003). Furthermore, sex-specific signaling along the HPA-axes following chronic sleep disruption and novel stress remains relatively unexplored, particularly in adolescent samples.

The HPA-axis continues to develop throughout adolescence (McCormick & Mathews, 2007). Adolescent exposure to chronic stress delays HPA-axis maturation (Romeo, 2010) especially in females (Gallucci et al., 1993; McCormick et al., 1995). The relationship between underdeveloped HPA axes, serotonergic functioning of dorsal raphe nuclei and depression is unclear. Originally, increased 5-HT signaling to the paraventricular nucleus of the hypothalamus from the dorsal raphe nucleus was found to stimulate adrenocorticotropin hormone and subsequent glucocorticoid release (L. Pan & Gilbert, 1992). However, these findings have been subject to debate since Welch, Farrar, Dunn and Saphier (Welch, Farrar, Dunn, & Saphier, 1993) reported 5-HT mediated inhibition of adrenocorticotropin hormone release in the paraventricular nucleus. Further investigation is necessary to define the exact relationship between the dorsal raphe nucleus and HPA-axis functionality.

The current study investigated the effects of chronic sleep disruption of regular sleep onset on cellular and behavioral correlates of depression and on its ability to sensitize the HPA axis in adolescent and adult male and female mice. To model the 4-hour sleep delay demonstrated in human adolescent development (Chaput et al., 2018; Crowley et al., 2007), adolescent mice were sleep disrupted for the first 4-hours of their rest phase. The effects of chronic sleep disruption were contrasted between 6-week-old adolescent and 10-week-old adult mice as the HPA axis of 6-week-old CD-1 mice is particularly sensitive to impaired functionality following stress exposure, an effect that normalizes in adulthood by week 10 (Holder & Blaustein, 2014; Pignatelli et al., 2006; Spinedi et al., 1997; Walker et al., 2001). We hypothesized that sleep-disrupted adolescent mice, particularly the adolescent females, would demonstrate depression-like behavior, increased 5-HT_{1A} expression in the dorsal raphe nuclei and decreased 5-HT_{1A} expression in projection areas. We also hypothesized that there would be increased glucocorticoid release, greater glucocorticoid receptor expression, and increased activity of glucocorticoid receptor immunoreactive cells in the hippocampus and medial prefrontal cortex of adolescent mice exposed to chronic sleep disruption. Furthermore,

we hypothesized that sleep disrupted male and female mice would display greater weight gain compared to rested counterparts.

2.0 Material and methods

2.1 Animals

Three-week-old male and female CD-1 mice (n=80; Charles River Laboratories, St-Constant, QC, Canada) were housed in groups of two or three per cage with *ab libitum* access to food (Envigo, Rodent Chow, Mississauga, Canada) and water. Cages were bedded with corn cob chips (Cat# 7097, Envigo) and included a cardboard hut (Cat# XKA2455, Ketchum) and one piece of nestlet (Cat# NES3600, Ancare). Housing rooms were maintained at 23 ± 2℃ with 60% humidity and a 12-hour light cycle (lights off/active phase from 8:00 PM to 8:00 AM). All experimental procedures were approved by the Animal Care Committee of the University of Ottawa according to the Canadian Council on Animal Care (CCAC) guidelines.

2.2 Timeline

At 6 or 10 weeks of age, male and female mice were either allowed to rest or subjected to chronic sleep disruption during the first 4 hours of their rest phase (8:00 AM to 12:00 PM) for 8 consecutive days (group n = 10). Mice allowed normal rest remained undisturbed until the forced swim test. The forced swim test was performed during the active phase of the light-dark cycle (10:00 PM to 12:00 AM) on the final day of chronic sleep disruption. Fourteen hours after final sleep disruption, control and experimental mice were subjected to a restraint stress for 30 minutes. Thirty minutes after the end of the restraint stress, mice were euthanized, and blood and brain were extracted. Control and experimental mice were euthanized at the same time of day. Weight was measured before and after chronic sleep disruption and analyzed as total individual change in mass (grams) during this period. Mice demonstrating weight loss exceeding 15% would be categorized as overstressed and removed from the experiment. No mice met this criterion.

2.3 Chronic sleep disruption

The chronic sleep disruption procedure was adapted from the “gentle manipulation sleep deprivation” protocol (Gross et al., 2015; S.-R. Yang, Sun, Huang,

Yao, & Qu, 2012) to ensure negligible impact of handling stress. Sleep restricted mice were moved to a separate holding area at the beginning of the first 4 hours (8:00 AM to 12:00 PM) of each rest phase for 8 consecutive days and were continuously observed by three experimenters. Wakefulness was operationally defined as constant locomotion or interactions with cage-mates (Tadavarty, Kaan, & Sastry, 2009). This alert state was maintained through gentle sensory stimulation (stroking the nape of the neck and back with a soft paintbrush) to induce arousal. Experimenters applied the stimulation when lethargy (reduction of locomotion), drooping eye lids, and/or decreased respiratory rates were observed. At the end of the 4-hour chronic sleep disruption session, mice were returned to the housing facility. Control mice were not manipulated during the 4-hour experimental period to avoid unintentional sleep disruption caused by handling. However, location of control mice cages was shuffled each day during the active phase of the light-dark cycle (7:00 am) to simulate transport of experimental mice to separate holding area.

2.4 Forced swim test

The forced swim test was used to measure the level of behavioral despair during the active phase of the light-dark cycle (10:00 PM to 12:00 AM) using the protocol described in more detail by Castagné et al. (2010). Testing was carried out under dim red light. Briefly, mice were allowed to acclimatize to the room for 1-hour prior to testing. Mice were individually placed in a 4 L beaker filled with 3 L of water (22°C +/- 2°C) for 5 minutes. Upon completion, mice were removed from the apparatus, dried and placed in recovery cages (30°C) for 5 minutes. The amount of time spent swimming (lateral movement), climbing (attempts at vertical climbing with no lateral movement), immobile (minimal movement to maintain balance only) as well as the latency to immobility (time elapsed from test initiation to first instance of immobility) were recorded using a video tracking software (EthoVision, Noldus, Leesburg, Virginia, USA). Behavior occurring within the final 4-minute period was scored by two experimenters blind to experimental conditions using the ODLog software (MACROPOD). Latency scores were added to improve the validity of the forced swim test and were recorded starting immediately after placing mice in the 4 L beakers (Castagné et al., 2009).

2.5 Novel restraint stressor, blood extraction, and intracardial perfusion

Sixty minutes prior to euthanasia, mice were restrained in ventilated plastic tubes for 30 minutes. Upon completion, mice were released into their home cage for 30 minutes (Ledoux, Sastre, Buda, Luppi, & Jouvet, 1996). Then, they were injected intraperitoneally with 500 mg/kg pentobarbital (Euthanyl, Virbac AH Inc., Fort Worth, TX, USA). After verifying the absence of pain reflexes, blood was extracted from the right heart ventricle with a winged infusion set. Blood samples were collected into two capillary tubes (Microvette CB300Z, Cat# 16.440.100, Sarstedt) and stored at 4^{o}C for 24 hours then centrifuged. Serum was extracted into 100µl tubes and stored at -80^{o}C until analysis. Following blood extraction, mice were intracardially perfused with 20 ml of 0.9% saline followed by 20 ml of 4% paraformaldehyde (PFA). Brains were extracted and post-fixed in 4% PFA for 2 hours. Brains were then placed in 30% sucrose for 14 hours. The sucrose solution was changed, and brains were placed in fresh 30% sucrose solution for a minimum of 48 hours. Brains were sliced into 4 series using an automated vibrating blade microtome (Leica VT1200) at a thickness of 40 µm. Brain sections were stored in a cryoprotectant solution at -20^{o}C.

2.6 ELISA

Blood serum was analysed for corticosterone using an enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Farmingdale, NY, Cat# ADI-900-097), according to the manufacturer's protocol and analyzed with a Biotek PowerWave XS2 Microplate Spectrophotometer using Gen 5 V2.0 software. Plates were coated with a donkey anti-sheep antibody (Enzo Life Sciences, Cat # 80-0045). Corticosterone detection is sensitive to a minimum of 27 pg/ml. Inter- and intra-assay variances of low, medium and high corticosterone concentrations are <10% except for low concentration inter-assay variance (~13.1%).

2.7 Immunofluorescence and immunohistochemistry

Free floating brain sections from 1 of the 4 series were rinsed in phosphate buffer solution and exposed to a donkey serum blocking solution. Tissue was incubated at 4^{o}C overnight (~16 hours) in rabbit anti-glucocorticoid receptor (1:600, Abcam/ab3671,

Cambridge, MA, USA) and guinea pig anti-c-Fos (1:450, Synaptic Systems, Gottingen, Germany) antibody solutions. Tissue was incubated in the dark for an additional 3 hours in secondary donkey anti-rabbit (1:1000, Alexa Fluor 594, Thermo Fisher Scientific, Waltham, MA, USA) and anti-guinea pig (1:1000, Alexa 488, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) antibody solutions.

An alternate series of sections was rinsed with tris-buffered saline (TBS; pH 7.6). Sodium citrate and glycine antigen retrieval was performed. Sections were washed in a goat serum-based blocking solution and incubated at 4^{o}C overnight (~16 hours) in a 5-HT_{1A} antibody (1:400, Abcam/ab85615, Cambridge, MA) solution. Sections were incubated in a secondary biotinylated goat anti-rabbit antibody (1:500, Vector/BA-1000, Burlingame, Ca) for 60 minutes followed by a 60-minute incubation in an avidin-biotin complex solution (Vector Vectastain ABC kit Elite Pk-6100 standard, Vector Laboratories, Burlingame, CA, USA). Targeted 5-HT_{1A} receptors were stained using 3, 3′-Diaminobenzidine (DAB) solution (DAB Peroxidase Substrate kit, SK-4100 Vector Laboratories, Burlingame, CA, USA).

2.8 Image capturing, scoring and colocalization

Images of the medial prefrontal cortex (+1.94 mm from Bregma), hippocampus (-1.94 mm from Bregma), and dorsal raphe nucleus (-4.60 mm from Bregma) were captured using a FLUOVIEW FV1000 confocal microscope (OLYMPUS). Two experimenters blind to treatment conditions manually cell-counted 5-HT_{1A}, glucocorticoid receptor and c-Fos immunoreactive cells using the Fiji (ImageJ) software. Simultaneous immunoreactivity to glucocorticoid receptor and c-Fos was scored with pixel colocalization analysis. This procedure is described in more detail by Dunn et al. (2011) and has been previously used to assess glucocorticoid receptor and c-Fos co-expression related to mood disorder (Lehner et al., 2009). Briefly, colocalization refers to several co-occurrence/correlation-based algorithms that compare the overlap and relative intensity of two fluorescent signals occupying individual pixels within a select area. Pixels are scored according to the fluorescent intensity of each signal and used to calculate a single correlation coefficient per image (FV10-ASW 3.0 software, Olympus). Greater coefficient values represent increased colocalization or greater

immunofluorescent overlap. The inverse is also true. Greater glucocorticoid receptor/c-Fos colocalization indicates increased co-expression of glucocorticoid receptor and c-Fos proteins indicating increased activity of cells implicated in the HPA-axis. Colocalization was calculated using the Mander's Overlap Coefficient equation due to its focus on co-occurrence and protection from intensity variation that arise from secondary antibody selection only.

Glucocorticoid receptor expression and glucocorticoid receptor/c-Fos colocalization was not investigated in the dorsal raphe nuclei. Multiple investigations indicate that glucocorticoids, particularly corticosterone, do not directly repress 5-HT_{1A} expression in the dorsal raphe nuclei via glucocorticoid receptor activation (de Kloet, Sybesma, & Reul, 1986; Meijer et al., 2000).

2.9 Statistical Analysis

Three-way (Age x Sex x Sleep) ANOVAs were used to evaluate forced swim test scores (duration of immobility, latency to immobility, duration of swimming and duration of climbing), receptor expression (5-HT_{1A}, glucocorticoid receptor, and c-Fos) and glucocorticoid receptor/c-Fos colocalization (n = 10). Omnibus and pairwise comparisons were significant at $p < 0.05$. Omnibus effect sizes were reported as partial eta squared (η_P^2). Pairwise comparisons were reported as differences between group means (MD), standard error values (SE) and p values. Pairwise comparisons were corrected according to the Bonferroni method. Outliers were identified by z-scores greater than +1.96 or less than -1.96 and Winsorized to the next extreme score (Anscombe, 1973). All statistical analyses were performed using SPSS (v.24, IBM).

3.0 Results

3.1 Forced swim test

3.1.1 Immobility duration

There was a main effect of Age ($F_{(1,72)} = 11.2$, $p < 0.01$, $\eta_P^2 = 0.13$) and a significant Age x Sleep interaction ($F_{(1,72)} = 4.1$, $p < 0.05$, $\eta_P^2 = 0.05$) for the duration of immobility behavior (Fig. 1A). Overall, adolescent mice displayed greater duration of immobility than adults (MD = 23.7, SE = 7.1, $p = 0.001$). Moreover, regardless of sex,

sleep disrupted adolescent mice displayed longer immobility duration than non-sleep disrupted adolescent (MD = 25.2, SE = 10.1, $p = 0.02$) and sleep disrupted adult counterparts (MD = 38.2, SE = 10.1, $p = 0.001$).

3.1.2 Latency to immobility

There were main effects of Age ($F_{(1,72)} = 10.0$, $p < 0.01$, $\eta_P^2 = 0.12$), Sex ($F_{(1,72)} = 6.5$, $p < 0.05$, $\eta_P^2 = 0.08$) and Sleep ($F_{(1,72)} = 15.2$, $p < 0.01$, $\eta_P^2 = 0.17$) and a significant Age x Sleep interaction ($F_{(1,72)} = 11.9$, $p < 0.01$, $\eta_P^2 = 0.14$) for the latency to immobility (Fig. 1B). Regardless of age and sleep condition, female mice displayed significantly shorter latency to immobility than male mice (MD = -10.5, SE = 4.1, $p = 0.01$). Regardless of sex, sleep disrupted adolescent mice displayed significantly shorter latency to immobility than non-sleep disrupted adolescent (MD = -30.1, SE = 5.8, $p < 0.001$) and sleep disrupted adult counterparts (MD = -27.1, SE = 5.8, $p < 0.001$).

3.1.3 Swimming duration

There was no significant effect of Age, Sex or Sleep on swimming behavior (Fig. 1C).

3.1.4 Climbing duration

There were main effects of Sex ($F_{(1,72)} = 28.5$, $p < 0.01$, $\eta_P^2 = 0.9$) and Sleep ($F_{(1,72)} = 5.5$, $p < 0.05$, $\eta_P^2 = 0.07$) and significant Age x Sleep ($F_{(1,72)} = 4.5$, $p < 0.05$, $\eta_P^2 = 0.06$) and Age x Sex x Sleep ($F_{(1,72)} = 7.4$, $p < 0.01$, $\eta_P^2 = 0.09$) interactions on climbing behavior (Fig. 1D). Overall, male mice displayed greater duration of climbing behavior than female mice (MD = 16.7, SE = 3.1, $p < 0.001$). Sleep disrupted mice also displayed greater duration of climbing behavior than non-sleep disrupted counterparts (MD = 7.3, SE = 3.1, $p = 0.02$). Regardless of sex, sleep disrupted adult mice displayed greater duration of climbing behavior than non-sleep disrupted adult (MD = 14.0, SE = 4.4, $p = 0.002$) and sleep disrupted adolescent counterparts (MD = 12.9, SE = 4.4, $p = 0.005$). Sleep disrupted adult females displayed greater duration of climbing behavior than non-sleep disrupted adult female (MD = 26.9, SE = 6.3, $p < 0.001$) and sleep disrupted adolescent female counterparts (MD = 25.6, SE = 6.3, $p < 0.001$). Non-sleep disrupted adolescent males displayed greater duration of climbing behavior than non-sleep

disturbed adolescent females (MD = 16.8, SE = 6.3, $p = 0.009$). Similarly, non-sleep disrupted adult males displayed greater duration of climbing behavior than non-sleep disturbed adult females (MD = 25.5, SE = 6.3, $p < 0.001$). However, sleep disrupted adolescent males displayed greater duration of climbing behavior than sleep disrupted adolescent female mice (MD = 25.0, SE = 6.3, $p < 0.001$). Climbing duration did not significantly differ between sleep disrupted adult males and sleep disrupted adult females.

3.2.0 Corticosterone

There were main effects of Sex ($F_{(1,79)} = 59.0$; $p < 0.01$, $\eta_P^2 = 0.45$) and Sleep ($F_{(1,79)} = 5.6$, $p < 0.05$, $\eta_P^2 = 0.07$) and significant Age x Sex ($F_{(1,72)} = 4.0$, $p < 0.05$, $\eta_P^2 = 0.05$) and Sex x Sleep ($F_{(1,72)} = 10.0$, $p < 0.01$, $\eta_P^2 = 0.12$) interactions (Fig. 1E). Overall, female mice displayed greater corticosterone concentrations than male mice (MD = 219.4, SE = 28.6, $p < 0.001$). Regardless of age and sex, sleep disrupted mice displayed greater corticosterone concentration than non-sleep disrupted mice (MD = 67.9, SE = 28.6, $p = 0.02$). Regardless of sleep condition, adult female mice displayed greater corticosterone concentrations than pubertal female mice (MD = 97.5, SE =38.9, $p = 0.01$). Regardless of age, sleep disrupted female mice displayed greater corticosterone concentrations than non-sleep disrupted female counterparts (MD = 158.3, SE =38.9, $p < 0.001$).

3.3.0 5-HT$_{1A}$ receptor expression

3.3.1 Dorsal raphe nuclei

There was a main effect of Sex ($F_{(1,63)} = 6.3$, $p < 0.05$, $\eta_P^2 = 0.09$) and a significant Sex x Sleep interaction ($F_{(1,63)} = 6.1$, $p < 0.05$, $\eta_P^2 = 0.09$) on the number of 5-HT$_{1A}$ expressing cells in the dorsal raphe nucleus (Fig. 3A). Overall, female mice displayed more 5-HT$_{1A}$ expressing cells in the dorsal raphe than males (MD = 82.5. SE = 33.0, $p = 0.02$). Regardless of age, sleep disrupted female mice displayed greater 5-HT$_{1A}$ expressing cells in the dorsal raphe than sleep disrupted male counterparts (MD = 164.2, SE = 46.5, $p < 0.001$).

3.3.2 CA1 region of the hippocampus

There was no effect of Age, Sex or Sleep on the number of 5-HT_{1A} expressing cells in the CA1 region of the hippocampus (Fig. 3B).

3.3.3 CA2 region of the hippocampus

There was a significant Age x Sleep interaction on the number of 5-HT_{1A} expressing cells in the CA2 region of the hippocampus ($F_{(1,58)} = 10.7$, $p < 0.01$, $\eta_P^2 = 0.16$) (Fig. 3C). Regardless of sex, sleep disrupted adolescent mice displayed greater 5-HT_{1A} expressing cells in the CA2 region compared to non-sleep disrupted adolescent counterparts (MD = 2.2, SE = 0.9, $p = 0.01$). In contrast, sleep disrupted adult mice displayed less 5-HT_{1A} expressing cells in the CA2 region compared to non-sleep disrupted adult counterparts (MD = -1.8, SE = 0.9, $p = 0.04$).

3.3.4 CA3 region of the hippocampus

There were main effects of Age ($F_{(1,58)} = 7.8$, $p < 0.01$, $\eta_P^2 = 0.12$) and Sleep ($F_{(1,58)} = 5.0$, $p < 0.05$, $\eta_P^2 = 0.08$) and significant Sex x Sleep ($F_{(1,58)} = 5.2$, $p < 0.05$, $\eta_P^2 = 0.08$) and Age x Sex x Sleep ($F_{(1,58)} = 4.3$, $p < 0.05$, $\eta_P^2 = 0.07$) interactions on the number of 5-HT_{1A} expressing cells in the CA3 region of the hippocampus (Fig. 3D). Regardless of sex and sleep disruption condition, adolescent mice expressed less 5-HT_{1A} immunoreactive cells in the CA3 region compared to adult mice (MD = -2.1, SE = 0.7, $p = 0.007$). Moreover, regardless of age and sex, sleep disrupted mice expressed less 5-HT_{1A} expressing cells in the CA3 than non-sleep disrupted control mice (MD = -1.7, SE = 0.7, $p = 0.03$). Sleep disrupted female mice displayed less 5-HT_{1A} expressing cells in the CA3 compared to non-sleep disrupted adolescent female (MD = -3.4, SE = 1.07, $p = 0.003$) and sleep disrupted male counterparts (MD = -3.0, SE = 1.0, $p = 0.005$). Sleep disrupted adolescent females displayed less 5-HT_{1A} expressing cells in the CA3 region compared to non-sleep disrupted adolescent females (MD = -4.9, SE = 1.5, $p = 0.002$), sleep disrupted adolescent males (MD = -4.4, SE = 1.5, $p = 0.005$) and sleep disrupted adult females (MD = -3.5, SE = 1.5, $p = 0.02$).

3.3.5 Anterior cingulate cortex

There was a main effect of Sleep ($F_{(1,56)} = 17.3$, $p < 0.01$, $\eta_P^2 = 0.24$) and a significant Age x Sex interaction ($F_{(1,56)} = 8.6$, $p < 0.01$, $\eta_P^2 = 0.13$) on the number of 5-HT_{1A} expressing cells in the anterior cingulate cortex (Fig. 3E). Overall, sleep disrupted mice expressed less 5-HT_{1A} immunoreactive cells in the anterior cingulate cortex than non-sleep disrupted control mice (MD = -20.9, SE = 5.0, $p < 0.001$). Regardless of sleep disruption condition, adult males displayed significantly more 5-HT_{1A} expressing cells in the anterior cingulate cortex than their adult female (MD = 19.3, SE = 6.5, $p = 0.01$) and adolescent male (MD = 24.2, SE = 7.3, $p = 0.002$) counterparts.

3.3.6 Prelimbic cortex

There were main effects of Sex ($F_{(1,56)} = 5.7$, $p < 0.05$, $\eta_P^2 = 0.09$) and Sleep ($F_{(1,56)} = 13.6$, $p < 0.01$, $\eta_P^2 = 0.20$) on the number of 5-HT_{1A} expressing cells in the prelimbic cortex (Fig. 3F). Overall, female mice expressed less 5-HT_{1A} immunoreactive cells in the prelimbic cortex compared to male mice (MD = -15.3. SE = 6.4, $p = 0.02$). Moreover, regardless of age and sex, sleep disrupted mice expressed less 5-HT_{1A} immunoreactive cells in the prelimbic cortex than non-sleep disrupted control mice (MD = -23.5, SE = 6.4, $p < 0.001$).

3.3.7 Infralimbic cortex

There was a main effect of Sleep on the number of 5-HT_{1A} expressing cells in the infralimbic cortex ($F_{(1,56)} = 11.0$, $p < 0.01$, $\eta_P^2 = 0.16$) (Fig. 3G), where sleep disrupted mice displayed less 5-HT_{1A} receptor expression than non-sleep disrupted control mice (MD = -35.7, SE = 10.8, $p = 0.002$) overall.

3.4.0 Glucocorticoid receptor expression

3.4.1 CA1 region of the hippocampus

There were main effects of Age ($F_{(1,71)} = 4.5$, $p < 0.05$, $\eta_P^2 = 0.06$) and Sleep ($F_{(1,71)} = 4.6$, $p < 0.05$, $\eta_P^2 = 0.06$) on the number of glucocorticoid receptor expressing cells in the CA1 region of the hippocampus (Fig. 4A). Overall, adolescent mice displayed less glucocorticoid receptor expressing cells in the CA1 region than adult mice (MD = -10.6, SE = 5.0, $p = 0.037$). Moreover, sleep disrupted mice displayed less glucocorticoid

receptor expressing cells in the CA1 region than non-sleep disrupted mice (MD = -10.6, SE = 5.0, $p = 0.035$).

3.4.2 CA2 region of the hippocampus

There was no significant effect of Age, Sex or Sleep on the number of glucocorticoid receptor expressing cells in the CA2 region of the hippocampus (Fig. 4B).

3.4.3 CA3 region of the hippocampus

There was a main effect of Age on the number of glucocorticoid receptor expressing cells in the CA3 region of the hippocampus ($F_{(1,71)} = 4.6$, $p < 0.05$, $\eta_P^2 = 0.06$) (Fig. 4C) with adolescent mice displaying less glucocorticoid receptor immunoreactive cells in the CA3 region than adult mice (MD = -5.0, SE = 2.4, $p = 0.036$).

3.4.4 Anterior cingulate cortex

There was no significant effect of Age, Sex or Sleep on the number of glucocorticoid receptor expressing cells in the anterior cingulate cortex (Fig. 4D).

3.4.5 Prelimbic cortex

There was a significant Age x Sex x Sleep interaction on the number of glucocorticoid receptor expressing cells in the prelimbic cortex ($F_{(1,52)} = 7.2$, $p < 0.01$, $\eta_P^2 = 0.12$) (Fig. 4E). Sleep disrupted adult females expressed less glucocorticoid receptor immunoreactive cells than non-sleep disrupted adult female (MD = -156.2, SE = 48.4, $p = 0.002$), sleep disrupted adult male (MD = -106.4, SE = 48.4, $p = 0.03$), and sleep disrupted adolescent female counterparts (MD = -121.6, SE = 48.4, $p = 0.02$).

3.4.6 Infralimbic cortex

There was no significant effect of Age, Sex or Sleep on the number of glucocorticoid receptor expressing cells in the infralimbic cortex (Fig. 4F).

3.5.0 C-Fos expression

3.5.1 Hippocampus

There was no significant effect of Age, Sex or Sleep on the number of c-Fos expressing cells in the hippocampal CA1, CA2 and CA3 regions of the hippocampus (Fig. 5A-C).

3.5.2 Anterior cingulate cortex

There was a main effect of Age on the number of c-Fos expressing cells in the anterior cingulate cortex ($F_{(1,58)} = 4.4$, $p < 0.05$, $\eta_P^2 = 0.07$) (Fig. 5D) with adolescent mice displaying more c-Fos immunoreactive cells in the anterior cingulate than adult mice (MD = 9.2, SE = 4.4, $p = 0.04$) overall.

3.5.3 Prelimbic cortex

There were main effects of Age ($F_{(1,58)} = 8.5$, $p < 0.01$, $\eta_P^2 = 0.13$) and Sleep ($F_{(1,58)} = 6.2$, $p < 0.05$, $\eta_P^2 = 0.10$) and a significant Age x Sleep interaction ($F_{(1,58)} = 9.4$, $p < 0.01$, $\eta_P^2 = 0.14$) on the number of c-Fos expressing cells in the prelimbic cortex (Fig. 5E). Overall, adolescent mice displayed more c-Fos expressing cells in the prelimbic cortex than adult mice (MD = 14.5, SE = 5.0, $p = 0.005$). Moreover, sleep disrupted mice displayed more c-Fos expressing cells in the prelimbic cortex than non-sleep disrupted control mice (MD = 12.4. SE = 5.0, $p = 0.02$). Regardless of sex, sleep disrupted adolescent mice displayed more c-Fos expressing cells in the prelimbic cortex than non-sleep disrupted adolescent (MD = 27.8, SE = 7.1, $p < 0.001$) and sleep disrupted adult counterparts (MD = 29.9, SE = 7.1, $p < 0.001$).

3.5.4 Infralimbic cortex

There was a significant Age x Sleep interaction on the number of c-Fos expressing cells in the infralimbic cortex ($F_{(1,58)} = 4.2$, $p < 0.05$, $\eta_P^2 = 0.07$) (Fig. 5F). Regardless of sex, sleep disrupted adolescent mice displayed more c-Fos expressing cells in the infralimbic cortex than non-sleep disrupted adolescent control mice (MD = 26.0, SE = 9.6, $p = 0.009$) and sleep disrupted adult mice (MD = 22.8, SE = 9.7, $p = 0.02$).

3.6.0 Glucocorticoid receptor and c-Fos colocalization

3.6.1 Hippocampus

There was no significant effect of Age, Sex or Sleep on glucocorticoid receptor/c-Fos co-expressing cells in the hippocampal CA1, CA2 and CA3 regions (Fig. 6A-C).

3.6.2 Anterior cingulate cortex

There was a significant Age x Sex x Sleep interaction on glucocorticoid receptor/c-Fos co-expressing cells in the anterior cingulate cortex ($F_{(1,54)} = 4.4$, $p < 0.05$, $\eta_P^2 = 0.08$) (Fig. 6D). Sleep disrupted adolescent females displayed a greater number of glucocorticoid receptor/c-Fos co-expressing cells in the anterior cingulate cortex than sleep disrupted adult female counterparts (MD = 0.14, SE = 0.07, $p = 0.04$).

3.6.3 Prelimbic cortex

There was a significant Age x Sex x Sleep interaction on glucocorticoid receptor/c-Fos co-expressing cells in the prelimbic cortex ($F_{(1,54)} = 7.9$, $p < 0.01$, $\eta_P^2 = 0.13$) (Fig. 6E). Sleep disrupted adolescent females displayed a greater number of glucocorticoid receptor/c-Fos co-expressing cells in the prelimbic cortex than non-sleep disturbed adolescent female (MD = 0.15, SE = 0.06, $p = 0.004$), sleep disrupted adolescent male (MD = 0.19, SE = 0.07, $p = 0.01$) and sleep disrupted adult female counterparts (MD = 0.23, SE = 0.07, $p = 0.001$). Moreover, sleep disrupted adult females displayed less glucocorticoid receptor/c-Fos co-expressing cells than non-sleep disrupted adult females (MD = -0.15, SE = 0.06, $p = 0.02$).

3.6.4 Infralimbic cortex

There was a significant Age x Sex x Sleep interaction for glucocorticoid receptor/c-Fos co-expressing cells in the infralimbic cortex ($F_{(1,54)} = 6.0$, $p < 0.05$, $\eta_P^2 = 0.10$) (Fig. 6F). Sleep disrupted adolescent females displayed a greater number of glucocorticoid receptor/c-Fos co-expressing cells in the infralimbic cortex than sleep disrupted adult females (MD = 0.16, SE = 0.07, $p = 0.032$).

3.7 Body weight

There were main effects of Age ($F_{(1,72)} = 27.8$, $p < 0.01$, $\eta_P^2 = 0.2798$) and Sleep ($F_{(1,72)} = 6.3$, $p < 0.01$, $\eta_P^2 = 0.08$) on percent body weight change (Fig. 1F). Regardless of sex and sleep disruption condition, adolescent mice gained more weight over the experimental period than adult mice (MD = 4.8, SE = 0.9, $p < 0.001$). Moreover, regardless of age and sex, sleep disrupted mice gained less weight over the experimental period than non-sleep disrupted control mice (MD = 2.3, SE = 0.9, $p = 0.01$).

4.0 Discussion

Adolescence is a critical period of development characterized by cellular reorganization and vulnerability to the onset of depression via exposure to chronic stress. Women are more susceptible to chronic HPA-axis activation and are twice as likely to be diagnosed with depression than men. However, the mechanisms underlying adolescent depression onset and sex differences in the prevalence rate were unclear. The current study was designed to investigate the effect of chronic sleep disruption on depression-like behavior, HPA axis sensitization, 5-HT_{1A} and glucocorticoid receptor expression and activation of cells immunoreactive to glucocorticoid receptor antibody in various brain regions in adolescent and adult male and female mice. The results demonstrate that chronic sleep disruption induces depression-like behavior, as measured by increased immobility duration and decreased latency to immobility, in both adolescent males and adolescent females compared to their non-sleep disrupted adolescent male and non-sleep disrupted adolescent female counterparts. Regardless of age, female mice exposed to chronic sleep disruption displayed greater 5-HT_{1A} expression in the dorsal raphe compared to male counterparts. Sleep disrupted adolescent females consistently displayed less 5-HT_{1A} expression than non-sleep disrupted adolescent females in the anterior cingulate, prelimbic, and infralimbic cortices, and in the CA3 region of the hippocampus. Sleep disrupted adolescent males displayed less 5-HT_{1A} than non-sleep disrupted adolescent males in the anterior cingulate and infralimbic cortices only. Sleep disrupted adolescent males and females displayed significantly greater c-Fos expression in the prelimbic cortex compared to their non-sleep disrupted adolescent counterparts. Sleep

disrupted adolescent females also displayed increased HPA-axis sensitivity compared to non-sleep disrupted adolescent females while adolescent males did not.

4.1 Chronic sleep disruption induced depression-like behavior in adolescent males and females.

Chronic sleep disruption elicited longer durations of immobility and shorter latencies to immobility in the forced swim test in adolescent male and female mice, indicating increased depression-like behavior (Castagné et al., 2010). This effect was limited to adolescent mice as adult male and female mice did not show an increase in depression-like behavior after chronic sleep disruption. These findings support our hypotheses regarding increased adolescent susceptibility to depressive behavior following chronic sleep disruption and are consistent with previous investigations linking adolescent stress to mood disorders in mice and humans (Chiba et al., 2012; Hankin et al., 1998). However, these findings only partially support our hypothesis because the effect was not limited to adolescent females. In fact, we did not find any difference in depression-like behavior between adolescent males and females following chronic sleep disruption, suggesting that both sexes are equally susceptible to the effects of chronic sleep disruption. If chronic sleep disruption contributes to the increased prevalence of depression in adolescent females compared to males, it does so through interaction with other factors like increased exposure or HPA-axis sensitivity. Human and CD-1 females typically enter puberty sooner than males and experience increased HPA-axis vulnerability to sensitization earlier, possibly at a time when brain reorganization associated with puberty is more vulnerable to disruption (Blakemore et al., 2010).

4.2 Mice demonstrate sex-dependent variations in 5-HT_{1A} receptor expression in the dorsal raphe after chronic sleep disruption.

Regardless of age, female mice displayed greater 5-HT_{1A} expression in the dorsal raphe than males following chronic sleep disruption. These results partially support our hypothesis and are consistent with previous work showing that male and female rodent models of depression often show decreased 5-HT production and increased 5-HT_{1A} expression in the dorsal raphe (Lopez et al., 1998). Increased expression of the 5-HT_{1A} autoreceptor reduces 5-HT production through a negative feedback loop and is associated

with increased depressive behavior (Burnet et al., 1994; De Montigny et al., 1984; Lopez et al., 1998). Chronic sleep disruption may directly increase depression-like behavior by mediating 5-HT signaling in the dorsal raphe in adolescent males and females. The proposed mechanism is consistent with previous findings showing that microinjections of melanin concentrating hormone, which regulates circadian rhythms and sleep patterns, into the dorsal raphe nuclei of male rats increases depression-like behavior (Lagos, Urbanavicius, Scorza, Miraballes, & Torterolo, 2011). Additionally, treatment with a selective 5-HT reuptake inhibitor blocks the depressive effect of melanin concentrating hormone, suggesting an interaction between the 5-HT_{1A} mediated 5-HT negative feedback loop, depression-like behavior and sleep disruption (Lagos et al., 2011). However, in our current study, we noted that sleep disrupted adolescent males displayed increased depression-like behavior without an increase in dorsal raphe 5-HT_{1A} expression whereas sleep disrupted adult females displayed increased dorsal raphe 5-HT_{1A} without increased depression-like behavior. Alternate or complementary mechanisms and signaling pathways are likely necessary to understand the dynamic relationships between adolescent development, sleep and depression.

4.3 Sleep disrupted mice express an age- and sex-specific decrease in 5-HT_{1A} receptor expression in the prefrontal cortex and hippocampus.

The effects of chronic sleep disruption on 5-HT_{1A} expression is mostly limited to adolescent mice. Sleep disrupted adolescent females display decreased 5-HT_{1A} expression in the anterior cingulate, prelimbic, and infralimbic cortices, and in the CA3 region of the hippocampus compared to non-sleep disrupted adolescent females. Sleep disrupted adolescent males display less 5-HT_{1A} expression than non-sleep disrupted adolescent males in the anterior cingulate and infralimbic cortices only. Additionally, sleep disrupted adolescent females display significantly less 5-HT_{1A} expression in the CA3 region of the hippocampus than all other comparison groups. These findings support our hypotheses and suggest sex-specific changes to adolescent 5-HT_{1A} expression in 5-HT projection areas following chronic sleep disruption. Our findings are also consistent with previous rodent models of depression that display decreased expression of 5-HT_{1A} receptors in 5-HT projection areas (Lopez et al., 1998). However, 5-HT_{1A} signaling and

the factors that influence 5-HT_{1A} expression may indirectly influence 5-HT production within the dorsal raphe nucleus through an alternate feedback loop (Burnet et al., 1994). Previous work has shown that, in adult male rats, increased serum corticosterone following chronic restraint stress is associated with a decrease in 5-HT_{1A} expression in the CA3 region of the hippocampus (Burnet et al., 1994; Lopez et al., 1998; Michelsen et al., 2007; Watanabe, Sakai, McEwen, & Mendelson, 1993) and in the prefrontal cortex (Watanabe et al., 1993). Additionally, adolescent male mice that display depressive behavior also express less 5-HT_{1A} in the prefrontal cortex (Garcia-Garcia et al., 2017). Increased corticosterone exposure of sleep disrupted adolescent females may partially explain why adolescent female mice express more consistent decreases of 5-HT_{1A} expression.

4.4 Chronic sleep disruption increased serum corticosterone concentration in adult and adolescent female mice in response to a novel stressor.

Regardless of age, sleep disrupted females displayed greater serum corticosterone release than non-sleep disrupted females. This effect was not observed in adolescent or adult male mice. Overall, females displayed greater serum corticosterone than male mice. These findings support our hypothesis related to increased susceptibility to HPA-axis sensitization to novel stress following chronic sleep disruption in females. We were unable to replicate previously reported increases in serum glucocorticoids of adult male rats following 8 days of 18-hour chronic sleep restriction (Meerlo et al., 2002) possibly due to shorter session duration. However, our findings are consistent with previous reports that adult female rats require less sleep than adult males before displaying increased serum glucocorticoids (Antunes et al., 2006; Meerlo et al., 2002). Our observations of sex-specific glucocorticoid release are consistent with previous reports demonstrating greater plasma corticosterone concentration in adult and pre-adolescent female rats following restraint stress compared to male counterparts (Romeo, Lee, & McEwen, 2004). Sex differences in serum corticosterone response to stress may be established by ovarian hormones. Chronic estrogen injections in female rats prolongs glucocorticoid release following a 5-second foot shock compared to ovariectomized female controls (Burgess & Handa, 1992). Increased estrogen in female mice may

sensitize the HPA-axis to restraint stress following chronic sleep disruption. Despite greater depression-like behavior, adolescent females had significantly less serum corticosterone than adult females. We previously observed similar age- and sex-specific effects in CD-1 mice after lipopolysaccharide injection (Girard-Joyal et al., 2015). The HPA-axes of adolescent females is underdeveloped compared to adult female mice, reducing responsiveness to novel stress and corticosterone release (Romeo, 2010; Romeo & McEwen, 2006). Further investigation is necessary to confirm HPA-axis sensitization to chronic sleep disruption in adolescent females. Continued investigations into estrogen, corticotrophin releasing and adrenocorticotropic hormones will help elucidate the underlying signaling pathways responsible for the female-specific increase in glucocorticoid release following chronic sleep disruption.

4.5 Glucocorticoid receptor expression in the CA1 region of the hippocampus decreases in all mice following chronic sleep disruption.

Regardless of age or sex, sleep disrupted mice displayed a significant decrease in glucocorticoid receptor expression in the CA1 region of the hippocampus. Adolescent female mice showed the greatest decrease in GR expression in the CA1 following chronic sleep disruption. These results are consistent with our hypothesis that chronic sleep disruption sensitizes the HPA-axis and that adolescent female mice are more susceptible to HPA-axis sensitization than adolescent male and adult male and female mice. Exposure to acute or chronic stress is associated with changes in GR expression in the brain and HPA-axis reactivity (Leistner & Menke, 2018). Our findings are consistent with previous observations of decreased hippocampal glucocorticoid receptor expression in the CA1 of adult male rats after tail shock and restraint stress (Jeon et al., 2012). The CA1 region of the hippocampus provides regulatory information to the HPA-axis after prolonged periods of stress (Jeon et al., 2012). A reduction in glucocorticoid receptor expression in the hippocampus can cause persistent activation of the HPA-axis, a hallmark of HPA-axis dysregulation commonly seen in depression (Leistner & Menke, 2018).

4.6 Exposure to chronic sleep disruption increases cellular activation in the prelimbic and infralimbic cortices in adolescent males and females.

Sleep disrupted adolescent males and females showed greater c-Fos expression in the prelimbic and infralimbic cortices compared to non-sleep disrupted adolescent and sleep disrupted adult counterparts. These findings support our hypothesis that adolescent mice exposed to chronic sleep disruption would display greater cellular activation following a novel stressor. While we are the first to show a greater increase in cellular activity following a restraint stress in sleep disrupted adolescent mice, our findings are consistent with recent research examining the effect of sleep disruption on cellular activity in the prelimbic and infralimbic cortices (Harkness et al., 2019). More specifically, adult male rats exposed to two 6-hour sessions of sleep disruption displayed increased cellular activation and oxidative stress in the prelimbic cortex and increased cellular activation in the anterior cingulate, prelimbic and infralimbic cortices following two 12-hour sessions (Harkness et al., 2019). Recent findings suggest that adult men and women diagnosed with major depression display increased activity in the prelimbic and infralimbic cortices while attempting to regulate negative emotions such as poor self-appraisal (Davey, Breakspear, Pujol, & Harrison, 2017). Increased c-Fos expression following chronic sleep disruption may indicate an underlying adolescent susceptibility to signaling regulating negative affect. Further research is necessary to elucidate the relationship between adolescent prelimbic activation and depression onset.

4.7 Chronic sleep disruption increases activation of glucocorticoid immunoreactive cells in the prelimbic cortex of adolescent female mice.

We also found that sleep disrupted adolescent females displayed greater glucocorticoid receptor/c-Fos co-expression in the prelimbic cortex compared to all other groups. Increased glucocorticoid receptor/c-Fos co-expression indicates a greater number of active glucocorticoid expressing-cells and possible HPA-axis sensitization (Lehner et al., 2009; Ostrander et al., 2003). These findings are consistent with our hypothesis that sleep disrupted adolescent females would be more susceptible to HPA-axis sensitization following a novel stressor. We are the first to report activation of glucocorticoid receptor immunoreactive cells in the prelimbic cortex following chronic sleep disruption and that

this effect is displayed in adolescent female mice only. These findings further support that cellular activity within the prelimbic cortex is susceptible to repeat sleep disruption (Harkness et al., 2019). Although chronic sleep disruption and restraint stress increased c-Fos expression in the prelimbic cortex similarly in adolescent male and female mice, it only increased glucocorticoid receptor/c-Fos co-expression in sleep disrupted adolescent females. These findings further support the existence of fundamental differences in signaling mechanisms underlying adolescent development, chronic sleep disruption, and depression between male and female groups.

4.8 Chronic sleep disruption may interrupt successful stress coping strategies in adult female mice

Only adult female mice exposed to chronic sleep disruption displayed longer climbing durations than their non-sleep disrupted controls. Climbing behavior in the forced swim test is used to assess stress-coping strategies (Commons, Cholanians, Babb, & Ehlinger, 2017). Rodents that display longer durations of climbing behavior fail to abandon inefficient stress-coping methods, depleting resources necessary for mental health (Heninger et al., 1996; Lustberg & Reynolds III, 2000). Interestingly, signaling from the prelimbic cortex initiates and maintains climbing behavior in adult male rats and is limited by inhibitory signaling from the infralimbic cortex (Fiore et al., 2015). Adult female mice also exposed to chronic sleep disruption displayed less glucocorticoid receptor expression and less glucocorticoid receptor/c-Fos co-expression in the prelimbic cortex than all other groups. We are the first to report a potential adult female susceptibility to impaired coping strategies following chronic sleep disruption and to suggest glucocorticoid receptors in the prelimbic cortex as a possible underlying mechanism. Decreased glucocorticoid expression may reduce infralimbic control over the prelimbic cortex and result in less climbing activity. As repeated and extended resource depletion is necessary before increasing depressive symptoms (Uehara, Sakado, Sakado, Sato, & Someya, 1999), sleep disrupted adult females may require more instances of stress exposure before displaying depressive symptoms. Further research into coping mechanisms, sleep disruption, and medial prefrontal functionality will be necessary to clarify the mechanisms responsible for this sex- and age-specific vulnerability.

4.9 Chronic sleep disruption influences body weight gain during adolescent development.

Chronic sleep disturbance reduced weight gain in adolescent and adult male and female mice compared to their age and sex appropriate control groups. These observations support our hypotheses and replicate previous rodent models of chronic sleep disruption (da Silva Rocha-Lopes et al., 2018; Koban et al., 2006; Vetrivelan et al., 2012). Reduced body weight gain is likely a result of mild glucocorticoid release following chronically delayed sleep onset (Meerlo et al., 2002). Daily administration of corticosterone to adolescent male C57BL/6N mice causes similar body weight loss (Shahanoor, Sultana, Baker, & Romeo, 2017). To verify the role of glucocorticoid release in weight loss following chronic sleep disruption, future investigations should consider recording daily corticosterone and body weight measurements.

Limitations and future directions

Chronically sleep disrupted adolescent mice were used to model the 4-hour sleep/wake shift observed in adolescent human development (Chaput et al., 2018; Crowley et al., 2007). However, the delay of sleep onset was not statistically confirmed. Efficacy of the "gentle manipulation" method to disrupt sleep has been validated elsewhere (S.-R. Yang et al., 2012). EEG and circadian measures were purposefully omitted to avoid the confounding effects of electrode implantation on initial stress assessment. Behavioral instances of drowsiness (eye drooping, lethargy, etc.) do not substitute for sleep onset assessment due to their qualitative nature. Additionally, the number of "gentle manipulations" per mouse was not recorded because the manipulation itself manifests negligible HPA-axis activation and is as stressful as an "air puff to the face" (Gross et al., 2015). Quantitative non-stressful measures would be necessary to establish a causal relationship between circadian rhythm, sleep development, HPA-axis activation and depressive phenotypes.

Depressive behavior was only assessed with the forced swim test. Given that the forced swim test is in itself stressful (Commons et al., 2017), less stressful measures may be necessary to clearly identify the interaction between sleep disruption, depressive onset and HPA-axis activation following novel restraint stress. Additionally, HPA-axis

activation after acute and chronic exposure to sleep disruption with and without novel stress exposure is necessary to fully elucidate the mechanism between sleep disruption and novel stress. As discussed earlier, we included an assessment of latency to immobility (Castagné et al., 2009) to supplement the immobility duration data. However, an examination of depression-like behavior using alternate measures like the tail suspension and sucrose preference tests could further strengthen our conclusions and should be considered in future research. Furthermore, chronic sleep disruption may increase immobility by decreasing locomotion, but it appears unlikely in the current study because average swimming duration did not differ significantly between groups. Nevertheless, a test of locomotion, like the open field test, should be included in future studies to investigate the effect of chronic sleep disruption on locomotion. The current study used a CD-1 mouse model because it is an outbred strain with high ecological validity (McCormick & Hodges, 2017). However, our observations could be strain- and/or species-specific. Future investigations should evaluate the effect chronic sleep disruption in different strains and species.

5.0 Conclusions

Adolescent development is characterized by a period of increased neural reorganization susceptible to modulation by repeat HPA-axis activation resulting in increased likelihood of developing depression. Exposure to small but repeated sessions of sleep disruption increases the likelihood of developing depression-like behavior in a rodent model of adolescent males and females. We have also identified that the HPA axis of adolescent females, and not adolescent males, exposed to chronic sleep disruption may become sensitized to novel stressors. Proper management of adolescent male and female sleep schedules could reduce the risk of depression onset in adolescent humans.

Conflicts of Interest

The authors report no declarations of interest.

Funding

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Acknowledgements

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References

See end of document.

Figures and Captions

See below.

Figure 1.

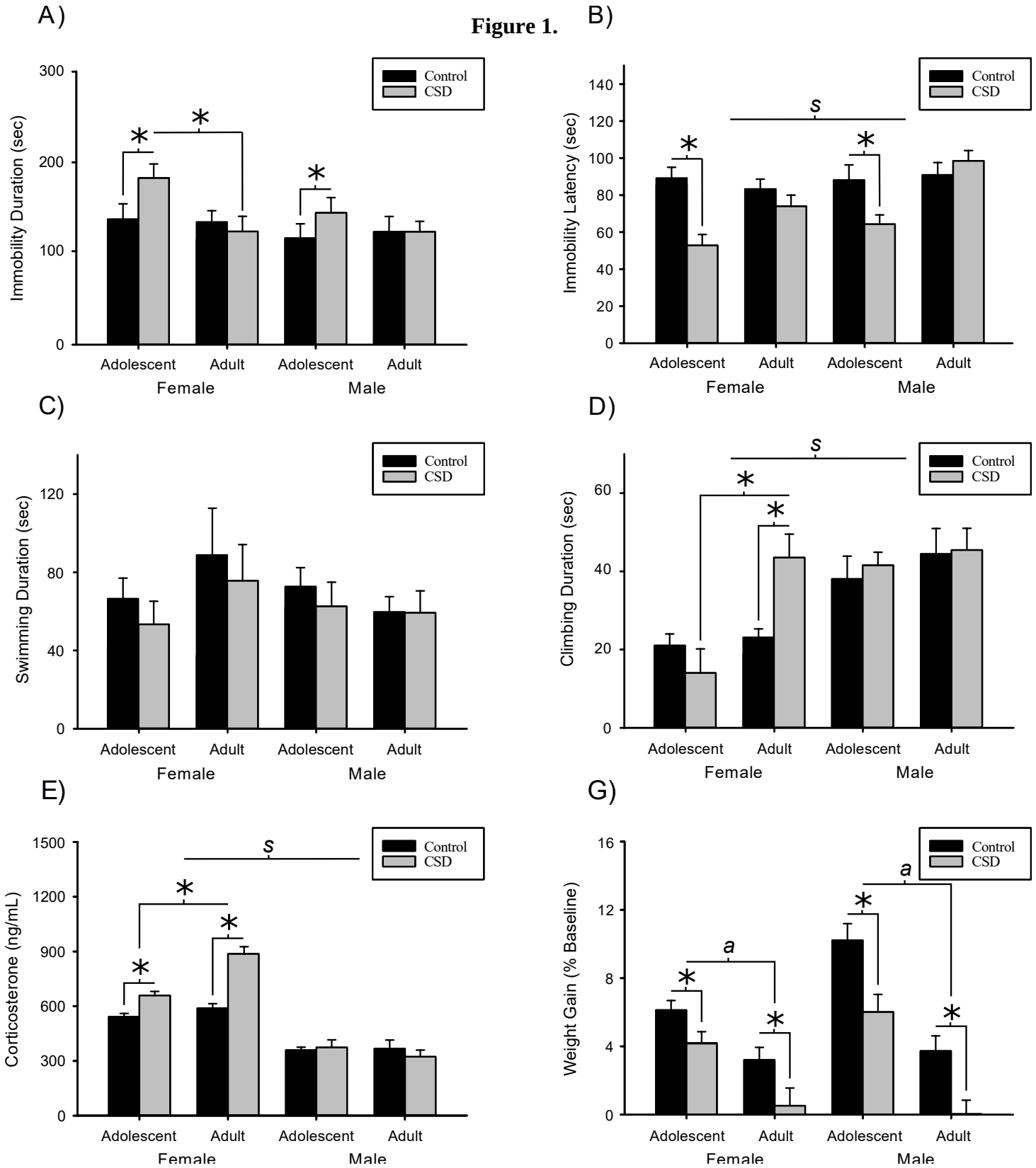
A)
Immobility Duration (sec)
300
200
100
0
Control
CSD
*
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male
B)
Immobility Latency (sec)
140
120
100
80
60
40
20
0
Control
CSD
s
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male
C)
Swimming Duration (sec)
120
80
40
0
Control
CSD
Adolescent
Adult
Adolescent
Adult
Female
Male
D)
Climbing Duration (sec)
60
40
20
0
Control
CSD
s
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male
E)
Corticosterone (ng/mL)
1500
1200
900
600
300
0
Control
CSD
s
*
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male
G)
Weight Gain (% Baseline)
16
12
8
4
0
Control
CSD
a
a
*
*
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male

Fig. 1. Mean (±SEM) (A) duration of immobility behavior (seconds), (B) latency to immobility behavior (seconds), (C) duration of swimming behavior (seconds), (D) duration of climbing behavior (seconds), (E) serum corticosterone concentration (ng/ml) after restraint, and (F) body weight change (%) from baseline in male and female mice exposed to either normal rest or to chronic sleep disruption (CSD). ***** = $p < .05$; *s* = main effect of sex ($p < .05$); *a* = main effect of age ($p < .05$).

Figure 2.

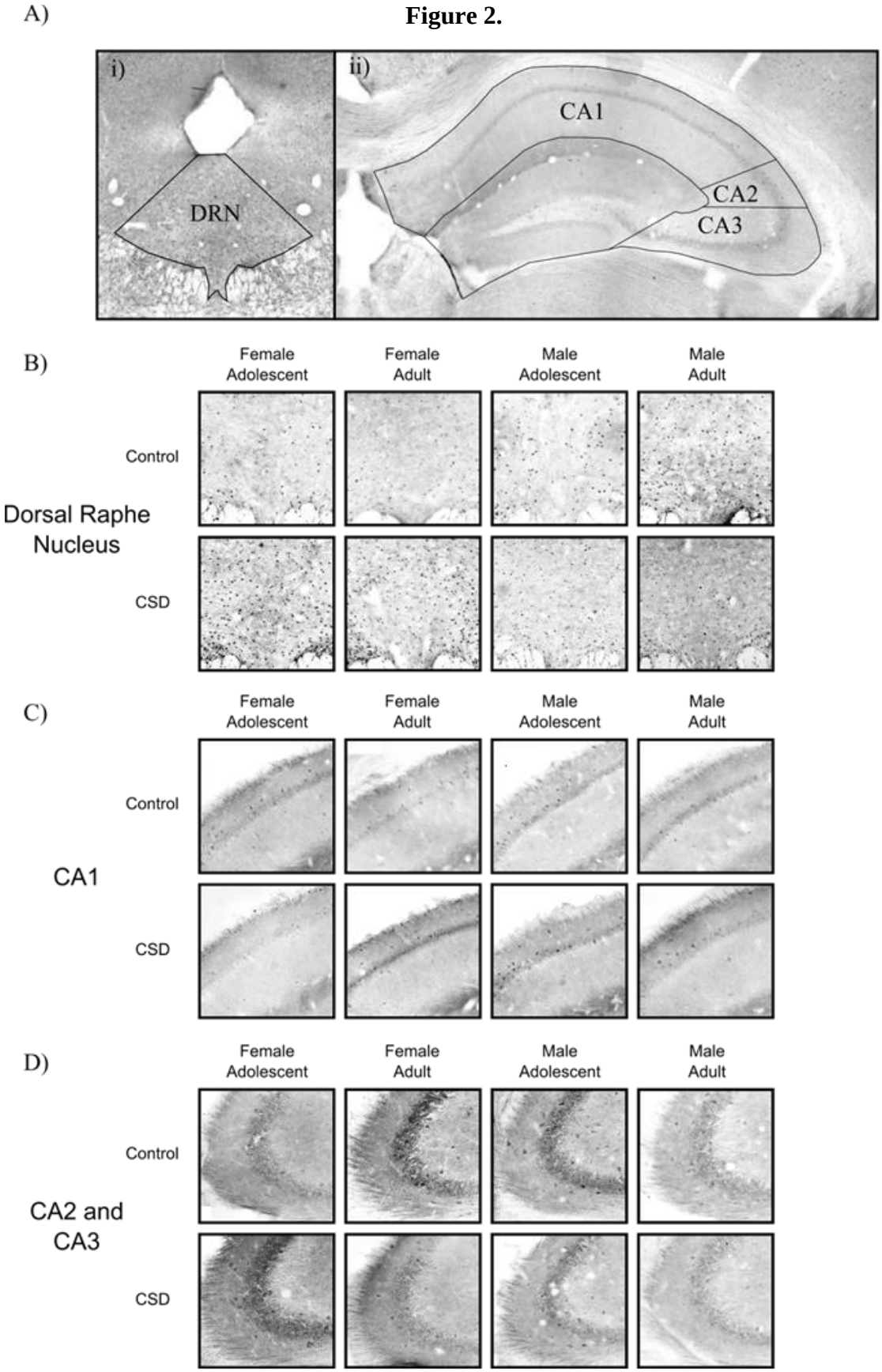
A)
i)
DRN
ii)
CA1
CA2
CA3
B)
Female Adolescent
Female Adult
Male Adolescent
Male Adult
Control
CSD
Dorsal Raphe Nucleus
C)
Female Adolescent
Female Adult
Male Adolescent
Male Adult
Control
CSD
CA1
D)
Female Adolescent
Female Adult
Male Adolescent
Male Adult
Control
CSD
CA2 and CA3

Fig. 2. Mouse brain images depicting (A) the boundaries of the dorsal raphe nucleus (DRN) and of the CA1, CA2, CA3 regions of the hippocampus. Representative photomicrographs of 5-HT_{1A} receptor expression in the (B) DRN, (C) CA1, and in the (D) CA2 and CA3 regions of the hippocampus in males and females exposed to either normal rest or chronic sleep disruption (CSD).

Figure 3.

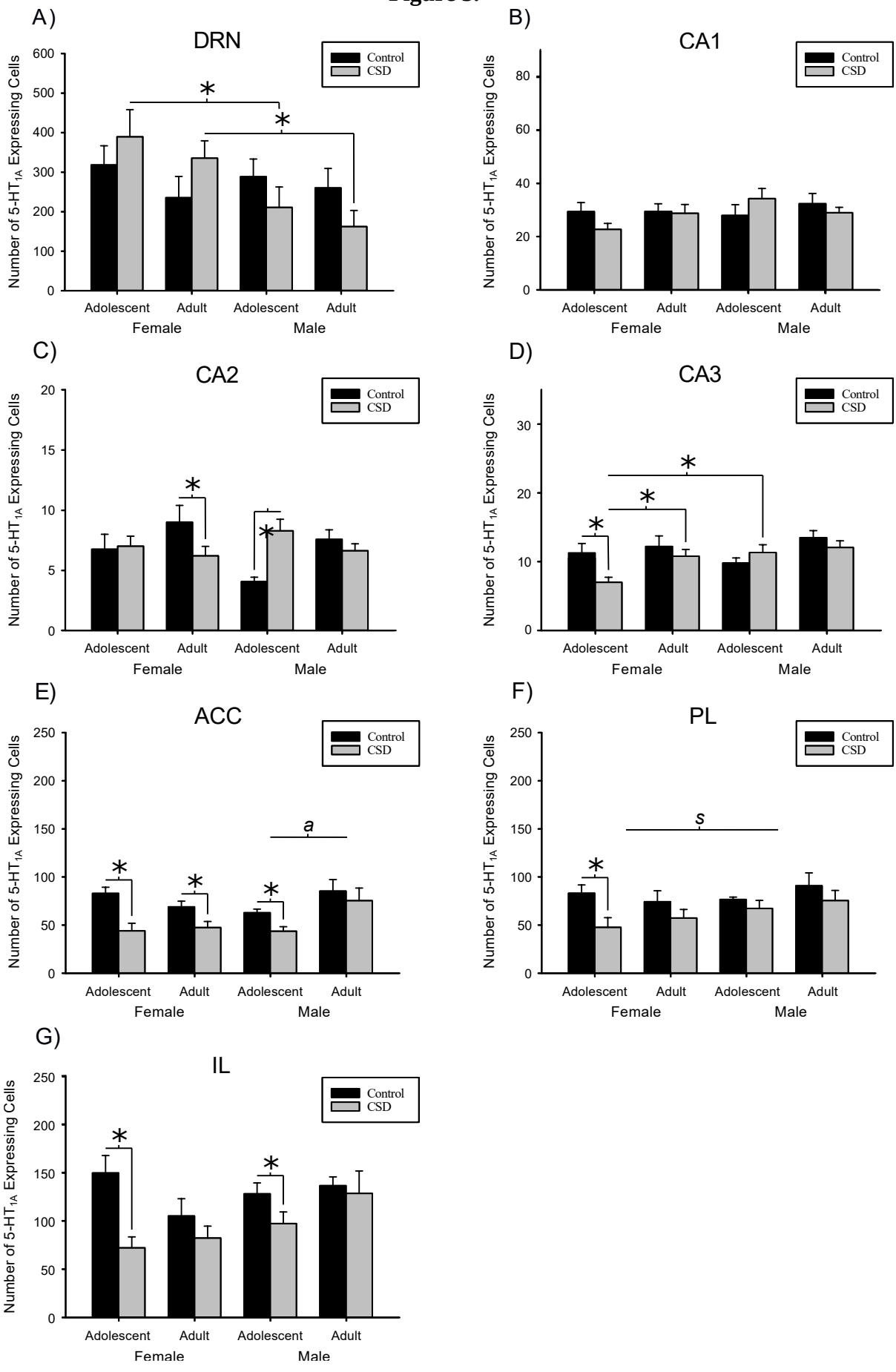
A)
DRN
B)
CA1
C)
CA2
D)
CA3
E)
ACC
F)
PL
G)
IL
Control
CSD
Number of 5-HT1A Expressing Cells
Adolescent
Adult
Female
Male
a
s

Fig. 3. Mean (±SEM) number of 5-HT_{1A} receptor-expressing cells in the (A) dorsal raphe (DRN), (B) CA1, (C) CA2, (D) CA3, (E) anterior cingulate cortex (ACC), (F) prelimbic cortex (PL), and (G) infralimbic cortex (IL) in male and female mice exposed to either normal rest (control) or to chronic sleep disruption (CSD). ***** = $p < .05$; *s* = main effect of sex ($p < .05$); *a* = main effect of age ($p < .05$).

Figure 4.

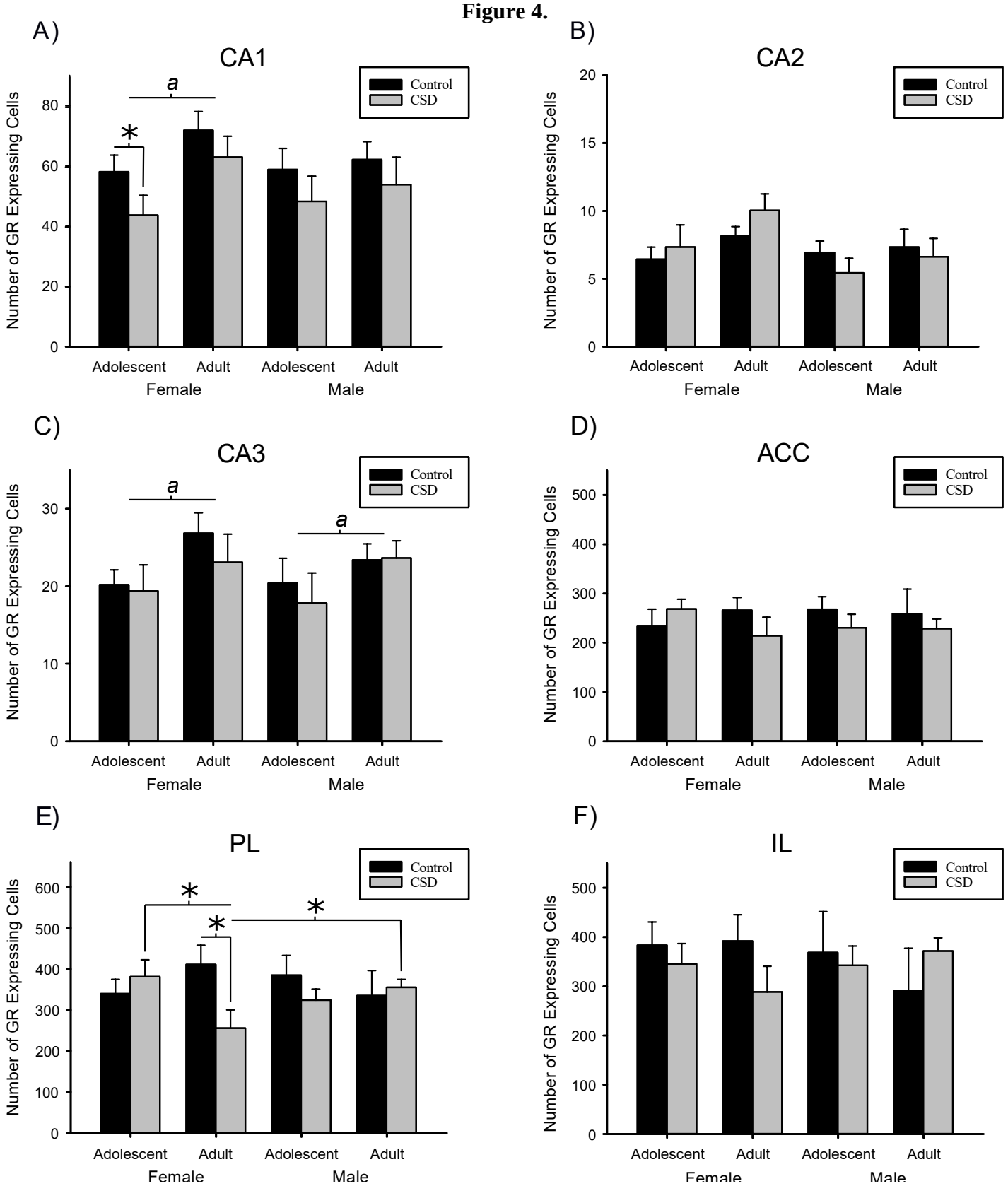
A)
CA1
Number of GR Expressing Cells
Control
CSD
a
*
Adolescent
Adult
Adolescent
Adult
Female
Male
B)
CA2
C)
CA3
a
a
D)
ACC
E)
PL
*
*
*
F)
IL

Fig. 4. Mean (±SEM) number of glucocorticoid receptor (GR)-expressing cells in the (A) CA1, (B) CA2, (C) CA3, (D) anterior cingulate cortex (ACC), (E) prelimbic cortex (PL), and (F) infralimbic cortex (IL) in male and female mice exposed to either normal rest or to chronic sleep disruption (CSD). ***** = $p < .05$; *s* = main effect of sex ($p < .05$); *a* = main effect of age ($p < .05$).

Figure 5.

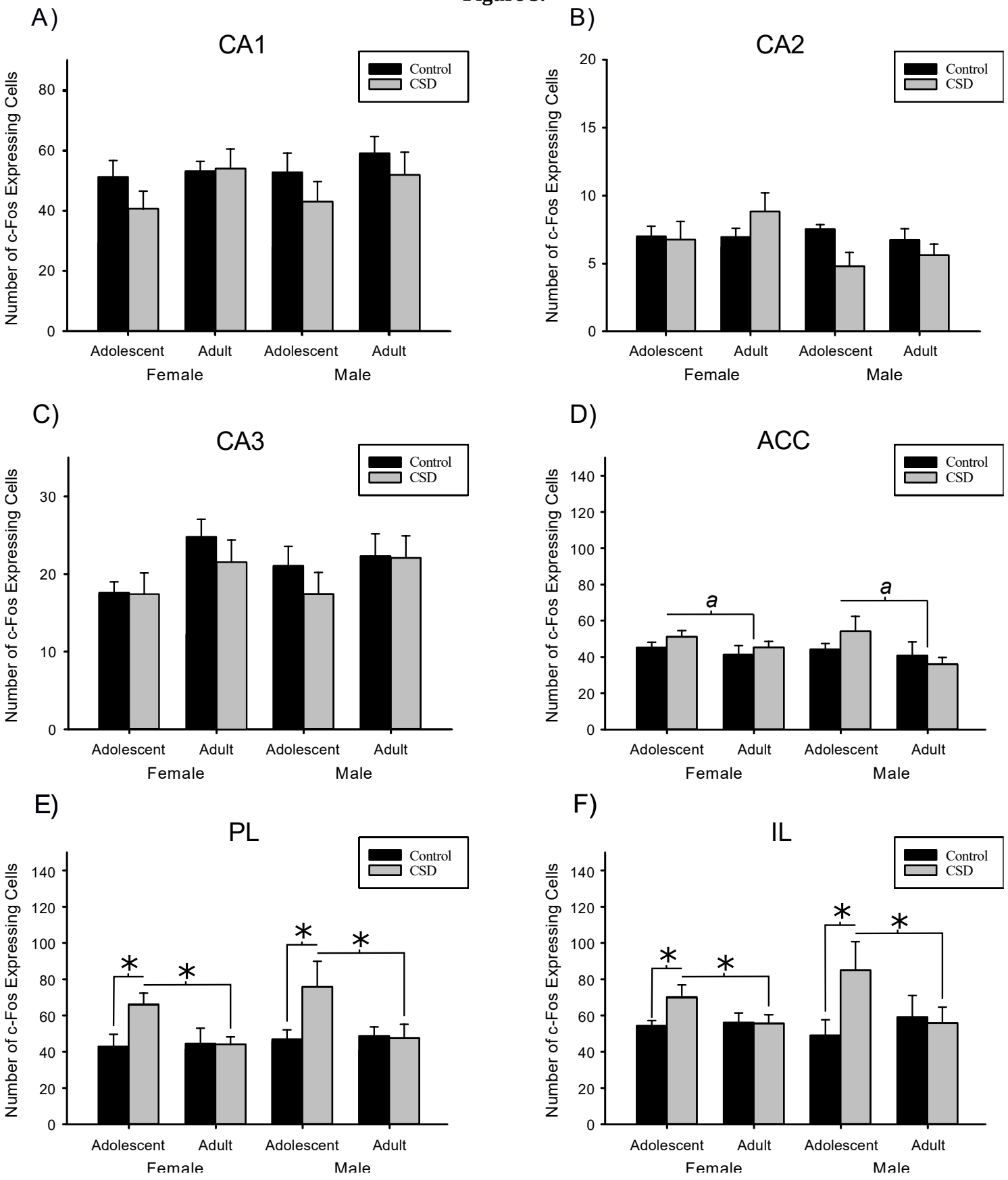
A)
CA1
Control
CSD
Number of c-Fos Expressing Cells
0
20
40
60
80
Adolescent
Adult
Adolescent
Adult
Female
Male
B)
CA2
Control
CSD
Number of c-Fos Expressing Cells
0
5
10
15
20
Adolescent
Adult
Adolescent
Adult
Female
Male
C)
CA3
Control
CSD
Number of c-Fos Expressing Cells
0
10
20
30
Adolescent
Adult
Adolescent
Adult
Female
Male
D)
ACC
Control
CSD
Number of c-Fos Expressing Cells
0
20
40
60
80
100
120
140
a
a
Adolescent
Adult
Adolescent
Adult
Female
Male
E)
PL
Control
CSD
Number of c-Fos Expressing Cells
0
20
40
60
80
100
120
140
*
*
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male
F)
IL
Control
CSD
Number of c-Fos Expressing Cells
0
20
40
60
80
100
120
140
*
*
*
*
Adolescent
Adult
Adolescent
Adult
Female
Male

Fig. 5. Mean (±SEM) number of c-Fos expressing cells in the (A) CA1, (B) CA2, (C) CA3, (D) anterior cingulate cortex (ACC), (E) prelimbic cortex (PL), and (F) infralimbic cortex (IL) in male and female mice exposed to either normal rest or to chronic sleep disruption (CSD). ***** = $p < .05$; *a* = main effect of age ($p < .05$).

Figure 6.

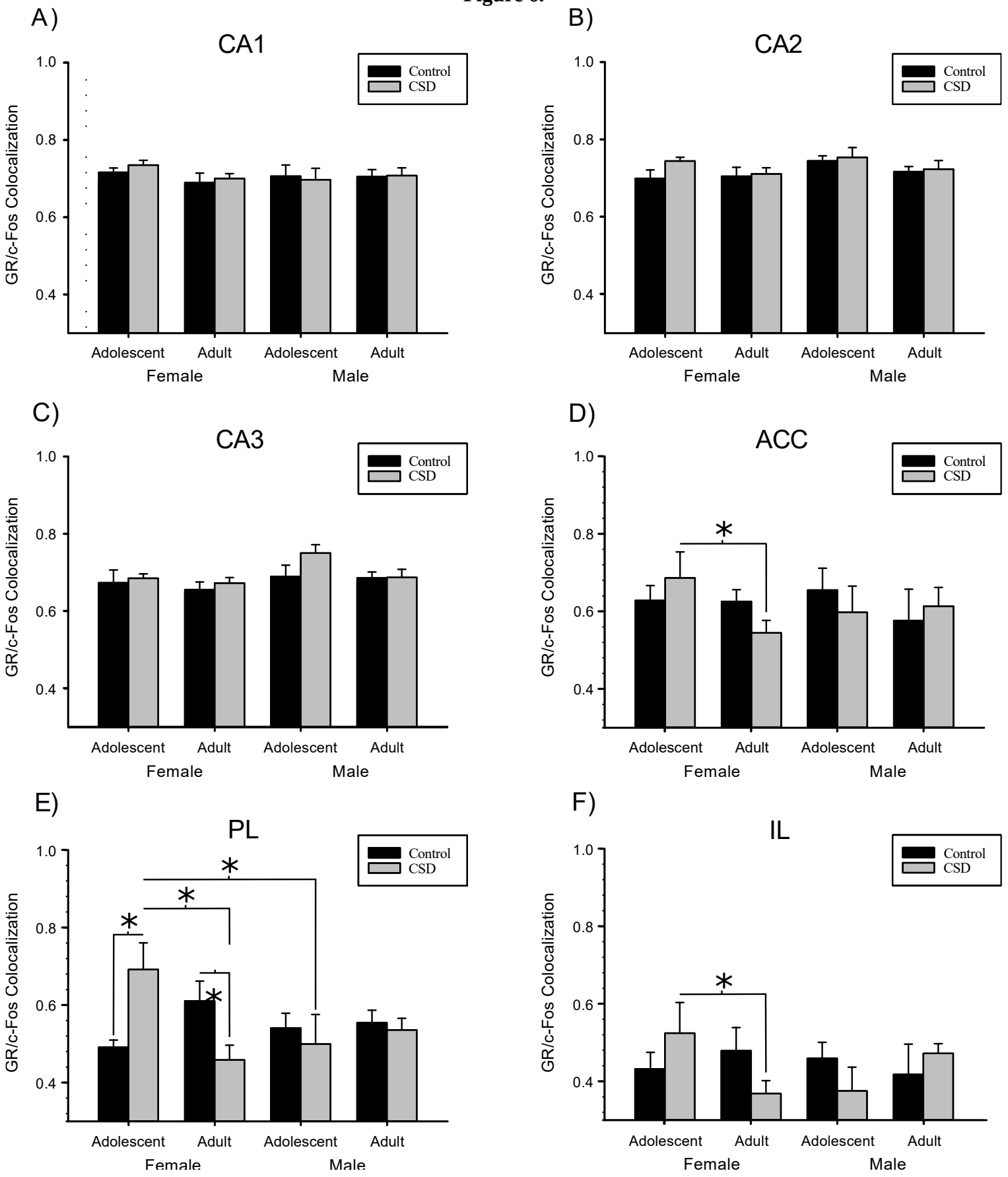
A)
CA1
B)
CA2
C)
CA3
D)
ACC
E)
PL
F)
IL
Control
CSD
GR/c-Fos Colocalization
1.0
0.8
0.6
0.4
Adolescent
Adult
Adolescent
Adult
Female
Male
*

Fig. 6. Mean (±SEM) Mander's overlap coefficient assessing the co-expression of glucocorticoid receptor (GR) and c-Fos in cells in the (A) CA1, (B) CA2, (C) CA3, (D) anterior cingulate cortex (ACC), (E) prelimbic cortex (PL), and (F) infralimbic cortex (IL) in male and female mice exposed to either normal rest or to chronic sleep disruption (CSD). Co-expression is measured using the overlap coefficient. ***** = $p < .05$.

Figure 7.

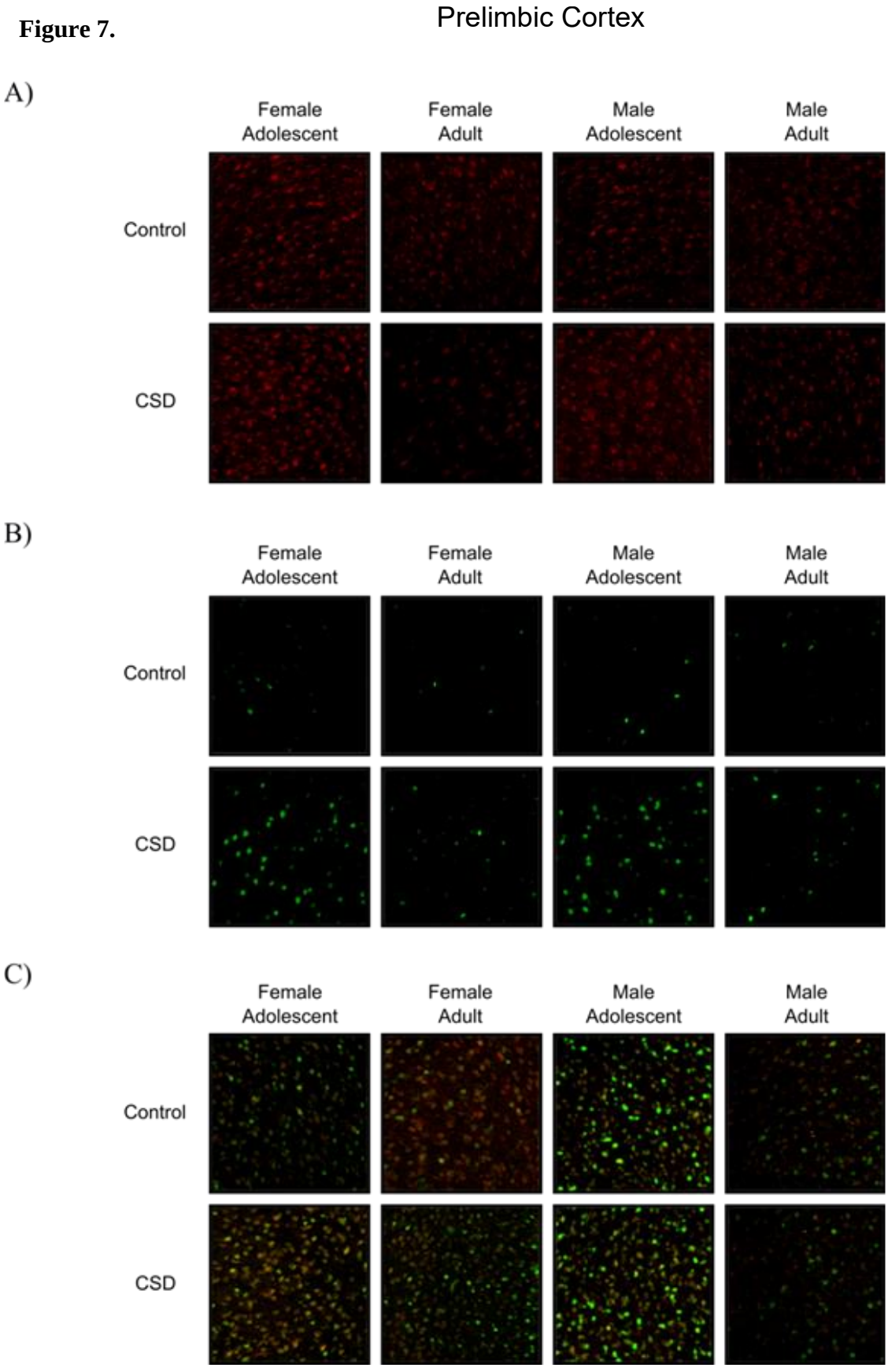
Prelimbic Cortex
A)
Female Adolescent
Female Adult
Male Adolescent
Male Adult
Control
CSD
B)
Female Adolescent
Female Adult
Male Adolescent
Male Adult
Control
CSD
C)
Female Adolescent
Female Adult
Male Adolescent
Male Adult
Control
CSD

Fig. 7. Representative photomicrographs of (A) glucocorticoid receptor expression, (B) c-Fos expression, and (C) glucocorticoid receptor/c-Fos co-expression in the prelimbic cortex in males and females exposed to either normal rest or to chronic sleep disruption (CSD).

Chapter 4: The effect of probiotic supplementation on sleep, depression-like behaviour, and central glucose and lactate metabolism in male and female pubertal mice exposed to chronic sleep disruption.

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Abstract

The prevalence of depression disorders significantly increases during puberty and adolescence. Chronic sleep disruption increases during adolescence and may lead to the onset of depression by reducing access to neuromodulating biomolecules like tryptophan, serotonin, and the glucose by-product L-lactate or by interrupting required rest. Probiotic treatment reduces depression symptoms, prolongs non-rapid eye movement (NREM) sleep, shortens sleep latency, and produces neuromodulating biomolecules. It was unclear whether probiotic treatment mitigates depression induced by chronic sleep disruption. Therefore, we investigated how pubertal chronic sleep disruption and probiotic intervention altered depression-like behaviour, sleep expression, and serotonin, tryptophan, glucose, and L-lactate concentration. Three-week-old male and female mice received cannula and electroencephalogram (EEG) electrode implants (N=120) and *ad libitum* access to probiotic mixtures Lacidofil® or Cerebiome®, or water for eighteen days. Mice were sleep-disrupted for the first 4 hours of their rest cycle or allowed normal rest for eleven days. Sleep and hippocampal L-lactate and glucose concentrations were measured over a 24-h period. Depression-like behaviour was evaluated using tail suspension and forced swim tests. Plasma and prefrontal cortical serotonin, tryptophan, glucose, and L-lactate concentrations were measured. Chronic sleep disruption increased depression-like behaviour, increased NREM duration in the early awake diurnal phase, and reduced prefrontal tryptophan and serotonin and hippocampal L-lactate and glucose concentrations. Both probiotics reduced depression-like behaviour, increased prefrontal tryptophan, and increased hippocampal L-lactate concentrations. Cerebiome increased prefrontal serotonin and hippocampal glucose concentrations, while Lacidofil increased NREM duration in the latter half of the rest phase. While both probiotic mixtures displayed similar antidepressant effects on sleep-disrupted samples, their mechanisms of action may involve different pathways.

Keywords: probiotics, depression, puberty, adolescence, sleep, L-lactate

1.0 Introduction

Mood disorders are the leading cause of disability and financial burden resulting from disease-related consequences (Friedrich, 2017; James et al., 2018). Depression alone affects over 280 million individuals worldwide (WHO, 2023). Clinically depressed individuals display at least 5 symptoms during the same two-week period that must include pervasive despair or anhedonia, and symptoms like significant weight change, disrupted sleep, poor concentration, and thoughts about dying or suicide (Association, 2013). Depression is further associated with a number of additional symptoms like delayed sleep latency and decreased or disturbed non-rapid eye movement (NREM) sleep (James et al., 2018; Jones, Gershon, Sitaram, & Keshavan, 1987). Despite progressive improvement in pharmacological treatments, the underlying biological mechanisms responsible for the onset of depression remain complex and unclear (Wolf & Hopko, 2008). Puberty and adolescence are periods of sex-specific reproductive maturation and are both associated with increased susceptibility to depression development (Anderson et al., 2006). For example, 1.79-2.09 % of preadolescent boys and 0.31-2.19 % of preadolescent girls between 11-13 years old display clinically relevant depression symptoms (Hankin & Abramson, 1999; Hankin et al., 1998). By 18 years of age, approximately 10.8 % of males and 23.2 % of females display clinically relevant symptoms of depression. Additionally, following puberty, females account for more than twice the number of cases of depression than males (Hankin et al., 1998). Investigating how depression develops during puberty may provide guidance on appropriate treatment and prevention therapies.

Puberty and adolescence are characterized by a maturation of stress response mechanisms (Blakemore et al., 2010; Crowley et al., 2007; McCormick & Mathews, 2007; Tanner, 1962). Exposure to chronic stress during puberty significantly alters the reactivity of underlying stress signaling pathways (Gallucci et al., 1993; McCormick et al., 1995; Romeo, 2010). For example, pubertal male rats exposed to 7 days of intermittent restraint stress display a more prolonged release of the stress hormone adrenocorticotropin hormone (ACTH) and increased cellular activation of the paraventricular nucleus (PVN) of the hypothalamus compared to adults (Romeo et al.,

2006; J. T. Smith & Clarke, 2007). Exposure to chronic stress during puberty and adolescence increases the likelihood of depression in adulthood (Herbison, Allen, Robinson, Newnham, & Pennell, 2017). In our own model of chronic sleep disruption, adolescent and adult female mice exposed to 7 days of "gentle manipulation" sleep disruption displayed increased corticosterone release following a novel stressor (Murack et al., 2021). In the same experiment, male and female pubertal mice exposed to chronic sleep disruption displayed increased depression-like symptoms in the forced swim test. However, the mechanisms responsible for the effect of pubertal sleep disruption on stress and mood dysregulation remains unclear.

Dysregulation of metabolites has been linked to the pathophysiology of depression (Carrard et al., 2018; Kraemer & McKinney, 1979; Murack & Messier, 2019; Naylor et al., 2012). The essential amino acid tryptophan and its derivative serotonin decrease in patients with major depression disorder and increase with pharmacological antidepressant treatment (Asberg et al., 1976; Lira et al., 2003; Marx et al., 2021; O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). Intraperitoneal injection of tryptophan in diabetic models of mice reduces depressive behaviour assessed by a forced swim test (Hilakivi-Clarke, 1991). Increased access to energy-providing molecules may also produce antidepressant effects (Carrard et al., 2018). L-lactate is a by-product of glucose metabolism (Bangsbo et al., 1993) that can be synthesized within astrocytes (Magistretti & Allaman, 2018) to produce adenosine triphosphate, a substrate for neuronal energy (F. Tang et al., 2014). L-lactate also can stimulate neuroplasticity because it increases brain-derived neurotrophic factor (BDNF) expression (J. Yang et al., 2014). Adult male mice display reduced depression-like behaviour after chronic intraperitoneal injections of sodium lactate with an efficacy comparable to Desipramine (Carrard et al., 2018). Both Desipramine and L-lactate injections were associated with increased hippocampal L-lactate and astrocyte activity in the hippocampus. Increasing the concentration of metabolites associated with antidepressant effects may help reduce pubertal and adolescent depression. Previous investigations into L-lactate antidepressant treatments are so far limited to intraperitoneal injections given L-lactate's poor palatability (Lowenbach et al., 1947; Murack & Messier, 2019). Alternate oral treatments are required to investigate the relation between L-lactate, sleep disruption, and depression.

Probiotic treatment is the oral supplementation of existing gut microbiota with bacterial strains associated with positive health benefits. The gut microbiota secretes biomolecules like tryptophan, serotonin, L-lactate, and over 150 alternate metabolites which could influence homeostasis and brain disease (Matsumoto et al., 2012). An increased consumption of foods fermented with lactic acid bacteria (LABs) has been associated with temporary increases in blood lactate concentration (Nakagawa et al., 2018; Rao, Rehman, Yu, & De Andino, 2018). Indeed, LABs are well known producers of lactic acid, classically used as starters in the dairy industry for that property. Supplementation with probiotic LABs may increase the host's access to psychologically relevant biomolecules (Kazemi et al., 2019; Uchida et al., 2004) and could be implemented as a palatable and gastric distress-free alternative to existing methods to manipulate physiological levels of blood and brain lactate. Early evidence shows that probiotic LAB supplementation is associated with reduced self-reported sleep disturbances (Nakagawa et al., 2018; Romijn, Rucklidge, Kuijer, & Frampton, 2017), decreased sleep latency and increased NREM duration in sleep disturbed mice (Lin et al., 2021), and reduced depression symptoms (Bested, Logan, & Selhub, 2013; Logan & Katzman, 2005; Tillmann et al., 2018).

The current project is designed to evaluate the anti-depressant effect of probiotic LABs on sleep disruption-induced depression in a pubertal mouse model (Murack et al., 2021). Several possible biological mechanisms for this antidepressant effect were investigated. Extracellular L- lactate and glucose concentration within the hippocampus was recorded simultaneously together with EEG and EMG activity to estimate sleep patterns (Carrard et al., 2018). The effect of chronic sleep disruption and probiotic treatment on concentrations of tryptophan, tryptophan's metabolite serotonin, and BDNF were also assessed as possible mechanisms. We hypothesized that sleep-disrupted animals would display increased depression-like behaviours and decreased expression of central glucose, central L-lactate, tryptophan, serotonin and BDNF which are associated with antidepressant effects in the brain and blood (Carrard et al., 2018; Murack et al., 2021; Murack & Messier, 2019). We also hypothesized that chronic sleep disruption would cause reduced NREM sleep in the rest phase and increased NREM in the early awake phase (Jones et al., 1987). Lastly, we hypothesized that treatment with probiotic

mixtures would disrupt the formation of the described depression-related sequelae of sleep disruption.

2.0 Methods

Animals

Three-week-old male and female CD-1 mice (N = 80; Charles River Laboratories, St-Constant, QC, Canada) were housed in groups of two per cage with *ad libitum* access to food (Envigo, Rodent Chow, Mississauga, Canada) and water until stereotaxic surgery. Cages included corn cob chip bedding (Cat# 7097, Envigo) and three pieces of nestlet (Cat# NES3600, Ancare). The vivarium was maintained at approximately 23 ℃ with 60 % humidity and a 12-hour light cycle (Zeitgeber time (ZT) 0 is 11:00 PM, ZT12 is 11:00 AM). Zeitgeber time (ZT) assigns "0" hour as the beginning of the awake phase determined by a light change (Daan & Merrow, 2002). ZT12 corresponds to the end of the awake phase and the initiation of the rest phase. All experimental procedures were approved by the Animal Care Committee of the University of Ottawa according to the Canadian Council on Animal Care (CCAC) guidelines.

Cannula, EEG, and electromyography (EMG) implantation

Implantation of guide cannula, EEG, and EMG electrodes proceeded as previously described by Wilson and Gifford (2005) and Mang and Franken (2012) at ZT7. Three-week-old mice received a 0.05 mg/kg subcutaneous injection of buprenorphine hydrochloride (Reckitt Benckiser Healthcare, Hull, North Humberside, UK) and 1 ml of 0.9 % saline (Hospira, Montreal, Canada) before surgery. Mice were anesthetised with 4 –5 % isoflurane (Fresenius Kabi Canada Ltd., Richmond Hill, ON) and secured in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Mice were kept under anesthesia on a heated pad (TP650, Gaymar Industries, Orchard Park, NY, USA) during the surgery procedure with 1–2.5 % isoflurane. Two cannulas (Pinnacle Technologies, Lawrence, KS, USA) were positioned above the right and left dorsal hippocampi (Posterior -2.18 mm, Lateral ± 1.8 mm and Dorsal -1.2 mm) (Murack et al., 2021; Paxinos & Franklin, 2019). Cannulas were fixed using a dental glue compound (C&A Metabond, Frontier Dental, Concord, ON, Canada). Four 0.10-inch

screws with wire leads (Cat # 8403, Pinnacle Technology) were positioned rostral to the two guide cannulas (EEG 1: Posterior -0.18 mm, Lateral ± 1.3 mm; EEG 2: Anterior 1.82 mm, Lateral 0.8 mm) and soldered to a head mount (Cat # 8402, Pinnacle Technology) (see Supplementary material 1). EMG wires extruding from the head mount were inserted into the posterior nuchal muscles. Post-surgical analgesia included application of a transdermal bupivacaine (Chiron Compounding Pharmacy Inc., Guelph, ON, Canada) and an injection of sustained-release buprenorphine (Chiron Compounding Pharmacy Inc). Mice were allowed to recover for 7 days.

Sham surgery

Six mice per group were tested on biosensor/EEG/EMG output. The remaining 4 mice per group composed the sham surgery group, who received bilateral cannula implantation, EEG lead wires, EMG insertion, recovery-time, probiotic treatment, sleep disruption, behavioural testing, and euthanasia procedures at the same time as the biosensor/EEG/EMG mice in their corresponding group. However, sham mice were not assessed for hippocampal metabolite concentration or sleep architecture.

Probiotic treatment

Mice received *ad libitum* access to either Lacidofil® probiotic mixture (Lallemand Health Solutions, Montreal, Quebec, Canada), Cerebiome® probiotic mixture (Lallemand Health Solutions), or continued access to water (n = 10). Lacidofil® is a mixture of *Lacticaseibacillus rhamnosus* strain R0011 and *Lactobacillus helveticus* strain R0052 in a ratio of 95:5 (Cowan, Callaghan, & Richardson, 2016). Cerebiome® is a mixture of *L. helveticus* strain R0052 and *Bifidobacterium longum* R0175 in a ratio of 90:10 (Wallace & Milev, 2021). Lyophilized probiotic containing products were hydrated in water at concentration of 10^9 colony forming units per millilitre in accordance with previous studies using the Lacidofil formula (Gareau et al., 2011). Probiotic and water solutions were replaced each day at ZT7. *Ad libitum* access to probiotic solutions and water continued for 18 days until euthanasia.

Chronic sleep disruption

The chronic sleep disruption procedure was adapted from the "gentle manipulation sleep deprivation" protocol previously described by Ledoux et al. (1996) and used in Murack et al. (2021) to minimize the impact of handling stress. Mice were moved to a separate holding area prior to sleep disruption exposure. Mice were gently manipulated for the first 4 hours (ZT12 to ZT16) of each rest phase for 11 consecutive days until euthanasia. Wakefulness was maintained through gentle sensory stimulation (brushing the nape and back with a soft paintbrush) to induce arousal. In this version of the gentle manipulation method, two experimenters were present to survey for signs of lethargy (reduction of locomotion) or drowsiness (drooping of eye lids). Gentle stimulation was applied by the experimenters when signs of lethargy or drowsiness were identified. Mice were returned to the housing facility at the end of each sleep disruption session. Control mice were not manipulated during the 4-hour experimental period to avoid unintentional sleep disruption.

Calibration

Hippocampal Biosensors

Biosensors were calibrated according to manufacturer recommendations before and after *in vivo* testing using the Sirenia Acquisition Software (Pinnacle Technology Inc.). Glucose and L-lactate biosensors were placed in 100 mM phosphate buffer (pH 7.4) at 23 °C (pre-testing) or 37 °C (post-testing) and tested for metabolite sensitivity by adding glucose (1 mM) and L-lactate (0.1 mM) to the buffer solution. Biosensor selectivity was tested by a single ascorbic acid (0.25 mM) addition to the phosphate buffer solution. Pre- and post-calibration curves were used to assess the sensitivity and selectivity of the glucose and L-lactate biosensors to account for factory- or procedure-related variations.

Pre-calibration curves displayed acceptable average sensitivity for glucose (5.44 nA/1 mM) and L-lactate (3.94 nA/0.1 mM) when compared to previous investigations (Béland-Millar et al., 2017). Glucose and lactate biosensors also displayed acceptable selectivity by showing negligible reactions to ascorbic acid (0.23 nA/ 0.25 mM and 0.37 nA/0.25 mM, respectively). Post-calibration curves displayed a linear decrease to average

glucose (3.94 nA/1 mM) and L-lactate (3.74 nA/ 0.1 mM) biosensor sensitivity. Glucose and lactate biosensors may have become less selective following hippocampal recordings as ascorbic acid had a greater impact on current following the post-calibration (0.49 nA/ 0.25 mM and 4.69 nA/0.25 mM, respectively). However, increased sensitivity to ascorbic acid may have resulted from variables like wear caused by the insertion and removal of the biosensors. Similar increases to ascorbic acid sensitivity have been reported in investigations with smaller recording durations (Béland-Millar et al., 2017).

EEG and EMG

EEG and EMG data was recorded using the Sirenia Acquisition Software (Pinnacle Technology Inc.). Using a mouse pre-amplifier, the EEG and EMG channels were individually tested with an artificial test source (Cat # 8249, Pinnacle Technology Inc.) before connecting to the head mounts. Unconnected EEG and EMG channels displayed a small amount of noise averaging less than ~0.01 µV. The pre-amplifier was then connected to the test source for 1 min and displayed sinusoidal waveforms when successful.

EEG, EMG, and Biosensor recordings

Mice were moved to a separate testing facility 24 hours prior to EEG, EMG, and Biosensor recordings and allowed to acclimate. Mice were lightly anaesthetized with isoflurane and the EEG/EMG head mount was attached to a pre-amplifier. Glucose and lactate biosensors were inserted into the dorsal hippocampus through the bilateral cannula and attached to the pre-amplifier. Mice were placed into a custom square polymethyl methacrylate cage with standard bedding (Teklad 7097 Corncob bedding, Envigo, Mississauga, Canada), 3 nestlets, food, and probiotics or water as appropriate. Simultaneous video, biosensor, EEG and EMG data was recorded immediately following attachment to preamplifier using the Sirenia Acquisition Software (Pinnacle Technology Inc.) and continued for approximately 24 hours until the end of the tail suspension test. Following completion of tail suspension test, mice were uncoupled from the pre-amplifier and biosensors and returned to the housing facilities.

Values for electric current (nA) recorded using the glucose and lactate biosensors were converted to concentration (mM) by multiplying current (nA) by a conversion factor

(mM/nA). Conversion factors were specific to each biosensor and calculated by averaging the incremental increases to current (nA) following addition of a known glucose or lactate concentration to a neutral buffer solution (see Calibration). Biosensor data was recorded at a sampling rate of 1 Hz but binned in 10-min epochs for analysis (Béland-Millar et al., 2017).

Signals for EEG and EMG were amplified and filtered (EEG: 0.5-100Hz, EMG = 10-300 Hz) at the head of the animal using a Pinnacle 8400-series preamplifier (Pinnacle Technology Inc.) and digitized with an adaptor at a sampling rate of 1 kHz. Sleep was divided into 5 second epochs, separated into the rest phase (ZT 18 to ZT 0) or awake phase (ZT0 to ZT6), and scored as NREM, REM, or Wake using the Sirenia Sleep Pro software (Pinnacle Technology Inc.). NREM, REM or Wake stages were assessed according to stage duration, number of bouts, average stage duration per bout, stage duration as a percentage of total time recorded, and stage duration as a percentage of sleep (NREM and REM only).

Tail suspension test

Following biosensor/EEG/EMG recordings, depression-like behaviour of mice was assessed with the tail suspension test during the active phase of the light-dark cycle (ZT10). Testing was carried out under dim red light. Adhesive tape was secured around the tail at three quarters of the distance from the tail's base (Castagné et al., 2010). Mice were suspended approximately 10 cm above a flat surface. Attempts at escape and immobility were recorded for 6 mins. After the 6 mins observation period, mice were returned to their housing facilities. Two experimenters blind to experimental conditions assessed the duration of immobility within each recording using the ODLog software (MACROPOD).

Forced swim test

Depression-like behaviour was reassessed with the forced swim test at ZT10. One hour prior to testing, mice were moved to the testing facilities to acclimate. Mice were placed in a 4 L container filled with 3 L of water (approximately 25°C) for 5 mins. The duration of time spent swimming (lateral movement), climbing (attempts at vertical

climbing with no lateral movement), and immobile (minimal movement to maintain balance only) was recorded for 5 mins using a video tracking software (EthoVision, Noldus, Leesburg, Virginia, USA). Upon completion, mice were removed from the container and placed in warm and dry recovery cages (30^{o}C) for 5 mins. Mice were then returned to the housing facilities. Behaviours were scored by two experimenters blind to conditions using the ODLog software (MACROPOD).

Euthanasia, tissue, and blood extraction

Mice were euthanized by intraperitoneal injection of 500 mg/kg of sodium pentobarbital at ZT9 (Euthanyl, Virbac AH Inc., Fort Worth, TX, USA). After verifying the absence of pain reflexes, blood was extracted from the heart through a cardiac puncture. Blood samples were placed in two capillary tubes (Lithium-Heparin Microvette CB 300, Cat # 16.443.100, Sarstedt) and stored at 4^{o}C for 2 hours then centrifuged. Plasma was then extracted into 100 µl tubes and stored at -80^{o}C until analysis. Following blood collection, the prefrontal cortex was extracted, flash frozen with liquid nitrogen, and stored at -80^{o}C. Remaining brain tissue was incubated in 4% paraformaldehyde solution for 14 days, frozen, sectioned at 14 µm using a cryostat. Biosensor implantation coordinates within the dorsal hippocampus were confirmed by staining sections with Cresyl Violet and referencing the Paxinos and Franklin (Paxinos & Franklin, 2019) stereotaxic atlas (see Supplementary material 1).

Tryptophan and tryptophan metabolite assays

Tryptophan (Cat # MBS1607987, MyBioSource, San Diego, CA, USA) and serotonin (Cat # FTEM1465, Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) concentrations were assessed in tissue homogenates and plasma extracted from the prefrontal cortex and peripheral blood, respectively, using an enzyme-linked immunoassay (ELISA) kit. BDNF (Cat # FTEM0020, Wuhan Fine Biotech Co., Ltd.) concentration in the prefrontal cortex was also assessed using an ELISA kit.

Timeline

Mice arrived at post natal day 21 and were implanted with cannulas and EEG and EMG electrodes at ZT7 on post-natal day 26 (see Supplementary material 1). Mice had *ad libitum* access to probiotics or water for 18 consecutive days beginning on post-natal

day 33. On post-natal day 40, mice were exposed to chronic sleep disruption for 11 days from ZT12 to ZT16. Glucose, lactate, EEG, and EMG measurements were recorded for ~24 hours on post-natal day 48 (ZT10 until ZT10 the following day). Depression behaviour was measured with the tail suspension test immediately before detachment from electrodes at ZT10 on post-natal day 49. Mice were assessed with the forced swim test on the subsequent day at ZT10. Mice were euthanized on post-natal day 51 at ZT9.

Data analysis

Extracellular glucose and lactate measurements were analyzed using a 4-way mixed ANOVA according to Sex (male/female), Sleep (chronic sleep disruption/rested), Probiotic treatment (Lacidofil/Cerebiome/water) and Time (Rest Phase: 5 min epochs from Z18 to Z0; Awake Phase: 5 min epochs from Z0 to Z6). The remaining measures were analyzed using a 3-way independent ANOVA according to Sex, Sleep, and Probiotic treatment. Significant main effects and interactions were assessed using pairwise comparisons corrected with the Bonferroni method. Omnibus and pairwise comparisons were considered significant at $p < 0.05$. Pairwise comparisons are reported as differences between group means (MD), standard error values (SE) and p values. Outliers were identified using box plot analysis and winsorized to the next extreme score (Anscombe, 1973). A Greenhouse-Geisser correction was applied to the mixed ANOVA if sphericity or homogeneity was violated. All statistical analyses were performed using SPSS (v.24, IBM). Non-significant results are included in Table 1 and 2.

3.0 Results

3.1 Tail suspension test

Figure 1A presents the results for the tail suspension test. There was a significant Sleep x Treatment interaction ($F_{(1,108)} = 3.5$, $p < 0.05$, $\eta_P^2 = 0.06$) for the duration of immobility during the tail suspension test. Sleep-disrupted mice displayed a greater duration of immobility than rested mice when treated with water (MD = 30.3, SE = 12.7, $p = 0.019$). Mice treated with water displayed a greater duration of immobility than mice treated with Cerebiome following sleep disruption (MD = 31.4, SE = 12.7, $p = 0.045$) only. Mice treated with water displayed a greater duration of immobility than mice treated with Lacidofil following sleep disruption (MD = 37.0, SE = 12.7, $p = 0.013$) only.

Both probiotics significantly reduced the effect of sleep disruption on immobility duration in the tail suspension test.

3.2 Forced swim test

3.2.1 Immobility Duration

Figure 1B presents the results for immobility duration recorded in the forced swim test. There were significant Sleep x Treatment ($F_{(2,108)} = 3.6$, $p < 0.05$, $\eta_P^2 = 0.06$) and Sex x Treatment ($F_{(2,108)} = 4.1$, $p < 0.05$, $\eta_P^2 = 0.07$) interactions for the duration of immobility during the forced swim test. Sleep-disrupted mice displayed greater duration of immobility than rested mice when treated with water (MD = 39.9, SE = 6.2, p <0.001), Cerebiome (MD = 29.1, SE = 6.2, p <0.001), or Lacidofil (MD = 16.2, SE = 6.2, p =0.011). Mice treated with water displayed greater duration of immobility than mice treated with Cerebiome when mice were rested (MD = 29.7, SE = 6.2, p <0.001) and when mice were sleep-disrupted (MD = 40.5, SE = 6.2, $p < 0.001$). Mice treated with water displayed greater duration of immobility than mice treated with Lacidofil when mice were rested (MD = 40.6, SE = 6.2, $p < 0.001$) and when mice were sleep disrupted (MD = 64.3, SE = 6.2, $p < 0.001$). Mice treated with Cerebiome displayed greater duration of immobility than mice treated with Lacidofil when mice were sleep disrupted (MD = 23.8, SE = 6.2, $p = 0.001$) only.

3.2.2 Swimming Duration

Figure 1C presents the results for swimming duration recorded in the forced swim test. There were main effects of Sleep ($F_{(1,108)} = 5.3$, $p < 0.05$, $\eta_P^2 = 0.047$) and Treatment ($F_{(2,108)} = 10.5$, $p < 0.01$, $\eta_P^2 = 0.163$) on the duration of swimming during the forced swim test. Overall, sleep-disrupted mice displayed shorter durations of swimming than rested mice (MD = -13.4, SE = 5.9, $p = 0.024$). On average, mice treated with water displayed shorter durations of swimming than mice treated with Cerebiome (MD = -27.9, SE = 7.2, $p = 0.001$) and Lacidofil (MD = -29.0, SE = 7.2, $p < 0.001$).

3.2.3 Behaviour Summary

Sleep disruption in male and female mice increased duration of immobility in the tail suspension and forced swim tests and decreased duration of swimming in the forced

swim test. Probiotic administration in sleep disrupted male and female mice showed reduced immobility and swimming duration when compared to water treated sleep disrupted male and female mice.

3.3 ELISA

3.3.1 Tryptophan Prefrontal Cortex

Figure 2A presents the results for tryptophan concentration in the prefrontal cortex. There was a significant Sleep x Treatment ($F_{(2,108)} = 5.2$, $p < 0.01$, $\eta_P^2 = 0.09$) interaction on tryptophan concentration in the prefrontal cortex. Sleep-disrupted mice displayed lower tryptophan concentration within the prefrontal cortex than rested mice treated with water (MD = -0.41, SE = 0.1, $p = 0.002$). Mice treated with water displayed lower tryptophan concentration within the prefrontal cortex than mice treated with Cerebiome following sleep disruption (MD = -0.33, SE = 0.1, $p = 0.034$) only. Mice treated with water displayed lower tryptophan concentration within the prefrontal cortex than mice treated with Lacidofil following sleep disruption (MD = -0.38, SE = 0.1, $p = 0.012$) only.

3.3.2 Serotonin Prefrontal Cortex

Figure 2C presents the results for serotonin concentration in the prefrontal cortex. There was a significant Sleep x Treatment ($F_{(2,108)} = 3.9$, $p < 0.05$, $\eta_P^2 = 0.07$) interaction on the concentration of serotonin in the prefrontal cortex. Sleep-disrupted mice displayed lower serotonin concentration within the prefrontal cortex than rested mice when treated with water (MD = -8.1, SE = 3.0, $p = 0.007$). Mice treated with water displayed lower serotonin concentration within the prefrontal cortex than mice treated with Cerebiome following sleep disruption (MD = -10.4, SE = 3.0, $p = 0.002$) only. Mice treated with Cerebiome displayed greater serotonin concentration within the prefrontal cortex than mice treated with Lacidofil following sleep disruption (MD = 8.4, SE = 3.0, $p = 0.015$) only.

3.3.3 BDNF Prefrontal Cortex

Figure 2E presents the results for BDNF concentration in the prefrontal cortex. There was a main effect of Sleep ($F_{(1,108)} = 25.0$, $p < 0.01$, $\eta_P^2 = 0.19$) on the

concentration of BDNF in the prefrontal cortex. Overall, sleep disrupted mice displayed greater BDNF concentration in the prefrontal cortex than rested mice (MD = 0.045, SE = 0.009, $p < 0.001$).

3.3.4 ELISA Summary

Sleep disrupted mice displayed reduced serotonin, reduced tryptophan, and increased BDNF concentration in the prefrontal cortex compared to rested mice. However, sleep disrupted mice treated with Cerebiome displayed increased tryptophan and serotonin compared to sleep disrupted mice treated with water. Sleep disrupted mice treated with Lacidofil increased tryptophan only.

3.4 Sleep (see Table 1, Table 2, and Supplementary material 2)

3.4.1 Sleep Latency

3.4.1.1 Rested

Figure 3A presents the results for sleep latency in rested mice. There was a significant Sex x Treatment ($F_{(2,30)} = 3.4$, $p < 0.05$, $\eta_P^2 = 0.19$) interaction on sleep latency in rested mice. Treatment with water resulted in greater sleep latency than treatment with Cerebiome in male (MD = 2923.3, SE = 1151.9, $p = 0.049$) and female mice (MD = 3680.8, SE = 1151.9, $p = 0.010$). Treatment with water resulted in greater sleep latency than treatment with Lacidofil in female mice (MD = 3960.8, SE = 1151.9, $p = 0.005$) only. Treatment with Lacidofil resulted in greater sleep latency than treatment with Cerebiome in male mice (MD = 2983.3, SE = 1151.9, $p = 0.044$) only.

3.4.1.2 Sleep-Disrupted

Figure 3B presents the results for sleep latency in sleep-disrupted mice. There was a significant Sex x Treatment ($F_{(2,30)} = 4.8$, $p < 0.05$, $\eta_P^2 = 0.24$) interaction on sleep latency of sleep-disrupted mice. Treatment with water resulted in shorter sleep latency than treatment with Cerebiome in male (MD = -1062.8, SE = 269.4, $p = 0.001$) and female mice (MD = -718.2, SE = 269.4, $p = 0.037$). Treatment with water resulted in shorter sleep latency than treatment with Lacidofil in female mice (MD = -704.2, SE = 269.4, $p = 0.042$) only. Treatment with Lacidofil resulted in shorter sleep latency than treatment with Cerebiome in male mice (MD = -1159.8, SE = 269.4, $p < 0.001$) only.

3.4.2 Awake Phase

3.4.1.1 NREM Duration

Figure 3C presents the results for NREM duration in the awake phase. There were significant main effects of Sex ($F_{(1,60)} = 5.3$, $p < 0.05$, $\eta_P^2 = 0.08$) and Sleep ($F_{(1,60)} = 4.3$, $p < 0.05$, $\eta_P^2 = 0.07$) on NREM duration in the awake phase. Overall, male mice displayed longer NREM duration in the awake phase than female mice (MD = 1281.9, SE = 554.5, $p = 0.024$). Overall, sleep-disrupted mice displayed longer NREM duration in the awake phase than rested mice (MD = 1151.7, SE = 554.5, $p = 0.042$).

3.4.2.2 NREM Bouts

Figure 3D presents the results for NREM bouts in the awake phase. There was a significant main effect of Sleep ($F_{(1,60)} = 5.8$, $p < 0.05$, $\eta_P^2 = 0.09$) on NREM duration in the awake phase. Overall, sleep-disrupted mice displayed more NREM bouts in the awake phase than rested mice (MD = 4.4, SE = 1.8, $p = 0.019$).

3.4.2.3 Wake Duration

Table 1 presents the results for wake duration in the awake phase. There were significant main effects of Sex ($F_{(1,60)} = 5.1$, $p < 0.05$, $\eta_P^2 = 0.08$) and Sleep ($F_{(1,60)} = 4.4$, $p < 0.05$, $\eta_P^2 = 0.07$) on wake duration in the awake phase. Overall, female mice displayed longer wake duration in the awake phase than male mice (MD = 1345.7, SE = 598.1, $p = 0.024$). Overall, rested mice displayed longer wake duration in the awake phase than sleep disrupted mice (MD = 1251.0, SE = 598.1, $p = 0.041$).

3.4.1.5 Wake Bouts

Figure 3E presents the results for Wake bouts in the awake phase. There was a significant main effect of Sleep ($F_{(1,60)} = 4.1$, $p < 0.05$, $\eta_P^2 = 0.06$) on the number of wake bouts in the awake phase. Overall, sleep-disrupted mice displayed more Wake bouts in the awake phase than rested mice (MD = 3.4, SE = 1.7, $p = 0.048$).

3.4.1.6 Average duration of Wake per Bout

Figure 3F presents the results for average duration of wake per bout in the awake phase. There was a significant Sex x Treatment ($F_{(2,60)} = 3.8$, $p < 0.05$, $\eta_P^2 = 0.11$)

interaction on wake duration per bout in the awake phase. Treatment with Lacidofil resulted in longer wake durations per bout in the awake phase than treatment with water (MD = 639.5, SE = 218.9, $p = 0.015$). Female mice displayed longer wake durations per bout in the awake phase than male mice when treated with Lacidofil (MD = 625.6, SE = 218.9, $p = 0.006$).

3.4.2 Rest Phase

3.4.2.1 NREM Duration

Figure 4A presents the results for NREM duration in the rest phase. There were significant main effects of Sex ($F_{(1,60)} = 4.8$, $p < 0.05$, $\eta_P^2 = 0.08$) and Treatment ($F_{(2,60)} = 3.7$, $p < 0.05$, $\eta_P^2 = 0.11$) on NREM duration in the rest phase. Overall, male mice displayed greater NREM duration in the rest phase than female mice (MD = 935.7, SE = 425.2, $p = 0.032$). Overall, mice treated with Lacidofil displayed greater NREM duration in the rest phase than mice treated with water (MD = 1406.7, SE = 520.8, $p = 0.027$).

3.4.2.2 NREM Bouts

Figure 4B presents the results for NREM bouts in the rest phase. There was a significant Sex x Treatment ($F_{(2,60)} = 5.8$, $p < 0.01$, $\eta_P^2 = 0.16$) interaction on NREM bouts in the rest phase. Male mice displayed fewer bouts of NREM in the rest phase than female mice when treated with water (MD = -7.7, SE = 2.0, $p < 0.001$). Treatment with water resulted in fewer bouts of NREM in the rest phase than treatment with Lacidofil in male mice (MD = -6.9, SE = 2.0, $p = 0.003$) only. Treatment with Lacidofil also resulted in more bouts of NREM in the rest phase than treatment with Cerebiome in male mice (MD = 5.3, SE = 2.0, $p < 0.027$) only.

3.4.2.3 Average duration of NREM per Bout

Figure 4C presents the results for the average duration of NREM per bout in the rest phase. There was a significant Sex x Treatment ($F_{(2,60)} = 4.5$, $p < 0.05$, $\eta_P^2 = 0.13$) interaction on NREM duration per bout in the rest phase. Male mice displayed greater NREM durations per bout in the rest phase than female mice when treated with water (MD = 174.8, SE = 51.1, $p = 0.001$) and Cerebiome (MD = 171.2, SE = 51.1, $p = 0.001$).

3.4.2.4 REM Duration

Figure 4D presents the results for REM duration in the rest phase. There was a significant Sex x Sleep ($F_{(1,60)} = 4.4$, $p < 0.05$, $\eta_P^2 = 0.07$) interaction on REM duration in the rest phase. Rest resulted in greater REM duration in the rest phase than sleep disruption in male mice (MD = 268.9, SE = 102.8, $p = 0.011$) only.

3.4.2.5 REM Bouts

Figure 4E presents the results for REM bouts in the rest phase. There was a main effect of Sleep ($F_{(1,60)} = 5.1$, $p < 0.05$, $\eta_P^2 = 0.08$) on REM bouts in the rest phase. Rested mice displayed more REM bouts in the rest phase than sleep-disrupted mice (MD = 1.9, SE = 0.8, $p = 0.028$).

3.4.2.6 Average duration of REM per Bout

Figure 4F presents the results for average duration of REM per bout in the rest phase. There was a significant Sleep x Treatment ($F_{(2,60)} = 3.4$, $p < 0.05$, $\eta_P^2 = 0.10$) interaction on REM duration per bout in the rest phase. Sleep disruption resulted in lower REM durations per bout in the rest phase than rest in mice treated with water (MD = -15.5, SE = 5.4, $p = 0.006$). Treatment with water resulted in lower REM durations per bout in the rest phase than treatment with Cerebiome following sleep disruption (MD = -20.4, SE = 5.4, $p = 0.001$).

3.4.2.7 Average duration of Wake per Bout

Table 1 presents the results for the average duration of Wake per bout in the rest phase. There was a significant Sex x Treatment ($F_{(2,60)} = 3.4$, $p < 0.05$, $\eta_P^2 = 0.10$) interaction on Wake duration per bout in the rest phase. Male mice displayed greater Wake durations per bout in the rest phase than female mice when treated with water (MD = 173.0, SE = 49.1, $p = 0.001$). Treatment with water resulted in greater Wake durations per bout in the rest phase than treatment with Lacidofil in male mice (MD = 185.8, SE = 49.1, $p = 0.001$) only. Treatment with Lacidofil also resulted in lower Wake durations per bout in the rest phase than treatment with Cerebiome in male mice (MD = -150.4, SE = 49.1, $p = 0.010$).

3.5 Extracellular hippocampal metabolites

3.5.1 Hippocampal Glucose: Awake Phase

Figure 5A presents the results for average hippocampal glucose concentration during the awake phase. There was a significant Sleep x Treatment ($F_{(2,60)} = 4.9$, $p < 0.05$, $\eta_P^2 = 0.14$) interaction on extracellular hippocampal glucose concentration in the awake phase. Sleep disruption resulted in lower extracellular hippocampal glucose concentration in the awake phase compared to rested mice treated with water (MD = -0.4, SE = 0.09, $p < 0.001$) and Lacidofil (MD = -0.2, SE = 0.09, $p = 0.006$). Treatment with Cerebiome resulted in greater extracellular hippocampal glucose concentration in the awake phase compared to mice treated with water following sleep disruption (MD = 0.4, SE = 0.09, $p < 0.001$). Treatment with Cerebiome resulted in greater extracellular hippocampal glucose concentration in the awake phase when compared to mice treated with Lacidofil following sleep disruption (MD = 0.3, SE = 0.09, $p = 0.001$).

3.5.2 Hippocampal Glucose: Rest Phase

Figure 5A presents the results for average hippocampal glucose concentration during the rest phase. There was a significant Sex x Sleep x Treatment ($F_{(2,60)} = 3.5$, $p < 0.05$, $\eta_P^2 = 0.10$) interaction on extracellular hippocampal glucose concentration in the rest phase. Rested female mice treated with water (MD = 0.4, SE = 0.1, $p = 0.010$) and Lacidofil (MD = 0.4, SE = 0.1, $p = 0.010$) displayed greater extracellular hippocampal glucose concentration in the rest phase than their sleep-disrupted counterparts. Sleep disrupted female mice treated with Cerebiome displayed greater extracellular hippocampal glucose concentration in the rest phase than sleep-disrupted mice treated with water (MD = 0.5, SE = 0.1, $p = 0.001$) and sleep-disrupted female mice treated with Lacidofil (MD = 0.5, SE = 0.1, $p = 0.002$).

3.5.3 Hippocampal L-Lactate: Awake Phase

Figure 5B presents the results for average hippocampal L-lactate concentration during the awake phase. There was a significant Sleep x Treatment ($F_{(2,60)} = 4.9$, $p < 0.05$, $\eta_P^2 = 0.14$) interaction on extracellular hippocampal lactate concentration in the awake phase. Sleep disruption resulted in lower extracellular hippocampal lactate concentration in the awake phase when compared to rested mice when treated with water (MD = -0.09, SE = 0.02, $p < 0.001$). Treatment with water resulted in lower extracellular hippocampal lactate concentration in the awake phase when compared to treatment with Lacidofil

following sleep disruption (MD = -0.07, SE = 0.02, $p < 0.001$). Treatment with water resulted in lower extracellular hippocampal lactate concentration in the awake phase when compared to treatment with Cerebiome following sleep disruption (MD = -0.07, SE = 0.02, $p < 0.001$).

3.5.4 Hippocampal L-Lactate: Rest Phase

Figure 5B presents the results for average hippocampal L-lactate concentration during the rest phase. There was a significant main effect of Sleep ($F_{(1,60)} = 4.1$, $p < 0.05$, $\eta_P^2 = 0.06$) on extracellular hippocampal lactate concentration in the rest phase. Overall, sleep-disrupted mice displayed lower extracellular hippocampal lactate concentration in the awake phase than rested mice (MD = -0.3, SE = 0.02, $p = 0.047$).

3.5.5 L-lactate and Glucose Summary

Sleep-disrupted mice treated with water displayed reduced hippocampal glucose and L-lactate concentrations compared to rested mice treated with water. Sleep-disrupted mice treated with Cerebiome displayed increased hippocampal glucose and L-lactate concentrations compared to sleep-disrupted mice treated with water during the awake phase. Sleep-disrupted mice treated with Lacidofil displayed increased hippocampal L-lactate concentrations compared to sleep disrupted mice treated with water during the awake phase.

4.0 Discussion

The prevalence of depression increases during puberty and continues to increase throughout adolescence (Anderson et al., 2006; Murack et al., 2021). Chronic sleep disruption increases depression-like behaviour in adolescent mice and rats (Martins et al., 2010; Murack et al., 2021). Supplementation with probiotic LABs may reduce sleep disturbances (Nakagawa et al., 2018; Romijn et al., 2017) and symptoms of depression (Bested et al., 2013; Logan & Katzman, 2005; Tillmann et al., 2018). However, it is unclear if probiotic LABs are an appropriate intervention for the depression-like behaviour induced by sleep disruption during puberty and adolescence. We hypothesized that sleep disruption would lead to depressive-like behavioural phenotypes and biomolecule concentrations and shift sleep into the early awake phase. We found that sleep-disrupted mice displayed increased immobility durations, decreased tryptophan,

serotonin, glucose, and L-lactate concentrations in the brain, and increased NREM activity during the awake phase (see Table 1 & 2). Cerebiome treatment decreased durations of immobility and increased tryptophan, serotonin, glucose, and L-lactate concentrations in the brain (see Table 2). Lacidofil treatment increased durations of immobility, increased tryptophan and L-lactate concentrations in the brain, and increased NREM duration during the rest phase. Cerebiome also reduced sleep latency in rested mice, and delayed sleep latency in sleep disrupted male and female mice, while Lacidofil reduced sleep latency in rested, and delayed sleep latency in sleep-disrupted female mice only (see Table 2).

Sleep-disrupted male and female mice treated with Cerebiome and Lacidofil did not show increases in the duration of immobility as observed in water treated mice. These findings support our hypothesis that probiotic treatment can reduce depression-like behaviour induced by chronic sleep disruption in adolescent mouse models. Our results are also consistent with previous investigations examining the antidepressive effects of probiotics (Bested et al., 2013; Q. F. Liu et al., 2020; Logan & Katzman, 2005; Tillmann et al., 2018). For example, young adult Swiss albino mice treated with a vehicle control and exposed to chronic restraint stress displayed significantly greater immobility duration in the forced swim and tail suspension tests than non-restrained mice treated with vehicle control and mice administered an 8-week *L. rhamnosus* LR5 and *B. lactis* BL3 treatment (Q. F. Liu et al., 2020). Adult outpatients diagnosed with moderate depression scored lower on a Hamilton Depression score after 6 weeks of *B. longum* and *L. rhamnosus* treatment (Ghorbani et al., 2018). There are many potential mechanisms for the antidepressant effect of probiotic treatment (Borovikova et al., 2000; Bravo et al., 2011; Gayathri & Rashmi, 2017), including increased concentration of mood regulating metabolites, increased access to energy substrates, and improved sleep. Despite similar antidepressant-like effects, our observations suggest that each probiotic mixture may involve different biological mechanisms.

Decreases in tryptophan concentration in the prefrontal cortex were not observed in sleep disrupted male and female mice if they received Cerebiome or Lacidofil treatment. However, only Cerebiome treatment showed recovery of serotonin

concentration in sleep disrupted mice. These observations supported our hypothesis that probiotic treatment would prevent decreases to tryptophan following sleep disruption. These observations only partially support our hypothesis that probiotic treatment would prevent decreases in serotonin concentration as only Cerebiome displayed this effect. Previous clinical investigations showed that an 8-week administration of the probiotic strains found in Cerebiome (*B. longum* and *L. helveticus*) and Lacidofil (*L. helveticus*) reduce tryptophan breakdown along the kynurenine pathway in humans with mild and moderate depression symptoms (Kazemi et al., 2019). These findings suggest that probiotic treatment may increase available tryptophan by inhibiting its catabolism, perhaps by reducing corticosteroid or inflammatory marker secretion that often increases tryptophan conversion along the kynurenine pathway (Baratta et al., 2018; Miura et al., 2008). The individual composition of probiotic formulations may have resulted in differing effects on serotonin concentration. Cerebiome contains higher concentration of *B. longum* and *L. helveticus* than Lacidofil. Thus, Cerebiome may have increased tryptophan concentration in the prefrontal cortex sufficiently to increase tryptophan synthesis to serotonin, resulting in greater antidepressive effect than Lacidofil (Hilakivi-Clarke, 1991; O'Mahony et al., 2015).

Our results also showed that chronic sleep disruption reduces extracellular hippocampal L-lactate concentration. This effect was not observed in male and female mice that were previously treated with either Cerebiome or Lacidofil. These findings support our hypothesis that probiotic treatment may improve access to alternate energy substrates in the brain during periods of depressive behaviour and reduced energy access. Probiotics may have increased hippocampal L-lactate through peripheral blood circulation. Individuals with surgically shortened intestines or who report high lactic acid probiotic diets display higher concentrations of lactic acid producing microorganisms in the gut and often predict increased blood lactate concentration (Nakagawa et al., 2018; Uchida et al., 2004). We observed increased peripheral blood L-lactate in male and female mice following an acute oral gavage of sugar water and 18 days of Cerebiome or Lacidofil administration when compared to mice with access to water only (see Supplementary material 3). Increasing concentration of lactic acid producing microorganisms in the gut may result in increased glucose conversion to lactate in the

gut, increasing L-lactate transfer across the gut lumen into the circulatory system. Furthermore, increasing blood L-lactate increases hippocampal L-lactate concentration and reduces depression-like behaviour. Adult male mice treated with chronic peripheral sodium lactate injections or Desipramine displayed similar increases to hippocampal L-lactate and astrocyte activity in the hippocampus and similar reductions to depressive behaviour (Carrard et al., 2018). Therefore, lactic acid probiotic treatment may produce sufficient L-lactate in peripheral blood to impact brain chemistry and reduce depression-like behaviour in specialized circumstances.

Decreased extracellular hippocampal glucose following sleep disruption were not observed in male and female mice that were previously treated with Cerebiome. This data only partially supported our hypothesis as Cerebiome, but not Lacidofil, increased hippocampal glucose in the brain following chronic sleep disruption. Previous investigations may support our observations of a strain-specific effect on hippocampal glucose concentration (Bonfili et al., 2020; Morgello, Uson, Schwartz, & Haber, 1995). In a mouse model of Alzheimer's disease, mice treated with a probiotic mixture similar to Cerebiome containing *L. helveticus* and multiple *Bifidobacterium* strains increased the expression of glucose transporting proteins GLUT1 and GLUT3 in the hippocampus (Bonfili et al., 2020). GLUT1 is found at the blood-brain barrier and in gray matter astrocytes responsible for regulation of glucose uptake at the blood brain barrier (Morgello et al., 1995). Cerebiome seems to increase transport of glucose across the blood brain barrier. It is unclear if *L. rhamnosus* found in Lacidofil has similar effects on GLUT1. Furthermore, GLUT1 deficient mice (Furuse et al., 2019) and humans (Tzadok et al., 2014) commonly display increased sleep disturbances. Our observations highlight the strain-specific effects different probiotic mixtures can display on central metabolism. Cerebiome-like formulations may be particularly efficient in moderating glucose dysregulation associated with sleep disruption.

Sleep-disrupted mice displayed decreased REM per bout in the rest phase and increased NREM in the awake phase when compared to rested mice. Sleep-disrupted male and female mice treated with Cerebiome did not display a significant decrease in REM duration per bout in the rest phase. Furthermore, Lacidofil treated mice displayed

increased NREM duration in the rest phase. These results support our hypothesis that probiotic treatment may partially reduce sleep disruption induced changes to sleep and depression-like behaviour. Previous literature suggests that diversifying gut flora reduces the impact of stress and may improve sleep quality. Adult male rats exposed to an inescapable tail shock stressor and a prebiotic diet since puberty displayed increased fecal *L. rhamnosus* concentration and increased time spent in REM compared to stressed rats given a regular diet (Thompson et al., 2017). Adult men and women treated for 4 weeks with *L. helveticus* MIKI-020 displayed greater self-reported calmness, motivation, and decreased fatigue as measured by the Visual Analogue scale (Nakagawa et al., 2018). Increasing lactic acid-producing gut flora may improve sleep by reducing the impact of stress. However, it is unclear why Cerebiome treatment effected sleep differently than Lacidofil. Nevertheless, our results indicate that certain probiotic strains can prevent changes to sleep following sleep disruption.

Probiotic treatment shortened sleep latency in rested mice. Conversely, sleep-disrupted mice treated with water displayed shorter sleep latency than sleep-disrupted mice treated with probiotics. These observations only partially support our hypothesis that probiotic treatment would reduce sleep latency as neither Cerebiome nor Lacidofil reduced sleep latency in sleep disrupted mice. Previous investigations provide contradicting results on the relation between lactic acid probiotics and sleep latency (Irwin et al., 2020; Murack & Messier, 2019). A meta-analysis reviewing fourteen studies of probiotic treatment and sleep in humans were unable to identify an effect of probiotic treatment on sleep latency using polysomnography recordings in healthy, depressed, or stressed adults (Irwin et al., 2020). Conversely, young adult C57BL/6J male mice stressed by introduction into a new environment display shorter sleep latency after 13 days of *Lactobacillus fermentum* PS150 (Lin et al., 2021). Based on these findings, the effect of probiotics on sleep latency may be limited to healthy adolescents or rodent samples. Furthermore, sleep-disrupted mice treated with probiotics may be more rested and so display a greater sleep latency than sleep-disrupted mice treated with water. Our observations of increased NREM duration and REM duration per bout following Lacidofil and Cerebiome, respectively, suggests that probiotic treatment may increase sleep pressure which may reduce daytime fatigue associated with depression in sleep-

disrupted mice. Further research is required to address the generalizability of these findings to adolescent men and women.

5.0 Conclusion

Depressive disorders are difficult to treat partly due to their many diverse symptoms (Wolf & Hopko, 2008). Lactic acid probiotics may be uniquely suited to treat the multi-modal syndrome of adolescent depression induced by chronic sleep disruption (Nakagawa et al., 2018; Romijn et al., 2017). Cerebiome and Lacidofil probiotic mixtures decrease adolescent depressive-behaviour inflated by chronic sleep disruption. Sleep disruption decreases the concentration of biomolecules associated with antidepressant effects like tryptophan in the prefrontal cortex and L-lactate in the hippocampus. However, sleep disrupted mice administered probiotics do not display the same decreased concentration of biomolecules. Furthermore, Cerebiome and Lacidofil selectively prevent the disruptive effects of sleep disruption on sleep architecture by increasing REM per bout and NREM duration in the rest phase, respectively. The underlying mechanisms responsible for the reduction of depression-related symptoms following lactic acid probiotic administration are unclear and required further investigation before serious consideration as an adjunct treatment for adolescent depression.

Conflicts of Interest

The authors report no declarations of interest.

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References

See end of document.

Tables, Figures, and Captions

See below.

Table 1

		Interactions (F value)				**Main effects (F value)**		
		Sex*Sleep*Tx	Sex*Sleep	Sex*Tx	Sleep*Tx	Sex	Sleep	Tx
Sleep Latency	Rested	N/A	N/A	**3.4***	N/A	**5.2***	N/A	**8.3****
	Sleep-Disrupted	N/A	N/A	**4.8***	N/A	0.2	N/A	**11.3*****
Late Rest Phase								
NREM	Duration	1.1	0.08	0.09	1.0	**4.8***	0.09	**3.7***
	Bouts	1.1	0.8	**5.8****	0.004	**7.0****	2.7	2.2
	Duration/ Bout	0.2	0.7	**4.5***	0.6	**14.0*****	0.9	0.6
Wake	Duration	0.6	0.05	0.4	0.8	**5.9***	0.1	**3.7***
	Bouts	0.4	0.02	**5.1****	0.6	**21.1*****	0.6	2.4
	Duration/ Bout	0.5	0.2	**3.4***	0.3	**8.7****	2.8	**5.5****
REM	Duration	0.6	**4.4***	1.0	0.3	0.3	2.6	2.0
	Bouts	0.5	1.8	2.5	0.3	1.2	**5.1***	1.5
	Duration/ Bout	0.7	3.3	0.6	**3.4***	0.1	3.4	**3.9***
Early Awake Phase								
NREM	Duration	2.0	0.3	0.9	1.7	**5.3***	**4.3***	0.05
	Bouts	0.4	0.4	1.5	1.7	0.6	**5.8***	0.5
	Duration/ Bout	1.0	0.01	0.8	0.6	2.1	0.2	1.5
Wake	Duration	2.1	0.03	0.9	1.9	**5.1***	**4.4***	0.1
	Bouts	0.1	0.4	0.2	1.5	0.2	**4.1***	0.2
	Duration/ Bout	0.1	0.4	**3.8***	0.9	3.5	1.1	1.1
REM	Duration	0.8	0.1	0.1	2.1	1.2	0.4	1.3
	Bouts	0.6	0.07	0.1	1.2	2.2	0.07	0.2
	Duration/ Bout	2.1	1.5	0.1	1.8	0.07	0.4	**3.9***

Table 1: The effect of sex, sleep disruption, and probiotic treatment on NREM, REM, and Wake duration (seconds), bouts, and duration per bout (average seconds/bout). *p<.050, **p<.010, ***p<.001.

Table 2

		Interactions (F value)				**Main effects (F value)**		
		Sex*Sleep*Tx	Sex*Sleep	Sex*Tx	Sleep*Tx	Sex	Sleep	Tx
Depressive Behaviour								
TST	Immobility	0.04	0.5	0.06	**3.5***	**14.3*****	0.2	1.6
FST	Immobility	0.5	0.08	**4.1***	2.5	0.5	**62.1*****	**73.2*****
	Swimming	0.8	0.2	0.01	0.88	2.4	**5.3***	**10.5*****
	Climbing	0.04	0.02	0.9	0.3	0.9	0.8	1.9
Metabolites								
Tryptophan	PFC	0.4	0.5	2.9	**5.2****	0.2	1.1	1.0
	Plasma	0.2	2.1	0.05	0.04	0.7	0.02	0.06
5-HT	PFC	0.04	0.03	0.4	**3.9***	0.08	3.4	**3.2***
	Plasma	0.06	1.7	0.7	1.3	1.3	0.7	1.8
BDNF	PFC	0.3	1.1	2.7	1.6	0.7	**25.0*****	1.8

Extracellular Hippocampal Metabolism											
	Interactions (F value)							**Main effects (F value)**			
	Sex*Sleep *Tx*Time	Sex* Sleep	Sex*Tx	Sex* Time	Sleep* Tx	Tx*Time	Sleep* Time	Sex	Sleep	Tx	Time
Glucose											
Late Rest Phase	1.1	0.02	0.2	0.7	**3.5***	1.2	0.7	0.002	**9.3****	**5.0****	0.9
Early Awake Phase	1.1	0.02	0.2	1.1	**4.9***	1.1	1.0	0.05	**16.5*****	**5.6****	1.4
L-Lactate											
Late Rest Phase	0.2	0.004	0.8	0.9	1.4	1.4	1.1	0.6	**4.1***	1.3	1.7
Early Awake Phase	1.2	0.06	1.3	1.4	**4.9***	1.7	0.9	0.5	**16.7*****	**4.5***	0.5

Table 2: The effect of sex, sleep disruption, and probiotic treatment on depression behaviour and peripheral and central metabolites associated with depression. *p<.050, **p<.010, ***p<.001.

Figure 1

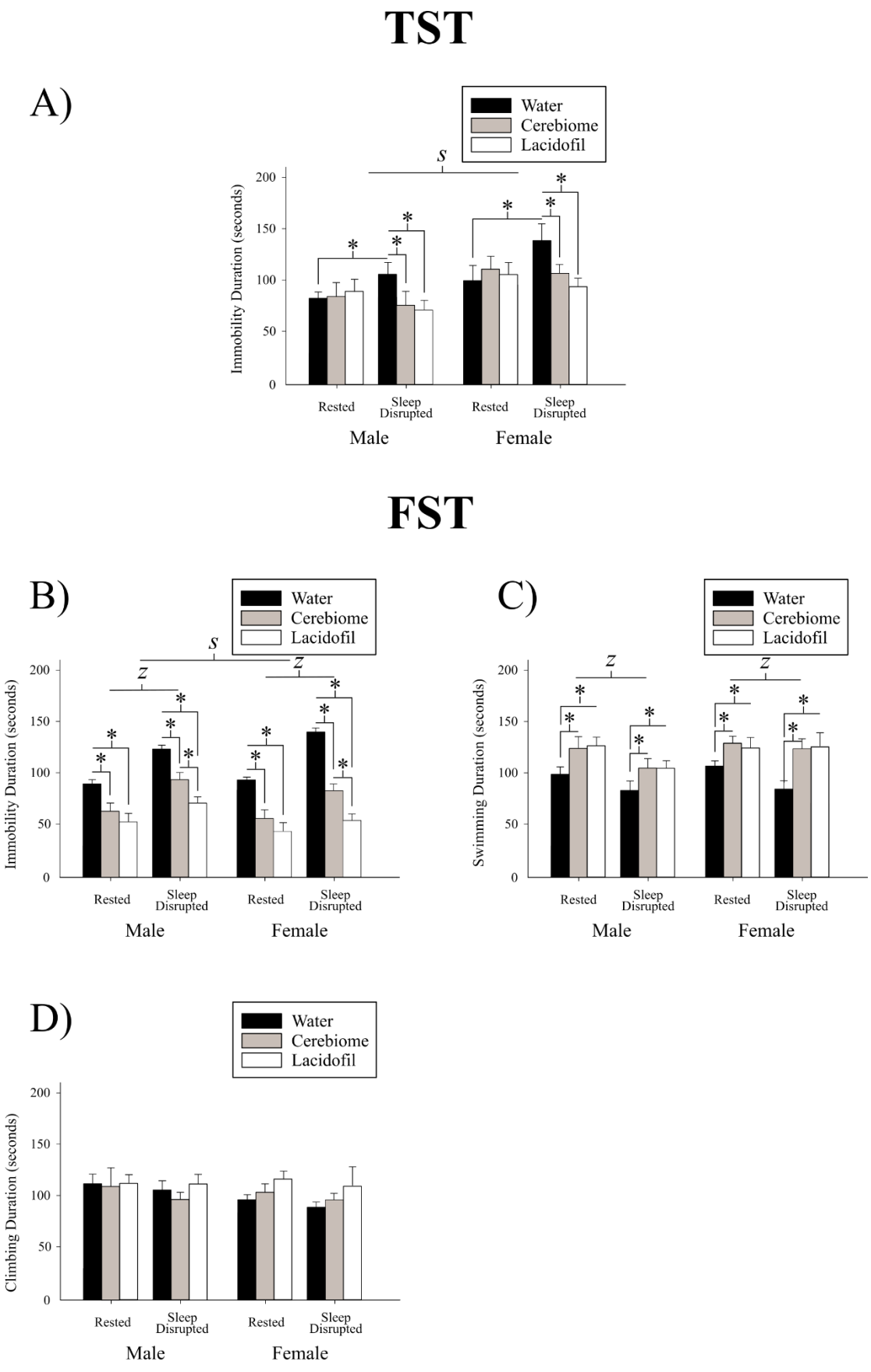

TST
A)
Water
Cerebiome
Lacidofil
S
Immobility Duration (seconds)
Rested
Sleep Disrupted
Male
Female
FST
B)
Z
Immobility Duration (seconds)
C)
Swimming Duration (seconds)
D)
Climbing Duration (seconds)

Fig. 1. Mean (±SEM) durations of immobile behaviour in the tail suspension test (A) and immobile (B), swimming (C), and climbing (D) behaviours in the forced swim test of rested and sleep-disrupted male and female mice treated with either water, Cerebiome or Lacidofil. * = significant group difference ($p < .05$)

Figure 2

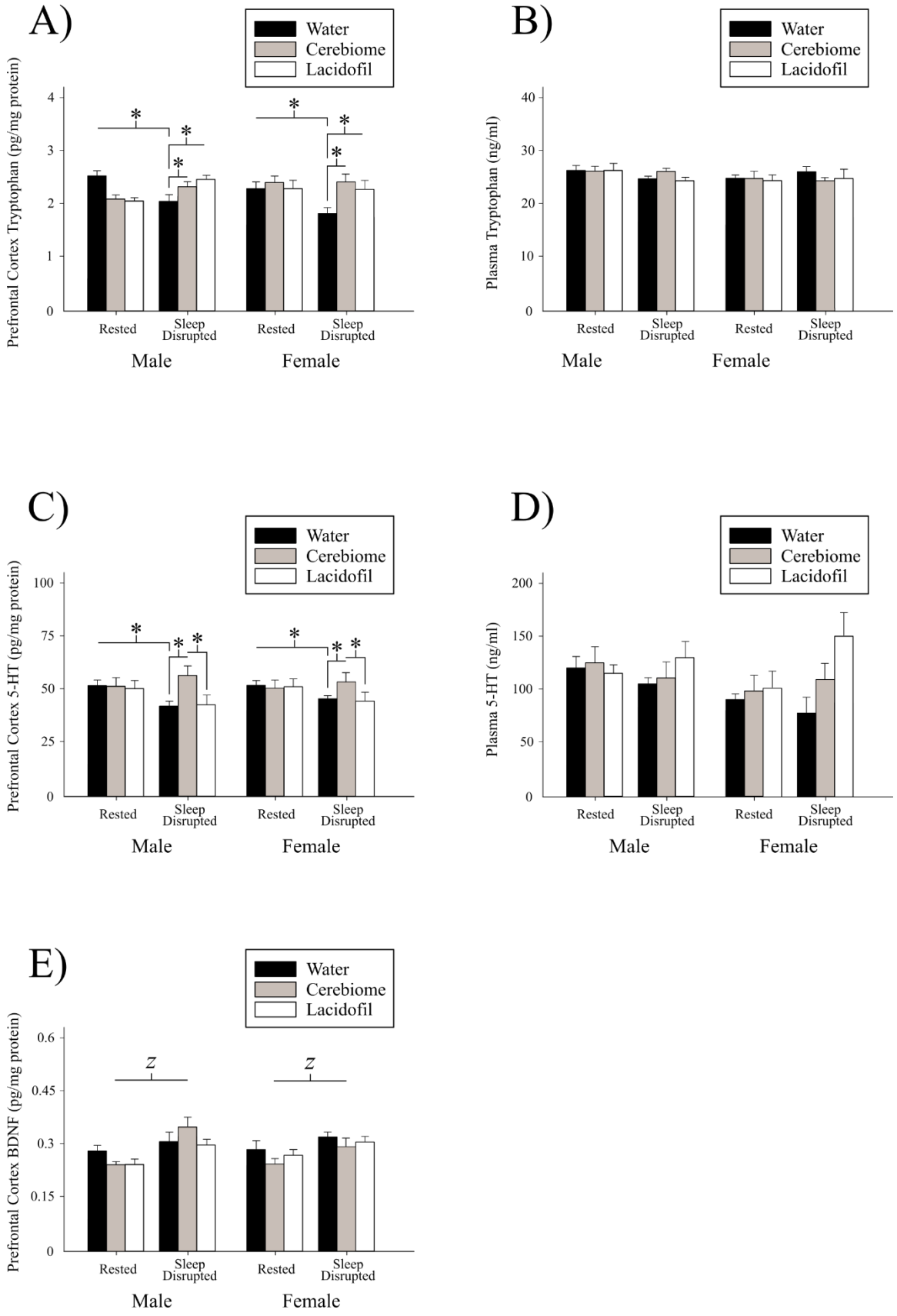

A)
Water
Cerebiome
Lacidofil
Prefrontal Cortex Tryptophan (pg/mg protein)
0
1
2
3
4
*
*
*
*
*
Rested
Sleep Disrupted
Rested
Sleep Disrupted
Male
Female
B)
Water
Cerebiome
Lacidofil
Plasma Tryptophan (ng/ml)
0
10
20
30
40
Rested
Sleep Disrupted
Rested
Sleep Disrupted
Male
Female
C)
Water
Cerebiome
Lacidofil
Prefrontal Cortex 5-HT (pg/mg protein)
0
25
50
75
100
*
*
*
*
*
Rested
Sleep Disrupted
Rested
Sleep Disrupted
Male
Female
D)
Water
Cerebiome
Lacidofil
Plasma 5-HT (ng/ml)
0
50
100
150
200
Rested
Sleep Disrupted
Rested
Sleep Disrupted
Male
Female
E)
Water
Cerebiome
Lacidofil
Prefrontal Cortex BDNF (pg/mg protein)
0
0.15
0.3
0.45
0.6
z
z
Rested
Sleep Disrupted
Rested
Sleep Disrupted
Male
Female

Fig. 2. Mean (±SEM) concentrations of tryptophan in the prefrontal cortex (A) and plasma (B), serotonin in the prefrontal cortex (C) and plasma (D), and BDNF in the prefrontal cortex of rested and sleep-disrupted males and females treated with either water, Cerebiome or Lacidofil. Prefrontal cortex concentrations were assessed as picograms of target per milligram of total protein extracted (A, C, and E). Plasma concentrations were assessed as nanograms of target per milliliter of plasma (B and D). * = significant group difference ($p < .05$)

Figure 3

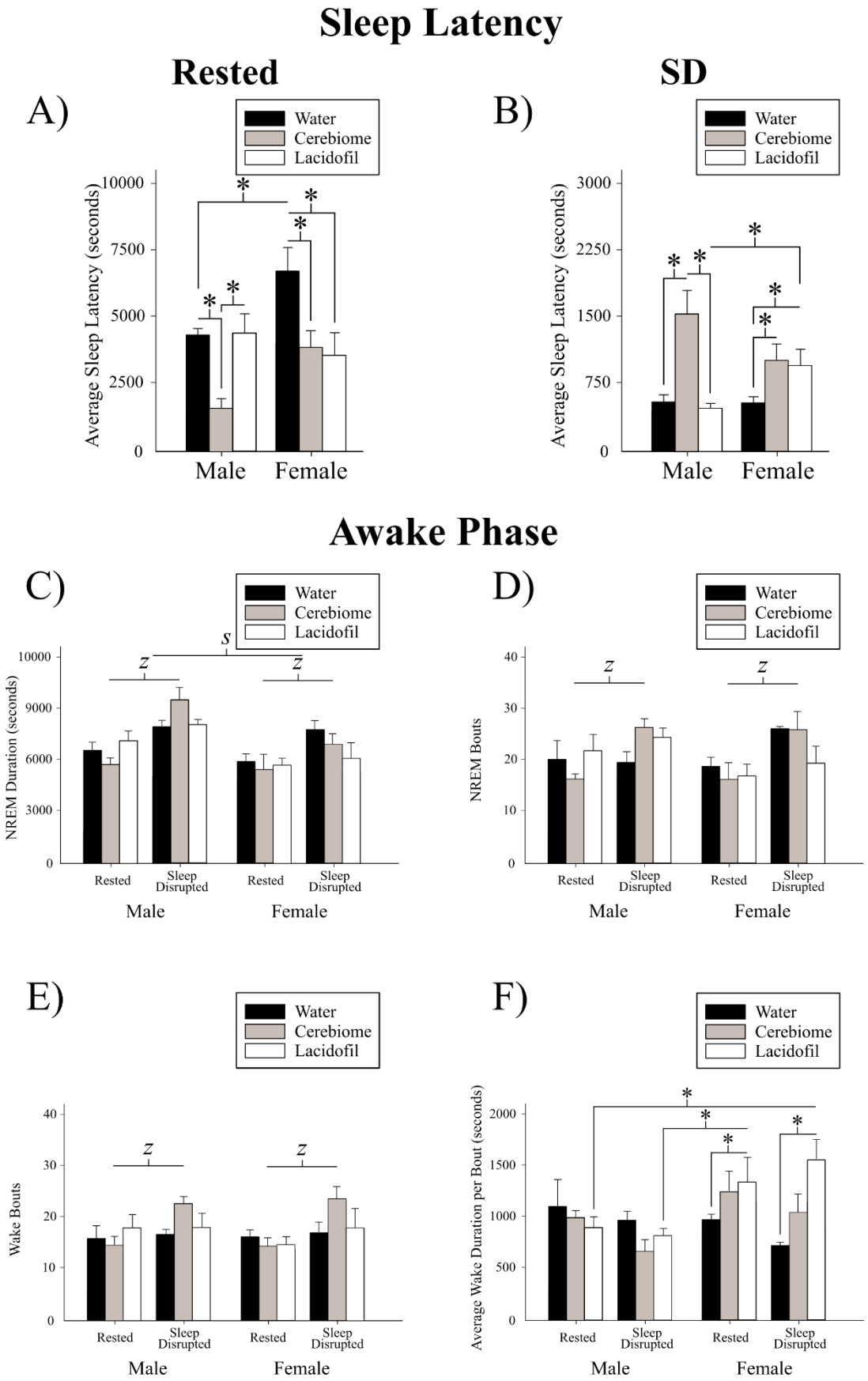

Sleep Latency
Rested
SD
A)
B)
Water
Cerebiome
Lacidofil
Average Sleep Latency (seconds)
10000
7500
5000
2500
0
3000
2250
1500
750
Male
Female
Awake Phase
C)
D)
E)
F)
NREM Duration (seconds)
NREM Bouts
Wake Bouts
Average Wake Duration per Bout (seconds)
Rested
Sleep Disrupted

Fig. 3. Latency to sleep in rested (A) and sleep-disrupted (B) male and female mice treated with either water, Cerebiome, or Lacidofil from Lights On (A) or following sleep disruption period (B). Duration of NREM (C), number of NREM bouts (D), number of Wake bouts (E) and average duration of Wake per bout (F) in male and female mice treated with either water, Cerebiome, or Lacidofil was assessed in the awake phase. * = significant group difference ($p < .05$)

Figure 4

Rest Phase

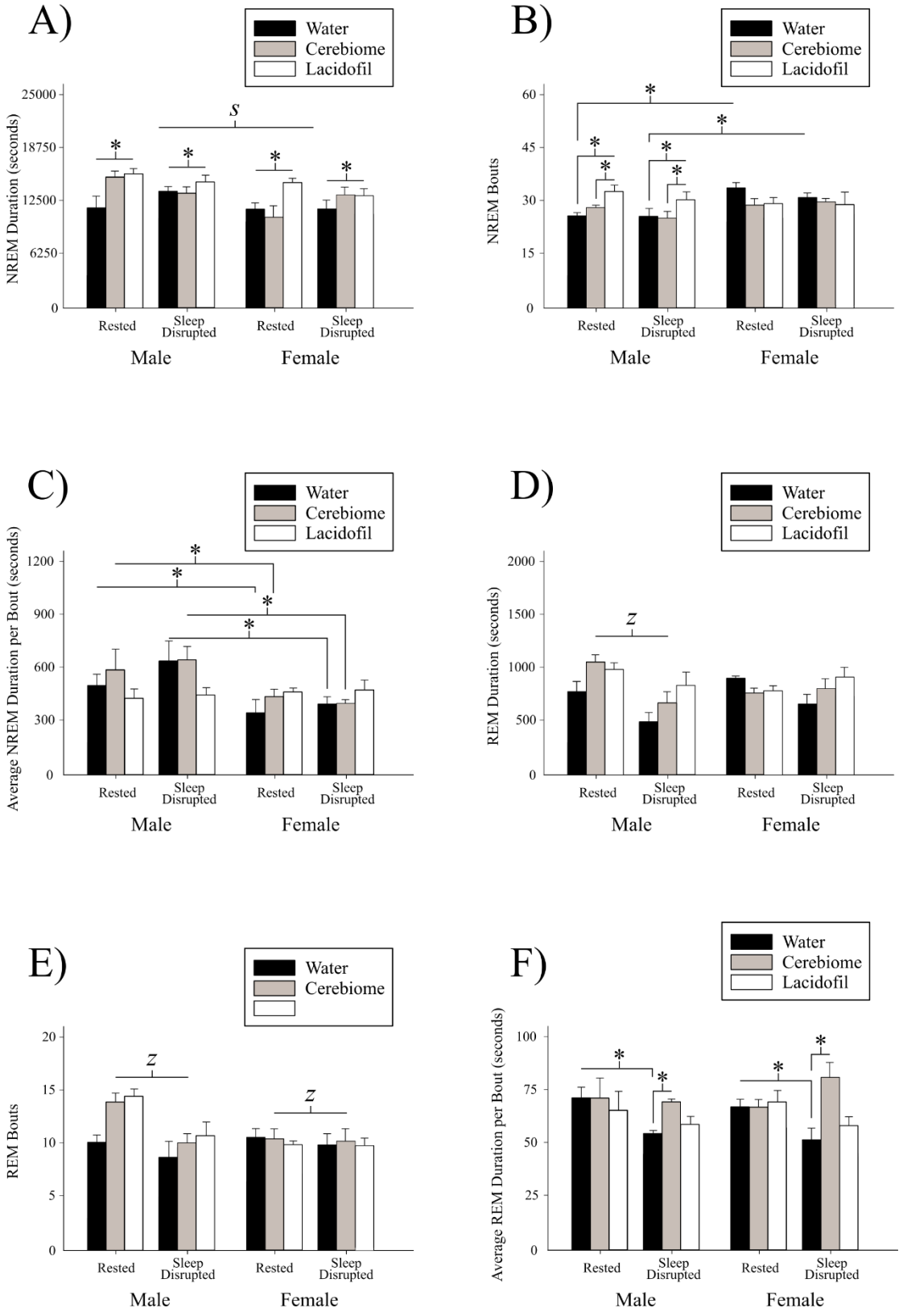
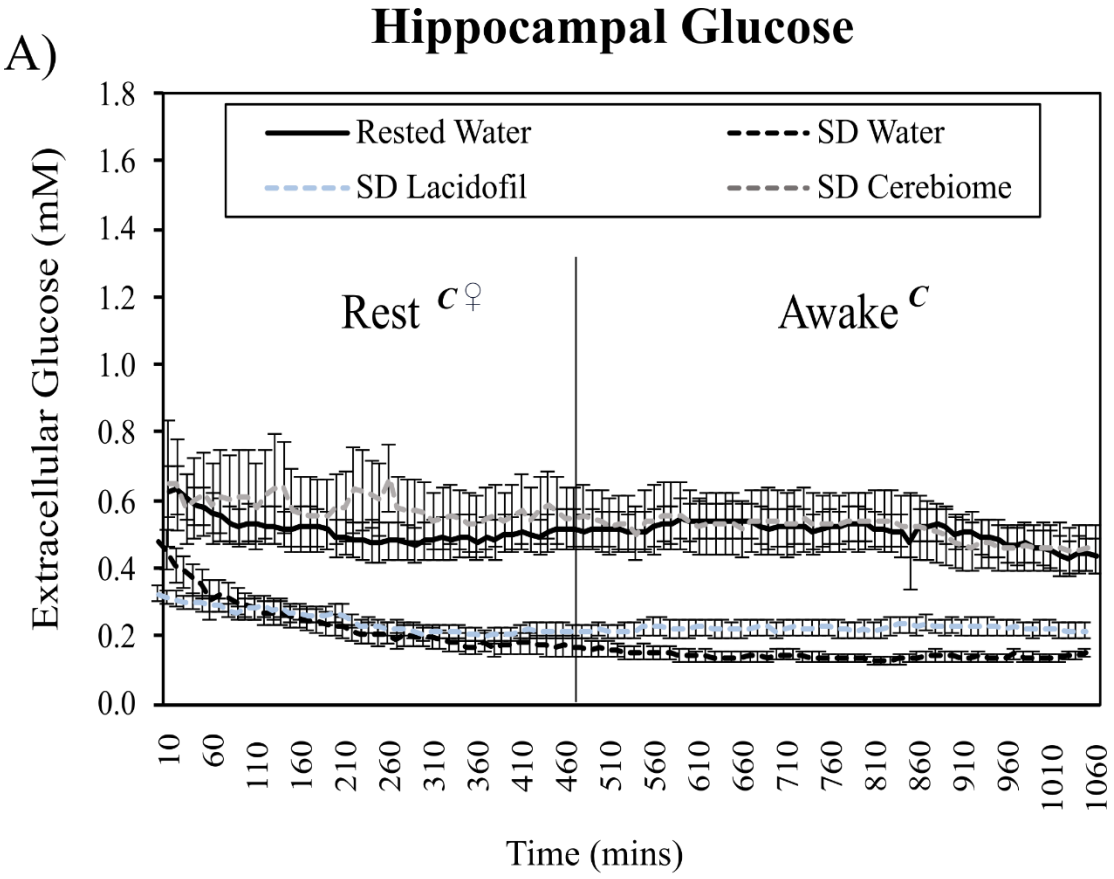


Fig. 4. Duration of NREM (A), number of NREM bouts (B), average NREM duration per bout (C), REM duration (D), REM bouts (E), and average REM duration per bout (F) in rested and sleep disrupted male and female mice treated with either water, Cerebiome, or Lacidofil were assessed in the rest phase. * = significant group difference ($p < .05$)

Figure 5


A)
Hippocampal Glucose
Extracellular Glucose (mM)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Rested Water
SD Water
SD Lacidofil
SD Cerebiome
Rest C ♀
Awake C
10 60 110 160 210 260 310 360 410 460 510 560 610 660 710 760 810 860 910 960 1010 1060
Time (mins)

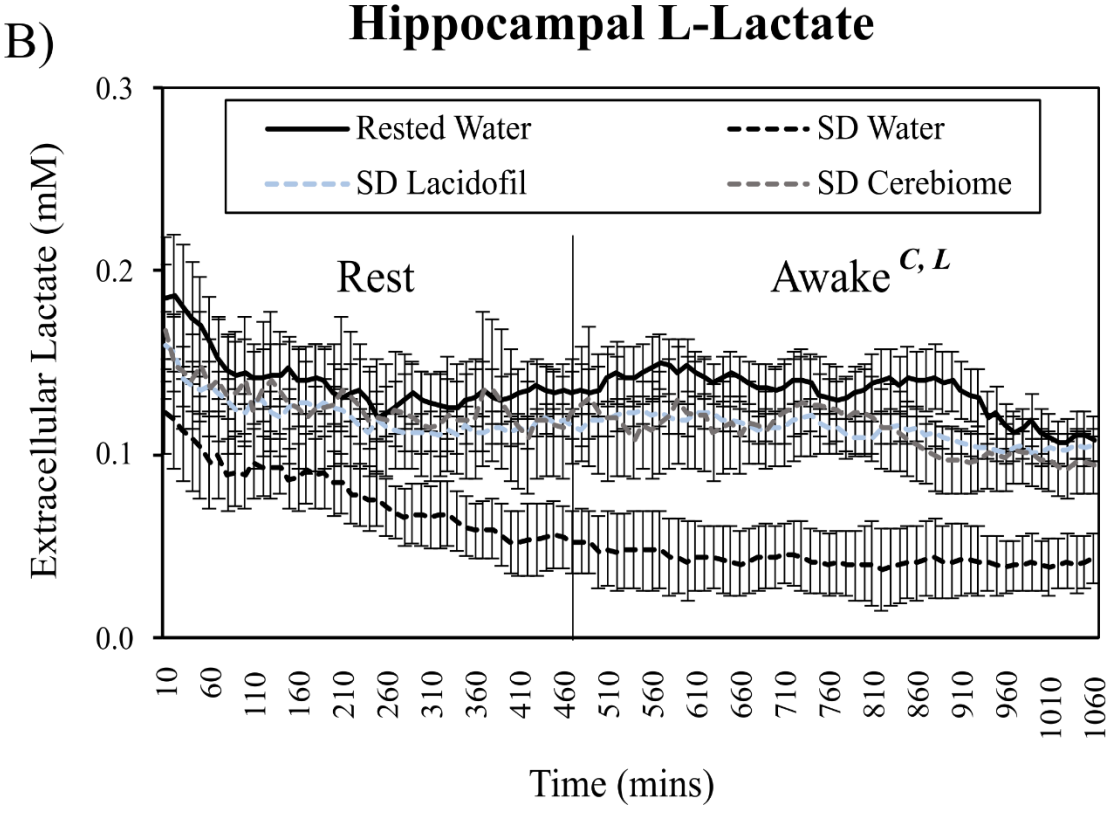
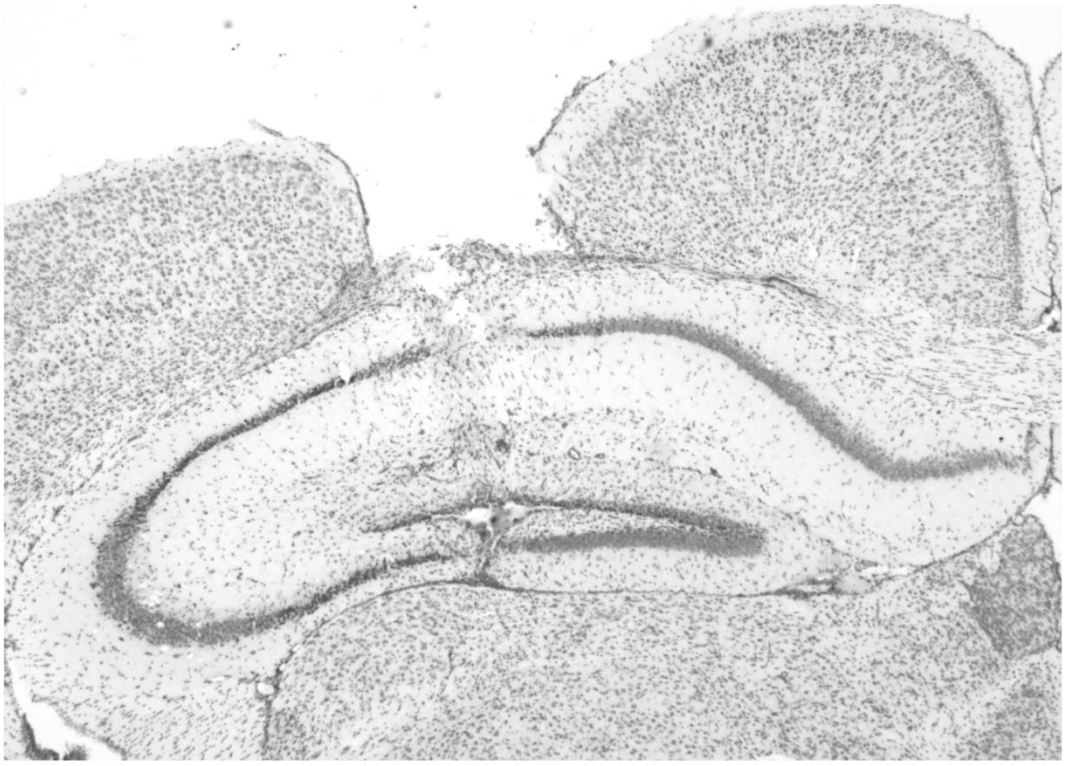

B)
Hippocampal L-Lactate
Extracellular Lactate (mM)
0.3
0.2
0.1
0.0
Rested Water
SD Water
SD Lacidofil
SD Cerebiome
Rest
Awake C, L
10 60 110 160 210 260 310 360 410 460 510 560 610 660 710 760 810 860 910 960 1010 1060
Time (mins)

Fig. 5. Mean (±SEM) concentration of extracellular hippocampal glucose (A) and L-lactate (B) in rested and sleep disrupted male and female mice treated with either water, Cerebiome, and Lacidofil were assessed during the rest and awake phases. * = significant group difference ($p < .05$), ***C*** = Cerebiome > Water, ***L*** = Lacidofil > Water, ***C*** ♀= Cerebiome treated females > water treated females

Supplementary material 1



Supplementary material 1: Histology verification of biosensor placement. Above is an image of a mouse brain slice taken following implantation of a biosensor in the CA1 of the hippocampus. Each surgical implantation was verified following plasma and tissue collection.

Supplementary material 2

1.0 Total Recordings: Rest + Awake Phases

1.1 NREM Duration

There was a significant main effect of Sex ($F_{(1,60)} = 9.0$, $p < 0.05$, $\eta_P^2 = 0.13$) on total NREM duration. Overall, male mice displayed greater total NREM duration than female mice (MD = 1950.4, SE = 649.1, $p = 0.004$).

1.2 NREM Bouts

There was a significant Sex x Treatment ($F_{(2,60)} = 5.3$, $p < 0.05$, $\eta_P^2 = 0.15$) interaction on total NREM bouts. Male mice treated with water displayed fewer total NREM bouts than male mice treated with Lacidofil (MD = -10.5, SE = 3.8, $p = 0.021$) and female mice treated with water (MD = -9.8, SE = 3.8, $p = 0.011$).

1.3 Average duration of NREM per Bout

There was a significant main effect of Sex ($F_{(1,60)} = 6.9$, $p < 0.05$, $\eta_P^2 = 0.10$) on total NREM duration per bout. Overall, male mice displayed greater total NREM duration per bout than female mice (MD = 54.7, SE = 20.7, $p = 0.011$).

1.4 Wake Duration

There was a significant main effect of Sex ($F_{(1,60)} = 12.7$, $p < 0.05$, $\eta_P^2 = 0.18$) on total Wake duration. Overall, female mice displayed greater total Wake duration than male mice (MD = 2396.8, SE = 672.7, $p = 0.001$).

1.5 Wake Bouts

There was a significant main effect of Sex ($F_{(1,60)} = 6.1$, $p < 0.05$, $\eta_P^2 = 0.09$) on total Wake bouts. Overall, female mice displayed greater total Wake bouts than male mice (MD = 5.1, SE = 2.1, $p = 0.016$).

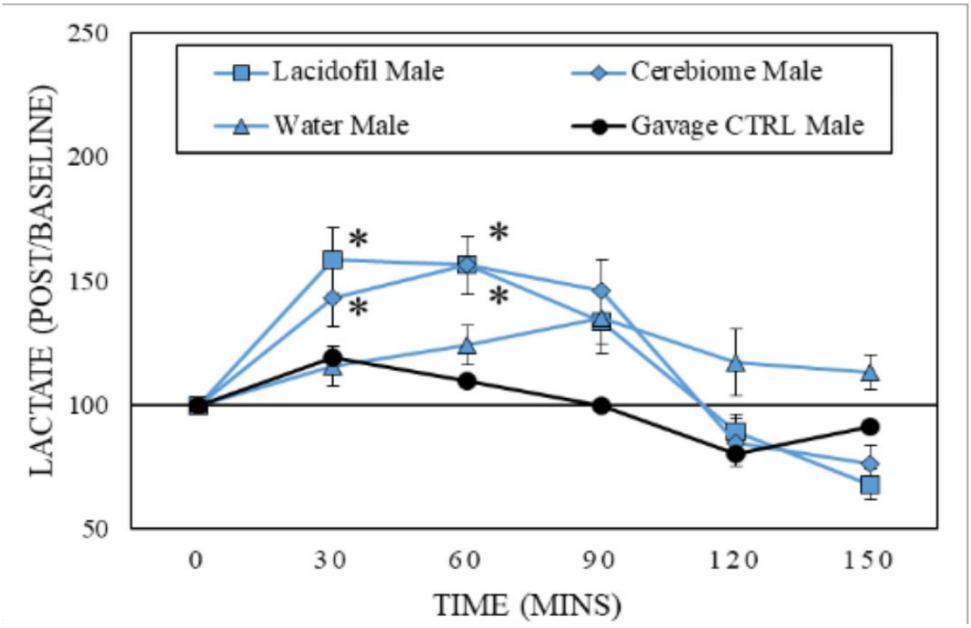
1.6 Average duration of Wake per Bout

There were significant Sex x Treatment ($F_{(2,60)} = 4.6$, $p < 0.05$, $\eta_P^2 = 0.13$) and Sleep x Treatment ($F_{(2,60)} = 3.7$, $p < 0.05$, $\eta_P^2 = 0.11$) interactions on total Wake duration per bout. Female mice treated with Lacidofil displayed greater total Wake duration per

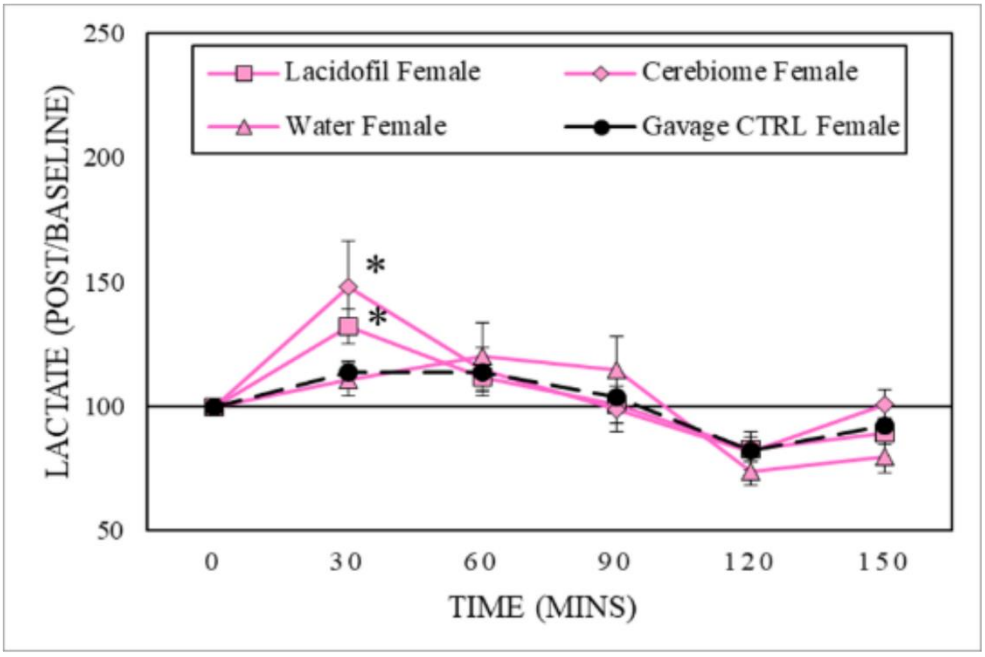
bout than male mice treated with Lacidofil (MD = 202.5, SE = 86.8, $p = 0.023$). Rested mice treated with Cerebiome displayed greater total Wake duration per bout than sleep disrupted mice treated with Cerebiome (MD = 212.8, SE = 86.8, $p = 0.017$).

Supplementary material 3

A)



B)



Supplementary material 3: Four week old male and female mice were treated with either Lacidofil, Cerebiome or water for 18 days. L-lactate concentration was measured from tail vein blood samples every 30 minsute following an oral gavage of 2g/kg glucose solution. L-lactate was assessed as concentration percent change from baseline.

Chapter 5: General Discussion

Depression is responsible for reducing quality-of-life (James et al., 2018; Weissman et al., 1993; Weissman & Klerman, 1977) and has been a focus for psychopathological research for nearly a century (López-Muñoz & Álamo, 2016; Lowenbach et al., 1947; Paykel, 2022). L-lactate administration has been shown to reduce depression and normalize impaired sleep (Béland-Millar et al., 2017; Carrard et al., 2021; Carrard et al., 2018; Magistretti & Allaman, 2018). Our objective was to examine the metabolite L-lactate and its relationship with depression and sleep. Our examinations included evaluations of oral treatments that increase L-lactate, identification of likely sources of stress-induced depression onset, and assessments of the potential antidepressant effects of L-lactate. We hypothesized one could safely increase L-lactate concentration in the blood and brain by an orally administered treatment (Lowenbach et al., 1947; Uchida et al., 2004). We hypothesized that significant sleep delay and disturbances could promote increased depression development during adolescence (Gallucci et al., 1993; McCormick et al., 1995; Romeo, 2010). Finally, we hypothesized we could increase L-lactate in sleep-disrupted pubertal groups through an oral treatment and reduce the onset of depressive behaviour, reduce sleep patterns associated with our model of adolescent depression, and moderate underlying signalling mechanisms associated with depression.

1. Summary of Results

In Chapter 2, oral administration of both Lowenbach's lactate beverage and medium chain triglyceride oil increased blood L-lactate in male mice immediately and up to a few hours following inoculation (Murack & Messier, 2019). These findings support our hypotheses that blood L-lactate concentration can be significantly influenced through oral treatments. Our initial findings suggest that L-lactate concentration can be manipulated through an orally administered intervention. Recent research associating L-lactate treatment with increased blood and brain L-lactate concentrations are limited to administration by injection (Béland-Millar et al., 2017; Carrard et al., 2021; Carrard et al., 2018). This is potentially problematic, as treatments using injection administration routes typically display decreased patient compliance and adherence to treatment

scheduling when compared to orally administered interventions (Alqahtani, Kazi, Alsenaidy, & Ahmad, 2021). Manipulation of L-lactate concentration through an orally administered treatment would increase the likelihood of treatment success and societal adoption. Our observations indicate that manipulation of blood L-lactate concentration and L-lactate associated behaviour is feasible with orally administered treatments.

Consumption of Lowenbach's lactate beverage or the medium chain triglyceride oil provided challenges that limited their usefulness as moderators of blood L-lactate concentration (Lowenbach et al., 1947; Murack & Messier, 2019). Lowenbach's lactate beverage increased blood L-lactate in a dose-dependent manner, but also proportionally increased sickness behaviour. The increased sickness behaviour we observed in mice likely mirrored the self-reported nausea described by human participants in the original Lowenbach lactate beverage study (Lowenbach et al., 1947). Lower concentrations of the lactate beverage displayed minimal sickness behaviour but did not significantly alter blood L-lactate concentration (Murack & Messier, 2019). Animals treated with higher concentrations of medium chain triglycerides displayed increased blood L-lactate without sickness behaviour. However, the most successful dosages (5ml/kg, and 10 ml/kg) were potentially too high for human use as they would theoretically require large volumes of oil consumption. Although we verified that blood L-lactate can be increased through orally administered interventions, we had yet to find a safe, palatable, and efficient treatment type.

Chapter 2 also evaluated the dose-dependent effect of sodium lactate injections on late day behaviour as an analogue for late day drowsiness (Murack & Messier, 2019). Mice were evaluated for running wheel activity at the end of their awake phase following chronic sodium lactate or saline injections. Mice displayed the quickest and greatest decrease to late day running wheel activity if administered the largest dosage of sodium lactate. Decreases to late day activity were less pronounced with lower concentration dosages of sodium lactate. These observations support our hypothesis that L-lactate based treatment may reduce sleep latency or sleep disturbances associated with depression. Increasing available lactate may reduce late day activity and perhaps instill "drowsiness" as reported by patients and control participants in the original lactate beverage study

(Lowenbach et al., 1947). L-lactate concentration has been identified as a potential biomarker for identifying differing stages of sleep (Naylor et al., 2012). However, it is unclear if L-lactate treatments can be used to manipulate sleep characteristics. Our observations suggest that sleep latency may be shortened by L-lactate treatment. Chapter 2 provided sufficient support for an interaction between L-lactate and sleep and justified further investigations.

Chapter 3 assessed the suitability of adolescent mouse models for investigations into sleep and depression development (Murack et al., 2021). Chapter 3 identified chronic sleep disruption, a common adolescent stressor, as a potential trigger for increased depression-like behaviour in adolescent male and female mouse samples. This supported our hypothesis that delayed or disturbed sleep would increase the likelihood of depression development in a population susceptible to mood disorder development. Increased depression-like behaviour in adolescent groups was accompanied by decreases to serotonin receptor expression throughout the brain and potential sensitization of underlying stress regulating pathways (Murack et al., 2021). Previous studies have reported associations between impaired or disturbed sleep and depression (Borbély & Wirz-Justice, 1982; Gillin et al., 1979). However, Chapter 3 identified associations between increased depression behaviour, modified serotonergic signaling, and a modified response to novel stress in a pubertal model exposed to sleep disruption. We reduced the likelihood of stress activation from physical manipulation by using the “gentle manipulation” method (Gross et al., 2015; S.-R. Yang et al., 2012). Use of the gentle manipulation method increased our confidence in the assertion that the observed modifications to stress reactivity, serotonergic signaling, and depressive behaviour resulted from sleep disruption rather than handling stress. These observations provide a model to which we can test the anti-depressant effects of L-lactate. We hypothesized that attenuation of the effects of sleep disruption on adolescent groups would reduce the likelihood of the development of depressive phenotypes.

Investigations outlined in Chapter 4 provided assessment of depressive behaviour, sleep patterns, and psychoactive brain molecules following oral treatment of L-lactate producing probiotics in mice depressed by chronic sleep disruption. Chronic sleep

disruption caused increases to depression-like behaviour, reductions of prefrontal tryptophan, prefrontal serotonin, hippocampal glucose, and hippocampal L-lactate concentration, and increased NREM duration in the early awake phase. Probiotic treatment reversed many of the effects associated with increased adolescent depression. These results support our hypothesis that L-lactate concentration may be increased by oral treatment with probiotics. These results also supported our hypothesis that sleep disruption induces depression in pubertal samples by replicating our observations on depressive behaviour from Chapter 3 (Murack et al., 2021). And finally, our observations support our hypothesis that an oral treatment associated with increasing L-lactate production will reduce depressive behaviour and improve sleep. Probiotic treatment effectively influenced multiple mechanisms associated with depression. However, it is unclear whether probiotic treatment reduced depression by increasing access to biomolecules associated with overall mental heath, increasing access to energy providing metabolites, moderating sleep, a combination of these pathways, or moderation by external factors. It is possible that L-lactate contributes to improved sleep and reduced depression following probiotic treatment as observed in previous investigations (Carrard et al., 2018; Lowenbach et al., 1947; Murack & Messier, 2019). However, the directionality of the relationship between L-lactate, sleep, and depression is not verified in Chapter 4. For example, probiotics may improve sleep and reduce depression through a mechanism separate from L-lactate and only increase L-lactate as a side effect. Further research is required to validate the relationship between L-lactate, sleep, and depression.

2. Assumptions and Limitations

Several assumptions and methodological limitations were mentioned throughout this thesis. For example, investigations in part 2 of Chapter 2 (Murack & Messier, 2019) used decreases of late day activity as an equivalent measure for increased “drowsiness” or as a potential indicator for shorter sleep latency. Late day activity as an analogous measure of drowsiness is limited as mice may have been active in other ways beyond the running wheel. In order to have a more precise measure of activity, additional measures such as the analysis of video recordings of all the mice movements or EEG and EMG simultaneous recordings would have provided more accurate measures of sleep and

activity (Murack & Messier, 2019). Visual recordings provided by an aerial camera could have provided further support for our assertions of reduced late day activity. However, decreased running wheel activity was still observed in a dose-dependent manner and indicates at least a reduction in a usually preferred activity following L-lactate treatment. We later improved our measurements of sleep onset by including EEG and EMG recordings in Chapter 4.

Another example of an untested assumption is that late day activity reduction could have been an artifact of muscle fatigue produced by body lactate increase. This assumption represents more of a popular myth than scientifically supported observations and so was not controlled for. Muscle fatigue was once thought to arise from increases to lactic acid and lactate concentration following extended muscle contractions. Evidence supporting lactic acid and L-lactate causing muscle fatigue has been mostly refuted as ATP depletion has been shown to be the likely explanation of muscle fatigue after intense exercise (D. Allen & Westerblad, 2001; Schurr, 2014; Westerblad et al., 2002).

Chapter 3 identified that adolescent mouse models are appropriate samples to investigate the effects of L-lactate on sleep and depression. Yet in our experiments, measures of depression were limited to the forced swim test (Murack et al., 2021). Latency to first immobility period also assesses depression-like behaviour and was introduced to improve on the limited verification of depression behaviour development (Castagné et al., 2009). We added the tail suspension test in Chapter 4 to increase confidence in our depressive measures. Similarly, we expanded our measures of stress regulation in Chapter 3 by adding assessment of cFos expression (Ledoux et al., 1996; Murack et al., 2021).

Expression of cFos is a marker of neuronal activation and was assessed in brain areas susceptible to glucocorticoid signalling and depression-associated changes (Ledoux et al., 1996; Murack et al., 2021). Our assumption was that changes in the selected areas represented changes to activity in brain areas responsible for stress regulation and/or depression. However, this assumption is limited in that signalling molecules other than glucocorticoid or serotonin receptor activation may have been responsible for the observed neuronal activity. We implemented colocalization analysis to compensate for

the limitation of this assumption. Colocalization is a correlation of the overlap of staining between cFos and glucocorticoid receptors. Still, no causation can be inferred without direct manipulation of the relevant signalling mechanisms.

Assessment of cFos often requires introduction of a stressor immediately prior to tissue collection (Ledoux et al., 1996). Introduction of a novel stressor masks baseline measures for stress regulation such as baseline corticosterone concentration. It is unclear whether our observations of glucocorticoid serum and receptors are a result of restraint stress, sleep disruption, or a combination of both sleep disruption and novel stress. The "gentle manipulation" method used to disrupt sleep in Chapter 3 has been show to limit the activation of stress-related brain changes (Gross et al., 2015; S.-R. Yang et al., 2012). Continued sleep disturbances may have introduced changes to the timing of the well-known circadian release of glucocorticoids (Van Reeth et al., 2000). Chapters 2-4 are limited in their assessment of sleep disruption's effect on the timing of glucocorticoid release and so provide an incomplete evaluation of the effect of sleep disruption on adolescent depression development.

Chapter 3 identified a depression-susceptible group that would allow for investigations into the relationship between depression, sleep, and L-lactate outlined in Chapter 2 (Murack et al., 2021; Murack & Messier, 2019). Lactic acid-producing probiotics were selected as an oral treatment for adolescent depression following sleep disruption due to its potential antidepressant (Bested et al., 2013; Logan & Katzman, 2005; Tillmann et al., 2018), sleep-improving (Nakagawa et al., 2018; Romijn et al., 2017), and L-lactate producing (Evivie et al., 2017; Klaenhammer & De Vos, 2011) effects. However, Chapter 2 did not include analysis of L-lactate concentration following probiotic treatment. We performed a trial prior to Chapter 4 and observed temporary increases to L-lactate concentration after a glucose tolerance test and following 18 days of *ad libitum* access Cerebiome and Lacidofil (Chapter 4: Supplementary Material). Gut microbiota may display significant and temporary conversion of sugars to L-lactate during periods of readily available food. Blood glucose concentration in probiotic-treated mice was lower during the glucose tolerance test compared to mice given water only. Decreased blood glucose is typical of increased glucose absorption (Béland-Millar et al.,

2017). The preliminary trial indicated that probiotics may increase access to L-lactate and glucose which could improve energy availability within the brain (Al-Salami et al., 2008; Hong et al., 2015; Nikbakht et al., 2018). Therefore, probiotics were introduced into Chapter 4 as a lactate-producing oral treatment.

An underlying assumption of Chapter 4 is that consumption of lactic acid bacteria increases concentration of these lactate-producing microorganisms in the gut microbiome. This was not directly verified through fecal assessment. However, previous studies have performed fecal analysis on our probiotic mixture. For example, Syrian hamsters administered Cerebiome for 21 days displayed significant increases of *L. helveticus* R0052 and *B. longum* R0175 in fecal samples following acute social defeat, repeat social defeat, or no stress exposure (Partrick et al., 2021). Only a small number of taxa were significantly altered by Cerebiome treatment. Cerebiome treatment increased proportions of the genus *Bifidobacterium* and decreased proportions of the phyla *Proteobacteria.* Thus increased *L. helveticus* R0052 and *B. longum* R0175 likely occurred in our CD-1 adolescent mice given similar treatments. However, unexpected effects of probiotic administration like the reduction of *Proteobacteria* gut flora may also contribute to the antidepressant effects of probiotics on host mice and cannot be accounted for given the lack of microbiome evaluation. Assessment of the effects of Lacidofil and Cerebiome on gut flora composition is currently being assessed in a separate study.

Another assumption of Chapter 4 was that the forced swim and tail suspension tests measured depression, and not fatigue following chronic sleep disruption. Increases to immobility duration is normally attributed to increased despair behaviour (Castagné et al., 2010). Increased fatigue following sleep disruption may have contributed to increased immobile durations. We observed a decrease to swimming duration in sleep disrupted mice compared to rested mice in Chapter 4 which could indicate fatigue contributed to immobility duration. However, sleep disrupted mice did not display a decreased swimming duration in Chapter 3 (Murack et al., 2021). Inclusion of locomotor measures such as the open field test in future investigations would help dissociate the influence of fatigue on immobile behaviour.

Furthermore, the assessment of sleep in Chapter 4 was limited to sleep onset and number and duration of typical sleep stages. While this allows for assessment of sleep presence within specific phases of the 24-hour cycle, it limits the assessment of the underlying sleep-related mechanisms responsible like circadian rhythm (Borbély, 1982; Borbély & Achermann, 1999). Sleep-disrupted mice on average displayed increased NREM in the early awake phase. However, it remains unclear how underlying endogenous circadian rhythms was affected (Boivin & Boudreau, 2014; Goichot et al., 1998). Evaluating the timing of circadian biomarker release could provide indication of whether circadian rhythm has shifted following sleep disruption. For example, both humans (Weibel & Brandenberger, 1998; Weibel et al., 1995) and mice (Sollars et al., 2014) display minimal stress hormone concentration early in the rest phase and peak stress hormone concentration near sleep termination. Furthermore, melatonin and body temperature also fluctuate throughout the 24-hour cycle (Baehr et al., 2000; Crowley et al., 2007). Delayed sleep phase would lead to increased stress hormone concentration during a larger percentage of time spent asleep. Increased concentrations of stress hormones may increase the number of sleep disturbances experienced. Chapter 4 demonstrated that sleep disrupted mice displayed a greater amount of Wake bouts which is indicative of a more disturbed sleep session. Misalignment between sleep/wake cycle and endogenous rhythms would ensure increased stress hormone concentration during sleep and may contribute to its disturbance. Further assessment of biomarkers for of endogenous circadian rhythm like cortisol/corticosterone, melatonin, and body temperature will be necessary to identify whether sleep disruption misaligns the sleep/wake cycle and circadian rhythm while increasing depression behaviour. Additional assessment of endogenous rhythms will also address whether probiotic treatment prevents misalignment of sleep/wake cycle and endogenous rhythm while reducing depression behaviour.

3. Implications of Results

Our observations discussed in Chapters 2-4 may include broader implications for depression-related literature. For example, an antidepressant effect of L-Lactate may partially explain the relationship between exercise and reduced depression. In their meta-

analysis of the adolescent depression and exercise, Xiang Wang et al. (2022) determined that physical exercise reduced depressive behaviour in clinically diagnosed and undiagnosed adolescents. Long durations of strenuous exercises such as aerobic and resistance training provided significant antidepressant effects in adolescents. This antidepressant effect of strenuous exercise had an average moderate effect size. However, the mechanisms underlying exercise's moderate antidepressant effect were unclear (Ross, VanDerwerker, Saladin, & Gregory, 2023). For example, serotonin is expected to decrease in the hippocampus and prefrontal cortex of individuals with depression (Q. Lu et al., 2019). Yet aerobic exercise does not significantly increase serotonin in participants with depression (Carneiro et al., 2017). Tryptophan similarly decreases during depression and displays an initial increase following acute aerobic exercise but regresses to near-baseline levels in older men following 16 weeks of training (Melancon, Lorrain, & Dionne, 2014). Aerobic exercise also does not appear to change resting BDNF levels despite decreases of BDNF often observed with depression (Dinoff, Herrmann, Swardfager, Gallagher, & Lanctot, 2018). Alternate pathways such as those involved with L-lactate may be responsible for the antidepressant effects of exercise.

High intensity exercise for extended durations requires anaerobic metabolism to fuel muscle contractions when oxygen is in limited supply (D. Allen & Westerblad, 2001; Schurr, 2014; Westerblad et al., 2002). Lactic acid and L-lactate are by-product of anaerobic metabolism. Our findings in Chapter 4 alongside previous investigations (Carrard et al., 2021; Carrard et al., 2018; Lowenbach et al., 1947) indicate that L-lactate is associated with an antidepressant mechanism. It is possible that transient increases to L-lactate concentration following exercise activate the same antidepressant mechanisms investigated in Chapters 2-4. It is interesting to note that low intensity exercises like yoga that involve a higher focus on mental exertion than muscular do not reliably display antidepressant effects comparable to aerobic or resistance-based exercises (Conboy, Noggle, Frey, Kudesia, & Khalsa, 2013; Xiang Wang et al., 2022). Yoga's lower intensity muscle movement and increased frequency of rest would decrease the body's reliance on anaerobic metabolism and resultant L-lactate formation. Yoga may display less of an antidepressant effect due to less L-lactate production following muscle contractions. Further research into the relation between aerobic and resistance exercise,

anaerobic metabolism, and depression may provide support for an L-lactate associated antidepressant effect.

Fasting is another lifestyle change that appears to increase available L-lactate and somewhat reduce depression symptoms (Stapel et al., 2022). Depressed in-patients exposed to 72 hours of food fasting displayed decreases in cognitive-affective depression symptoms as measured by the Beck's Depression Inventory-2 (Stapel et al., 2022). Antidepressant effects were mostly observed in moderate and severe cognitive-affective symptoms that displayed resistance to alternate treatment. The mechanisms underlying the antidepressant effect of fasting are disputed. Measurements of metabolic markers, cortisol, and BDNF are not reliable predictors of fasting's antidepressant effects (Stapel et al., 2022). Alternatively, plasma L-lactate increases during fasting-induced ketosis (J. W. Pan, Rothman, Behar, Stein, & Hetherington, 2000) and could contribute to the antidepressant effect of fasting. Fasting may increase L-lactate concentration by shifting metabolism from a pyruvate-centric to ketone-centric processes (J. W. Pan et al., 2000). Manipulation of the underlying L-lactate signaling pathways during fasting may reveal an L-lactate based mechanism for the antidepressant effect of fasting.

4. Concluding remarks

Studies presented in Chapters 2-4 were designed to clarify mechanisms relating lactate, sleep, and depression. Chapters 3 and 4 demonstrated that sleep-disrupted adolescent groups display increased depressive behaviour, sensitization of the HPA response to novel stress, and disrupted tryptophan and serotonin signalling pathways when compared to rested mice (Murack et al., 2021). Chapter 4 demonstrated reduced L-lactate and glucose concentration in the extracellular space of the hippocampus following sleep disruption. Lactic acid probiotic treatment of sleep-disrupted mice normalized depressive behaviour, tryptophan/serotonin/L-lactate/glucose concentration within the brain, and increased NREM sleep in the rest phase. Of the three oral treatments investigated in Chapters 2-4, only probiotic treatment improved access to L-lactate, sleep duration, and depressive behaviour without visible side effects. Chapters 3-4 indicate a susceptibility of adolescent samples to sleep disruption and depression development and highlight the importance of early intervention.

We cannot conclude that L-lactate is solely responsible for the amelioration of depression following pubertal probiotic treatment. Directionality from treatment to the antidepressant effect was not included in Chapters 2-4 investigations. Probiotics may have reduced depression symptoms by increasing access to L-lactate, by improving tryptophan/serotonin concentration, by improving sleep, by some combination of these or by an extenuating mechanism. To validate our theories on L-lactate mediated probiotic treatment of depression, mechanisms associating L-lactate with probiotic's antidepressant effects must be further investigated. Future directions of lactate/depression research should include direct L-lactate assessment following probiotic treatment while compensating for potential interference from alternate antidepressant pathways. Inhibition of these pathways while maintaining probiotic's antidepressant effects could indicate a direct role of L-lactate in depression reduction. Alternatively, manipulation of L-lactate uptake or absorption would more directly relate L-lactate with the antidepressant effect of probiotics. However, this may prove problematic given the production and reliance of brain cells on L-lactate for ATP production (Magistretti & Allaman, 2018). Primarily, future investigations should include goals of safe L-lactate inhibition to verify the antidepressant effect of increased L-lactate.

The direction of upcoming L-lactate and depression research should also include investigations into the mechanisms responsible for L-lactate's action on depression reduction. Inflammation of the central nervous system may link depression development, sleep disruption, and L-lactate treatment (Leproult, Holmbäck, & Van Cauter, 2014; Mac Giollabhui, Ng, Ellman, & Alloy, 2021; Osimo et al., 2020). Multiple investigations suggest that depression development may be caused by an increased expression of inflammatory factors within the central nervous system (Mac Giollabhui et al., 2021; Osimo et al., 2020). Chronic delays in sleep and increased circadian shifts increase expression of inflammatory factors (Leproult et al., 2014) and may be responsible for the observed increases to adolescent depression following sleep disruption (Murack et al., 2021). Furthermore, inflammatory marker expression is reduced in hippocampal microglia following L-lactate treatment (Liang, Liu, Deng, Li, & Zhao, 2022). Inflammation pathways could align our observations concerning adolescent sleep disruption, depression development, and L-lactate-based antidepressant effects. We

hypothesized that L-lactate-based treatments of depression fit well within modern models of depression and should be considered in future depression investigations of sleep and pubertal development.

References

Aalling, N. N., Nedergaard, M., & DiNuzzo, M. (2018). Cerebral Metabolic Changes During Sleep. *Current Neurology and Neuroscience Reports*, *18*(9), 57.

Adriaanse, M., Dekker, J., Heine, R., Snoek, F. J., Beekman, A., Stehouwer, C., . . . Pouwer, F. (2008). Symptoms of depression in people with impaired glucose metabolism or Type 2 diabetes mellitus: The Hoorn Study. *Diabetic Medicine*, *25*(7), 843-849.

Aguilera, G., Kiss, A., Liu, Y., & Kamitakahara, A. (2007). Negative regulation of corticotropin releasing factor expression and limitation of stress response. *Stress*, *10*(2), 153-161.

Al-Salami, H., Butt, G., Fawcett, J. P., Tucker, I. G., Golocorbin-Kon, S., & Mikov, M. (2008). Probiotic treatment reduces blood glucose levels and increases systemic absorption of gliclazide in diabetic rats. *European Journal of Drug Metabolism and Pharmacokinetics*, *33*(2), 101-106.

Alban, L., Dahl, A., Hansen, A. K., Hejgaard, K., Jensen, A. L., Kragh, M., . . . Steensgaard, P. (2001). The welfare impact of increased gavaging doses in rats. *Animal Welfare*, *10*(3), 303-314.

Albeck, D. S., McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., Nikulina, J., McEwen, B. S., & Sakai, R. R. (1997). Chronic social stress alters levels of corticotropin-releasing factor and arginine vasopressin mRNA in rat brain. *Journal of Neuroscience*, *17*(12), 4895-4903.

Allaman, I., Fiumelli, H., Magistretti, P. J., & Martin, J.-L. (2011). Fluoxetine regulates the expression of neurotrophic/growth factors and glucose metabolism in astrocytes. *Psychopharmacology*, *216*(1), 75-84.

Allen, D., & Westerblad, H. (2001). Role of phosphate and calcium stores in muscle fatigue. *The Journal of Physiology*, *536*(3), 657-665.

Allen, N. B., Barrett, A., Sheeber, L., & Davis, B. (2006). Pubertal development and the emergence of the gender gap in mood disorders: A developmental and evolutionary synthesis. *Mood and anxiety disorders in women*, 1-19.

Alqahtani, M. S., Kazi, M., Alsenaidy, M. A., & Ahmad, M. Z. (2021). Advances in oral drug delivery. *Frontiers in Pharmacology, 12*, 618411.

Amiel, S. A., Caprio, S., Sherwin, R. S., Plewe, G., Haymond, M. W., & Tamborlane, W. V. (1991). Insulin resistance of puberty: a defect restricted to peripheral glucose metabolism. *The Journal of Clinical Endocrinology & Metabolism*, *72*(2), 277-282.

Anderson, S. E., Cohen, P., Naumova, E. N., & Must, A. (2006). Association of depression and anxiety disorders with weight change in a prospective community-based study of children followed up into adulthood. *Archives of Pediatrics and Adolescent Medicine*, *160*(3), 285-291.

Anderwald, C., Gastaldelli, A., Tura, A., Krebs, M., Promintzer-Schifferl, M., Kautzky-Willer, A., . . . Bischof, M. G. (2011). Mechanism and effects of glucose absorption during an oral glucose tolerance test among females and males. *The Journal of Clinical Endocrinology & Metabolism*, *96*(2), 515-524.

Anscombe, F. J. (1973). Graphs in statistical analysis. *The American Statistician*, *27*(1), 17-21.

Antunes, I. B., Andersen, M. L., Baracat, E. C., & Tufik, S. (2006). The effects of paradoxical sleep deprivation on estrous cycles of the female rats. *Hormones and Behavior*, *49*(4), 433-440.

Armario, A., Hidalgo, J., & Giralt, M. (1988). Evidence that the pituitary-adrenal axis does not cross-adapt to stressors: comparison to other physiological variables. *Neuroendocrinology*, *47*(3), 263-267.

Armitage, R. (1996). Effects of antidepressant treatment on sleep EEG in depression. *Journal of Psychopharmacology*, *10*(1_suppl), 22-25.

Aronoff, S. L., Berkowitz, K., Shreiner, B., & Want, L. (2004). Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectrum*, *17*(3), 183-190.

Arslanian, S., & Suprasongsin, C. (1997). Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. *The Journal of Clinical Endocrinology & Metabolism*, *82*(10), 3213-3220.

Arslanian, S. A., & Kalhan, S. C. (1994). Correlations between fatty acid and glucose metabolism: potential explanation of insulin resistance of puberty. *Diabetes*, *43*(7), 908-914.

Asberg, M., Thoren, P., Traskman, L., Bertilsson, L., & Ringberger, V. (1976). " Serotonin depression"--a biochemical subgroup within the affective disorders? *Science*, *191*(4226), 478-480. doi:https://doi.org/10.1126/science.1246632

Association, A. P. (2013). *Diagnostic and statistical manual of mental disorders* (*DSM-5®*): American Psychiatric Pub.

Aston-Jones, G., & Bloom, F. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *Journal of Neuroscience, 1*(8), 876-886.

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism*, *21*(10), 1133-1145.

Aubert, A., & Costalat, R. (2005). Interaction between astrocytes and neurons studied using a mathematical model of compartmentalized energy metabolism. *Journal of Cerebral Blood Flow and Metabolism*, *25*(11), 1476-1490.

Avolio, E., Fazzari, G., Zizza, M., De Lorenzo, A., Di Renzo, L., Alò, R., . . . Canonaco, M. (2019). Probiotics modify body weight together with anxiety states via pro-inflammatory factors in HFD-treated Syrian golden hamster. *Behavioural Brain Research*, *356*, 390-399.

Azogu, I., de la Tremblaye, P. B., Dunbar, M., Lebreton, M., LeMarec, N., & Plamondon, H. (2015). Acute sleep deprivation enhances avoidance learning and spatial memory and induces delayed alterations in neurochemical expression of GR, TH, DRD1, pCREB and Ki67 in rats. *Behavioural Brain Research*, *279*, 177-190.

Bach, A. C., & Babayan, V. K. (1982). Medium-chain triglycerides: an update. *The American Journal of Clinical Nutrition*, *36*(5), 950-962.

Baehr, E. K., Revelle, W., & Eastman, C. I. (2000). Individual differences in the phase and amplitude of the human circadian temperature rhythm: with an emphasis on morningness–eveningness. *Journal of Sleep Research*, *9*(2), 117-127.

Bagarolli, R. A., Tobar, N., Oliveira, A. G., Araújo, T. G., Carvalho, B. M., Rocha, G. Z., . . . Prada, P. O. (2017). Probiotics modulate gut microbiota and improve insulin sensitivity in DIO mice. *The Journal of Nutritional Biochemistry, 50*, 16-25.

Bagherzadeh-Yazdi, M., Bohlooli, M., Khajeh, M., Ghamari, F., Ghaffari-Moghaddam, M., Poormolaie, N., . . . Sheibani, N. (2021). Acetoacetate enhancement of glucose mediated DNA glycation. *Biochemistry and Biophysics Reports*, *25*, 100878.

Bangsbo, J., Johansen, L., Graham, T., & Saltin, B. (1993). Lactate and H+ effluxes from human skeletal muscles during intense, dynamic exercise. *The Journal of Physiology*, *462*(1), 115-133.

Bankman, I. N., Sigillito, V. G., Wise, R. A., & Smith, P. L. (1992). Feature-based detection of the K-complex wave in the human electroencephalogram using neural networks. *IEEE Transactions on Biomedical Engineering*, *39*(12), 1305-1310.

Baratta, A. M., Buck, S. A., Buchla, A. D., Fabian, C. B., Chen, S., Mong, J. A., & Pocivavsek, A. (2018). Sex differences in hippocampal memory and kynurenic acid formation following acute sleep deprivation in rats. *Scientific Reports, 8*(1), 6963.

Barrea, L., Pugliese, G., Frias-Toral, E., Napolitano, B., Laudisio, D., Aprano, S., . . . Muscogiuri, G. (2022). Is there a relationship between the ketogenic diet and sleep disorders? *International Journal of Food Sciences and Nutrition, 73*(3), 285-295.

Baxter, L. R., Schwartz, J. M., Phelps, M. E., Mazziotta, J. C., Guze, B. H., Selin, C. E., . . . Sumida, R. M. (1989). Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Archives of General Psychiatry*, *46*(3), 243-250.

Bayat, A., Azizi-Soleiman, F., Heidari-Beni, M., Feizi, A., Iraj, B., Ghiasvand, R., & Askari, G. (2016). Effect of cucurbita ficifolia and probiotic yogurt consumption on blood glucose, lipid profile, and inflammatory marker in type 2 diabetes. *International Journal of Preventive Medicine,* 7.

Beck-Friis, J., Kjellman, B., Aperia, B., Unden, F., Von Rosen, D., Ljunggren, J. G., & Wetterberg, L. (1985). Serum melatonin in relation to clinical variables in patients with major depressive disorder and a hypothesis of a low melatonin syndrome. *Acta Psychiatrica Scandinavica, 71*(4), 319-330.

Béland-Millar, A., Larcher, J., Courtemanche, J., Yuan, T., & Messier, C. (2017). Effects of Systemic Metabolic Fuels on Glucose and Lactate Levels in the Brain Extracellular Compartment of the Mouse. *Frontiers in Neuroscience*, *11*, 7.

Belda, X., Fuentes, S., Daviu, N., Nadal, R., & Armario, A. (2015). Stress-induced sensitization: the hypothalamic–pituitary–adrenal axis and beyond. *Stress*, *18*(3), 269-279.

Benbadis, S. R. (2015). What type of EEG (or EEG-video) does your patient need? *Expert Review of Neurotherapeutics*, *15*(5), 461-464.

Benedetti, F., Fresi, F., Maccioni, P., & Smeraldi, E. (2008). Behavioural sensitization to repeated sleep deprivation in a mice model of mania. *Behavioural Brain Research*, *187*(2), 221-227. doi:10.1016/j.bbr.2007.09.012

Beneke, R., Hutler, M., Jung, M., & Leithauser, R. M. (2005). Modeling the blood lactate kinetics at maximal short-term exercise conditions in children, adolescents, and adults. *Journal of Applied Physiology*, *99*(2), 499-504.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). Biochemistry: New York: WH Freeman.

Berry, R. B., Brooks, R., Gamaldo, C., Harding, S. M., Lloyd, R. M., Quan, S. F., . . . Vaughn, B. V. (2017). AASM scoring manual updates for 2017 (version 2.4): American Academy of Sleep Medicine.

Berry, R. B., Brooks, R., Gamaldo, C. E., Harding, S. M., Marcus, C., & Vaughn, B. V. (2012). The AASM manual for the scoring of sleep and associated events. *Rules, Terminology and Technical Specifications, Darien, Illinois, American Academy of Sleep Medicine*, *176*, 2012.

Berthet, C., Lei, H., Thevenet, J., Gruetter, R., Magistretti, P. J., & Hirt, L. (2009). Neuroprotective role of lactate after cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*, *29*(11), 1780-1789.

Bes, F., Jobert, M., Muller, L., & Schulz, H. (1996). The diurnal distribution of sleep propensity: experimental data about the interaction of the propensities for slow-wave sleep and REM sleep. *Journal of Sleep Research*, *5*(2), 90-98.

Bested, A. C., Logan, A. C., & Selhub, E. A. (2013). Intestinal microbiota, probiotics and mental health- from Metchnikoff to modern advances- Part I – autointoxication revisited. *Gut Pathogens, 5*(1), 1-16. doi:10.1186/1757-4749-5-5

Blackburn-Munro, G., & Blackburn-Munro, R. (2001). Chronic pain, chronic stress and depression: coincidence or consequence? *Journal of Neuroendocrinology, 13*(12), 1009-1023.

Blakemore, S. J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Human Brain Mapping, 31*(6), 926-933.

Blaustein, J. D. (2008). Progesterone and progestin receptors in the brain: the neglected ones. *Endocrinology, 149*(6), 2737-2738.

Boiteux, A., & Hess, B. (1974). *Oscillations in glycolysis, cellular respiration and communication*. Paper presented at the Faraday symposia of the chemical Society.

Boivin, D. B., & Boudreau, P. (2014). Impacts of shift work on sleep and circadian rhythms. *Pathologie Biologie, 62*(5), 292-301.

Bonaventura, J. M., Sharpe, K., Knight, E., Fuller, K. L., Tanner, R. K., & Gore, C. J. (2015). Reliability and accuracy of six hand-held blood lactate analysers. *Journal of Sports Science & Medicine, 14*(1), 203.

Bonfili, L., Cecarini, V., Gogoi, O., Berardi, S., Scarpona, S., Angeletti, M., . . . Eleuteri, A. M. (2020). Gut microbiota manipulation through probiotics oral administration restores glucose homeostasis in a mouse model of Alzheimer's disease. *Neurobiology of Aging, 87*, 35-43.

Borbély, A. A. (1982). A two process model of sleep regulation. *Human Neurobiology, 1*(3), 195-204.

Borbély, A. A., & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *Journal of Biological Rhythms, 14*(6), 559-570.

Borbély, A. A., Baumann, F., Brandeis, D., Strauch, I., & Lehmann, D. (1981). Sleep deprivation: effect on sleep stages and EEG power density in man. *Electroencephalography and Clinical Neurophysiology, 51*(5), 483-493.

Borbély, A., Tobler, I., & Wirz-Justice, A. (1981). Circadian and sleep dependent processes in sleep regulation: outline of a model and implications for depression. *Sleep Research, 10*, 19.

Borbély, A., & Wirz-Justice, A. (1982). Sleep, sleep deprivation and depression. *Human Neurobiology*, *1*(205), 10.

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., . . . Tracey, K. J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature*, *405*(6785), 458-462.

Bosker, F. J., Klompmakers, A., & Westenberg, H. G. (1997). Postsynaptic 5-HT1A receptors mediate 5-hydroxytryptamine release in the amygdala through a feedback to the caudal linear raphe. *European Journal of Pharmacology*, *333*(2-3), 147-157. doi:https://doi.org/10.1016/s0014-2999(97)01141-2

Bourdon, A. K., Spano, G. M., Marshall, W., Bellesi, M., Tononi, G., Serra, P. A., . . . Cirelli, C. (2018). Metabolomic analysis of mouse prefrontal cortex reveals upregulated analytes during wakefulness compared to sleep. *Scientific Reports*, *8*(1), 11225. doi:10.1038/s41598-018-29511-6

Brant, D., & Kavanau, J. L. (1964). 'Unrewarded'exploration and learning of complex mazes by wild and domestic mice. *Nature*, *204*(4955), 267.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., . . . 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*, *108*(38), 16050-16055.

Brietzke, E., Mansur, R. B., Subramaniapillai, M., Martinez, V. B., Vinberg, M., González-Pinto, A., . . . McIntyre, R. S. (2018). Ketogenic diet as a metabolic therapy for mood disorders: Evidence and developments. *Neuroscience and Biobehavioral Reviews*, *94*, 11-16.

Brooks, G. A. (2020). Lactate as a fulcrum of metabolism. *Redox biology*, *35*, 101454.

Burgess, L. H., & Handa, R. J. (1992). Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. *Endocrinology*, *131*(3), 1261-1269.

Burnet, P. W., Michelson, D., Smith, M. A., Gold, P. W., & Sternberg, E. M. (1994). The effect of chronic imipramine administration on the densities of 5-HT1A and 5-HT2 receptors and the abundancies of 5-HT receptor and transporter mRNA in

the cortex, hippocampus and dorsal raphe of three strains of rat. *Brain Research, 638*(1-2), 311-324. doi:https://doi.org/10.1016/0006-8993(94)90664-5

Calfa, G., Kademian, S., Ceschin, D., Vega, G., Rabinovich, G. A., & Volosin, M. (2003). Characterization and functional significance of glucocorticoid receptors in patients with major depression: modulation by antidepressant treatment. *Psychoneuroendocrinology, 28*(5), 687-701.

Caprio, S., Cline, G., Boulware, S., Permanente, C., Shulman, G., Sherwin, R., & Tamborlane, W. (1994). Effects of puberty and diabetes on metabolism of insulin-sensitive fuels. *American Journal of Physiology-Endocrinology and Metabolism, 266*(6), E885-E891.

Carneiro, L. S., Mota, M. P., Vieira-Coelho, M. A., Alves, R. C., Fonseca, A. M., & Vasconcelos-Raposo, J. (2017). Monoamines and cortisol as potential mediators of the relationship between exercise and depressive symptoms. *European Archives of Psychiatry and Clinical Neuroscience, 267*, 117-121.

Carpenter, J. S., Robillard, R., & Hickie, I. B. (2015). Variations in the sleep–wake cycle from childhood to adulthood: chronobiological perspectives. *ChronoPhysiology and Therapy, 5*, 37-49.

Carr, G. V., & Lucki, I. (2011). The role of serotonin receptor subtypes in treating depression: a review of animal studies. *Psychopharmacology, 213*(2), 265-287.

Carrard, A., Cassé, F., Carron, C., Burlet-Godinot, S., Toni, N., Magistretti, P. J., & Martin, J.-L. (2021). Role of adult hippocampal neurogenesis in the antidepressant actions of lactate. *Molecular Psychiatry, 26*(11), 6723-6735.

Carrard, A., Elsayed, M., Margineanu, M., Boury-Jamot, B., Fragnière, L., Meylan, E., ... Martin, J. (2018). Peripheral administration of lactate produces antidepressant-like effects. *Molecular Psychiatry, 23*(2), 392-399.

Carskadon, M. A. (1990). Patterns of sleep and sleepiness in adolescents. *Pediatrician, 17*(1), 5-12.

Carskadon, M. A. (2011). Sleep in adolescents: the perfect storm. *Pediatric Clinics of North America, 58*(3), 637.

Carskadon, M. A., & Acebo, C. (2005). Intrinsic circadian period in adolescents versus adults from forced desynchrony. In *Sleep* (Vol. 28, pp. A71-A71). One Westbrook

Corporate Center STE 920, Westchester, IL 60154 USA: AMER ACADEMY SLEEP MEDICINE.

Carskadon, M. A., Acebo, C., Richardson, G. S., Tate, B. A., & Seifer, R. (1997). An approach to studying circadian rhythms of adolescent humans. *Journal of Biological Rhythms*, *12*(3), 278-289.

Carvalho, A. F., Rocha, D. Q., McIntyre, R. S., Mesquita, L. M., Koehler, C. A., Hyphantis, T. N., . . . Berk, M. (2014). Adipokines as emerging depression biomarkers: a systematic review and meta-analysis. *Journal of Psychiatric Research*, *59*, 28-37.

Casanueva, F. F., & Dieguez, C. (1999). Neuroendocrine regulation and actions of leptin. *Frontiers in Neuroendocrinology*, *20*(4), 317-363.

Casazza, J. P., Felver, M., & Veech, R. (1984). The metabolism of acetone in rat. *Journal of Biological Chemistry*, *259*(1), 231-236.

Castagné, V., Moser, P., Roux, S., & Porsolt, R. D. (2010). Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Current Protocols in Pharmacology*, *49*(1), 5-8. doi:https://doi.org/10.1002/0471141755.ph0508s38

Castagné, V., Porsolt, R. D., & Moser, P. (2009). Use of latency to immobility improves detection of antidepressant-like activity in the behavioral despair test in the mouse. *European Journal of Pharmacology*, *616*(1-3), 128-133.

Chakravarty, E. F., Hubert, H. B., Lingala, V. B., & Fries, J. F. (2008). Reduced disability and mortality among aging runners: a 21-year longitudinal study. *Archives of Internal Medicine*, *168*(15), 1638-1646.

Chang, H. T., Olson, L. K., & Schwartz, K. A. (2013). Ketolytic and glycolytic enzymatic expression profiles in malignant gliomas: implication for ketogenic diet therapy. *Nutrition & Metabolism*, *10*(1), 1-6.

Chaput, J.-P., Dutil, C., & Sampasa-Kanyinga, H. (2018). Sleeping hours: what is the ideal number and how does age impact this? *Nature and science of sleep*, *10*, 421.

Chaudhry, R., & Varacallo, M. (2018). Biochemistry, glycolysis.

Checkley, S. (1996). The neuroendocrinology of depression and chronic stress. *British Medical Bulletin*, *52*(3), 597-617.

Chen, J. J., Gendy, E., Leu, S., Zaldivar, F., Gallassetti, P., Nussbaum, E., . . . Random-Aizik, S. (2018). Age, sex and weight effects on lactate and leukocyte response to exercise in children and adolescents. *European journal of sports & exercise science, 6*(1), 1.

Cheng, M. Y., Bullock, C. M., Li, C., Lee, A. G., Bermak, J. C., Belluzzi, J., . . . Zhou, Q.-Y. (2002). Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature*, *417*(6887), 405-410.

Chiba, S., Numakawa, T., Ninomiya, M., Richards, M. C., Wakabayashi, C., & Kunugi, H. (2012). Chronic restraint stress causes anxiety-and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *39*(1), 112-119. doi:https://doi.org/10.1016/j.pnpbp.2012.05.018

Chuquet, J., Quilichini, P., Nimchinsky, E. A., & Buzsáki, G. (2010). Predominant enhancement of glucose uptake in astrocytes versus neurons during activation of the somatosensory cortex. *Journal of Neuroscience*, *30*(45), 15298-15303.

Clark, C. T., Weissbach, H., & Udenfriend, S. (1954). 5-Hydroxytryptophan decarboxylase: preparation and properties. *Journal of Biological Chemistry*, *210*(1), 139-148.

Clarkson, J., & Herbison, A. E. (2006). Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology*, *147*(12), 5817-5825.

Clary, J., Feron, V., & Van Velthuijsen, J. (1998). Safety assessment of lactate esters. *Regulatory Toxicology and Pharmacology*, *27*(2), 88-97.

Cobos, E. J., Ghasemlou, N., Araldi, D., Segal, D., Duong, K., & Woolf, C. J. (2012). Inflammation-induced decrease in voluntary wheel running in mice: a nonreflexive test for evaluating inflammatory pain and analgesia. *PAIN®*, *153*(4), 876-884.

Cohen, E. R., Ugurbil, K., & Kim, S.-G. (2002). Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. *Journal of Cerebral Blood Flow and Metabolism*, *22*(9), 1042-1053.

Collins-Nakai, R. L., Noseworthy, D., & Lopaschuk, G. D. (1994). Epinephrine increases ATP production in hearts by preferentially increasing glucose metabolism. *American Journal of Physiology-Heart and Circulatory Physiology*, *267*(5), H1862-H1871.

Combs, D. J., Dempsey, R. J., Maley, M., Donaldson, D., & Smith, C. (1990). Relationship between plasma glucose, brain lactate, and intracellular pH during cerebral ischemia in gerbils. *Stroke*, *21*(6), 936-942.

Commons, K. G., Cholanians, A. B., Babb, J. A., & Ehlinger, D. G. (2017). The rodent forced swim test measures stress-coping strategy, not depression-like behavior. *ACS Chemical Neuroscience*, *8*(5), 955-960.

Conboy, L. A., Noggle, J. J., Frey, J. L., Kudesia, R. S., & Khalsa, S. B. S. (2013). Qualitative evaluation of a high school yoga program: feasibility and perceived benefits. *Explore*, *9*(3), 171-180.

Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of Neurobiology*, *60*(2), 236-248.

Corey, M., Edwards, L., Levison, H., & Knowles, M. (1997). Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis. *The Journal of pediatrics*, *131*(6), 809-814.

Cosgrove, K. P., Hunter, R. G., & Carroll, M. E. (2002). Wheel-running attenuates intravenous cocaine self-administration in rats: sex differences. *Pharmacology Biochemistry and Behavior*, *73*(3), 663-671.

Cost, K. T., Crosbie, J., Anagnostou, E., Birken, C. S., Charach, A., Monga, S., . . . Burton, C. L. (2021). Mostly worse, occasionally better: impact of COVID-19 pandemic on the mental health of Canadian children and adolescents. *European Child and Adolescent Psychiatry*, *31*(4), 671-684.

Cowan, C. S., Callaghan, B. L., & Richardson, R. (2016). The effects of a probiotic formulation (Lactobacillus rhamnosus and L. helveticus) on developmental trajectories of emotional learning in stressed infant rats. *Translational psychiatry*, *6*(5), e823-e823.

Crowley, S. J., Acebo, C., & Carskadon, M. A. (2007). Sleep, circadian rhythms, and delayed phase in adolescence. *Sleep Medicine*, *8*(6), 602-612.

Crowley, S. J., Wolfson, A. R., Tarokh, L., & Carskadon, M. A. (2018). An update on adolescent sleep: New evidence informing the perfect storm model. *Journal of Adolescence, 67*, 55-65.

Crozier, G. L. (1988). Medium-chain triglyceride feeding over the long term: the metabolic fate of [14C] octanoate and [14C] oleate in isolated rat hepatocytes. *The Journal of Nutrition, 118*(3), 297-304.

d'Anglemont de Tassigny, X., & Colledge, W. H. (2010). The role of kisspeptin signaling in reproduction. *Physiology, 25*(4), 207-217.

d'Anglemont de Tassigny, X., Fagg, L. A., Dixon, J. P., Day, K., Leitch, H. G., Hendrick, A. G., . . . Carlton, M. B. (2007). Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. *Proceedings of the National Academy of Sciences, 104*(25), 10714-10719.

da Silva Rocha-Lopes, J., Machado, R. B., & Suchecki, D. (2018). Chronic REM sleep restriction in juvenile male rats induces anxiety-like behavior and alters monoamine systems in the amygdala and hippocampus. *Molecular Neurobiology, 55*(4), 2884-2896.

Daan, S., & Merrow, M. (2002). External time–internal time (Vol. 17, pp. 107-109): Sage Publications Sage CA: Thousand Oaks, CA.

Dalla, C., Antoniou, K., Drossopoulou, G., Xagoraris, M., Kokras, N., Sfikakis, A., & Papadopoulou-Daifoti, Z. (2005). Chronic mild stress impact: are females more vulnerable? *Neuroscience, 135*(3), 703-714. doi:https://doi.org/10.1016/j.neuroscience.2005.06.068

Dallmant, M., & Yates, F. (1969). Dynamic asymmetries in the corticosteroid feedback path and distribution metabolism-binding elements of the adrenocortical system. *Annals of the New York Academy of Sciences, 156*(2), 696-721.

Danilenko, K. V., Plisov, I. L., Hebert, M., Krauchi, K., & Wirz-Justice, A. (2008). Influence of timed nutrient diet on depression and light sensitivity in seasonal affective disorder. *Chronobiology International, 25*(1), 51-64. doi:10.1080/07420520801903976

Dash, M. B., Tononi, G., & Cirelli, C. (2012). Extracellular levels of lactate, but not oxygen, reflect sleep homeostasis in the rat cerebral cortex. *Sleep, 35*(7), 909-919.

Davey, C. G., Breakspear, M., Pujol, J., & Harrison, B. J. (2017). A brain model of disturbed self-appraisal in depression. *American Journal of Psychiatry*, *174*(9), 895-903.

De Crescenzo, F., Lennox, A., Gibson, J., Cordey, J., Stockton, S., Cowen, P., & Quested, D. (2017). Melatonin as a treatment for mood disorders: a systematic review. *Acta Psychiatrica Scandinavica*, *136*(6), 549-558.

De Groot, M., Anderson, R., Freedland, K. E., Clouse, R. E., & Lustman, P. J. (2001). Association of depression and diabetes complications: a meta-analysis. *Psychosomatic Medicine*, *63*(4), 619-630.

de Kloet, R., Sybesma, H., & Reul, H. M. (1986). Selective control by corticosterone of serotonin receptor capacity in raphe-hippocampal system. *Neuroendocrinology*, *42*(6), 513-521. doi:https://doi.org/10.1159/000124496

De Montigny, C., Blier, P., & Chaput, Y. (1984). Electrophysiologically-identified serotonin receptors in the rat CNS: effect of antidepressant treatment. *Neuropharmacology*, *23*(12), 1511-1520. doi:https://doi.org/10.1016/0028-3908(84)90095-9

Demakakos, P., Zaninotto, P., & Nouwen, A. (2014). Is the association between depressive symptoms and glucose metabolism bidirectional? Evidence from the English Longitudinal Study of Ageing (ELSA). *Psychosomatic Medicine*, *76*(7), 555.

Demyttenaere, K., Bruffaerts, R., Posada-Villa, J., Gasquet, I., Kovess, V., Lepine, J., . . . Polidori, G. (2004). Prevalence, severity, and unmet need for treatment of mental disorders in the World Health Organization World Mental Health Surveys. *Journal of the American Medical Association*, *291*(21), 2581-2590. doi:https://doi.org/10.1001/jama.291.21.2581

Detke, M., Wieland, S., & Lucki, I. (1995). Blockade of the antidepressant-like effects of 8-OH-DPAT, buspirone and desipramine in the rat forced swim test by 5HT 1A receptor antagonists. *Psychopharmacology*, *119*(1), 47-54.

Dhiman, R. K., Rana, B., Agrawal, S., Garg, A., Chopra, M., Thumburu, K. K., . . . Chawla, Y. K. (2014). Probiotic VSL# 3 reduces liver disease severity and

hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology*, *147*(6), 1327-1337.

Dienel, G. A. (2012). Brain lactate metabolism: the discoveries and the controversies. *Journal of Cerebral Blood Flow and Metabolism*, *32*(7), 1107-1138.

Dijk, D.-J., Groeger, J. A., Stanley, N., & Deacon, S. (2010). Age-related reduction in daytime sleep propensity and nocturnal slow wave sleep. *Sleep*, *33*(2), 211-223.

DiMicco, J., Abshire, V., Hankins, K., Sample, R., & Wible Jr, J. (1986). Microinjection of GABA antagonists into posterior hypothalamus elevates heart rate in anesthetizes rats. *Neuropharmacology*, *25*(9), 1063-1066.

Dinoff, A., Herrmann, N., Swardfager, W., Gallagher, D., & Lanctot, K. L. (2018). The effect of exercise on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF) in major depressive disorder: A meta-analysis. *Journal of Psychiatric Research, 105*, 123-131.

Diop, L., Guillou, S., & Durand, H. (2008). Probiotic food supplement reduces stress-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. *Nutrition Research*, *28*(1), 1-5.

Dorkins, H. (1982). Suxamethonium-the development of a modern drug from 1906 to the present day. *Medical History*, *26*(2), 145-168.

Drevets, W. C., Thase, M. E., Moses-Kolko, E. L., Price, J., Frank, E., Kupfer, D. J., & Mathis, C. (2007). Serotonin-1A receptor imaging in recurrent depression: replication and literature review. *Nuclear Medicine and Biology*, *34*(7), 865-877.

Drouin, J., Sun, Y., Chamberland, M., Gauthier, Y., De Lean, A., Nemer, M., & Schmidt, T. (1993). Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *The EMBO Journal*, *12*(1), 145-156.

Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nature Medicine*, *22*(3), 238-249.

Dunn, K. W., Kamocka, M. M., & McDonald, J. H. (2011). A practical guide to evaluating colocalization in biological microscopy. *American Journal of Physiology: Cell Physiology*, *300*(4), C723-C742. doi:https://doi.org/10.1152/ajpcell.00462.2010

Dutta, S. K., Verma, S., Jain, V., Surapaneni, B. K., Vinayek, R., Phillips, L., & Nair, P. P. (2019). Parkinson's disease: the emerging role of gut dysbiosis, antibiotics, probiotics, and fecal microbiota transplantation. *Journal of Neurogastroenterology and Motility*, *25*(3), 363.

Edgar, D. M., Kilduff, T. S., Martin, C. E., & Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior*, *50*(2), 373-378.

Eikelis, N., Esler, M., Barton, D., Dawood, T., Wiesner, G., & Lambert, G. (2006). Reduced brain leptin in patients with major depressive disorder and in suicide victims. *Molecular Psychiatry*, *11*(9), 800-801.

Evivie, S. E., Huo, G.-C., Igene, J. O., & Bian, X. (2017). Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. *Food & Nutrition Research*, *61*(1), 1318034.

Fairchild, G., Leitch, M., & Ingram, C. (2003). Acute and chronic effects of corticosterone on 5-HT1A receptor-mediated autoinhibition in the rat dorsal raphe nucleus. *Neuropharmacology*, *45*(7), 925-934.

Feizollahzadeh, S., Ghiasvand, R., Rezaei, A., Khanahmad, H., & Hariri, M. (2017). Effect of probiotic soy milk on serum levels of adiponectin, inflammatory mediators, lipid profile, and fasting blood glucose among patients with type II diabetes mellitus. *Probiotics and antimicrobial proteins*, *9*(1), 41-47.

Ferreira, J. C., Rolim, N. P., Bartholomeu, J. B., Gobatto, C. A., Kokubun, E., & Brum, P. C. (2007). Maximal lactate steady state in running mice: effect of exercise training. *Clinical and Experimental Pharmacology and Physiology*, *34*(8), 760-765.

Festing, M. F. (1977). Wheel activity in 26 strains of mouse. *Laboratory Animals*, *11*(4), 257-258.

Fiore, V. G., Mannella, F., Mirolli, M., Latagliata, E. C., Valzania, A., Cabib, S., . . . Baldassarre, G. (2015). Corticolimbic catecholamines in stress: a computational model of the appraisal of controllability. *Brain Structure and Function*, *220*(3), 1339-1353.

Fleming, J. E., Offord, D. R., & Boyle, M. H. (1989). Prevalence of childhood and adolescent depression in the community: Ontario Child Health Study. *The British Journal of Psychiatry*, *155*(5), 647-654.

Franco, A. J., Chen, C., Scullen, T., Zsombok, A., Salahudeen, A. A., Di, S., . . . Tasker, J. G. (2016). Sensitization of the hypothalamic-pituitary-adrenal axis in a male rat chronic stress model. *Endocrinology*, *157*(6), 2346-2355.

Freckmann, G., Link, M., Schmid, C., Pleus, S., Baumstark, A., & Haug, C. (2015). System accuracy evaluation of different blood glucose monitoring systems following ISO 15197: 2013 by using two different comparison methods. *Diabetes Technology & Therapeutics*, *17*(9), 635-648.

Freminet, A., & Poyart, C. (1975). Lactate-glucose interrelations, glucose recycling and the Cori cycle in normal fed rats. *Pflügers Archiv*, *361*(1), 25-31.

Frey, P. A., & Arabshahi, A. (1995). Standard Free Energy Change for the Hydrolysis of the α,β-Phosphoanhydride Bridge in ATP. *Biochemistry*, *34*(36), 11307-11310.

Friedrich, M. J. (2017). Depression is the leading cause of disability around the world. *Journal of the American Medical Association*, *317*(15), 1517-1517.

Frisco, M. L., Houle, J. N., & Lippert, A. M. (2013). Weight change and depression among US young women during the transition to adulthood. *American Journal of Epidemiology*, *178*(1), 22-30.

Funes, S., Hedrick, J. A., Vassileva, G., Markowitz, L., Abbondanzo, S., Golovko, A., . . . Gustafson, E. L. (2003). The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochemical and Biophysical Research Communications*, *312*(4), 1357-1363.

Furuse, T., Mizuma, H., Hirose, Y., Kushida, T., Yamada, I., Miura, I., . . . Onoe, H. (2019). A new mouse model of GLUT1 deficiency syndrome exhibits abnormal sleep-wake patterns and alterations of glucose kinetics in the brain. *Disease Models & Mechanisms*, *12*(9), dmm038828.

Fuxe, D. (1964). Evidence for existence of monoamine-containing neurons in central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica*, *62*, 1-55.

Gallucci, W. T., Baum, A., Laue, L., Rabin, D. S., Chrousos, G. P., Gold, P., & Kling, M. A. (1993). Sex differences in sensitivity of the hypothalamic-pituitary-adrenal axis. *Health Psychology*, *12*(5), 420.

Gandhi, R., Hayley, S., Gibb, J., Merali, Z., & Anisman, H. (2007). Influence of poly I: C on sickness behaviors, plasma cytokines, corticosterone and central monoamine activity: moderation by social stressors. *Brain, Behavior, and Immunity*, *21*(4), 477-489.

Garcia-Garcia, A. L., Meng, Q., Canetta, S., Gardier, A. M., Guiard, B. P., Kellendonk, C., . . . Leonardo, E. D. (2017). Serotonin signaling through prefrontal cortex 5-HT1A receptors during adolescence can determine baseline mood-related behaviors. *Cell reports*, *18*(5), 1144-1156.

Garcia, C. K., Goldstein, J. L., Pathak, R. K., Anderson, R. G., & Brown, M. S. (1994). Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell*, *76*(5), 865-873.

Gareau, M. G., Wine, E., Rodrigues, D. M., Cho, J. H., Whary, M. T., Philpott, D. J., . . . Sherman, P. M. (2011). Bacterial infection causes stress-induced memory dysfunction in mice. *Gut*, *60*(3), 307-317.

Garza, J. C., Guo, M., Zhang, W., & Lu, X.-Y. (2012). Leptin restores adult hippocampal neurogenesis in a chronic unpredictable stress model of depression and reverses glucocorticoid-induced inhibition of GSK-3β/β-catenin signaling. *Molecular Psychiatry*, *17*(8), 790-808.

Gayathri, D., & Rashmi, B. (2017). Mechanism of development of depression and probiotics as adjuvant therapy for its prevention and management. *Mental Health & Prevention*, *5*, 40-51.

Ge, X., Conger, R. D., & Elder Jr, G. H. (2001). Pubertal transition, stressful life events, and the emergence of gender differences in adolescent depressive symptoms. *Developmental Psychology*, *37*(3), 404.

Gervasoni, D., Darracq, L., Fort, P., Souliere, F., Chouvet, G., & Luppi, P. H. (1998). Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. *European Journal of Neuroscience*, *10*(3), 964-970.

Gezginc, Y., Topcal, F., Comertpay, S., & Akyol, I. (2015). Quantitative analysis of the lactic acid and acetaldehyde produced by Streptococcus thermophilus and Lactobacillus bulgaricus strains isolated from traditional Turkish yogurts using HPLC. *Journal of Dairy Science*, *98*(3), 1426-1434.

Ghorbani, Z., Nazari, S., Etesam, F., Nourimajd, S., Ahmadpanah, M., & Razeghi Jahromi, S. (2018). The effect of synbiotic as an adjuvant therapy to fluoxetine in moderate depression: a randomized multicenter trial. *Archives of Neuroscience*, *5*(2).

Giedke, H., & Schwärzler, F. (2002). Therapeutic use of sleep deprivation in depression. *Sleep Medicine Reviews*, *6*(5), 361-377.

Gilboa, A., Sekeres, M., Moscovitch, M., & Winocur, G. (2014). Higher-order conditioning is impaired by hippocampal lesions. *Current Biology*, *24*(18), 2202-2207.

Gillin, J. C., Duncan, W., Pettigrew, K. D., Frankel, B. L., & Snyder, F. (1979). Successful separation of depressed, normal, and insomniac subjects by EEG sleep data. *Archives of General Psychiatry*, *36*(1), 85-90.

Gillin, J. C., Duncan, W. C., Murphy, D. L., Post, R. M., Wehr, T. A., Goodwin, F. K., . . . Bunney Jr, W. E. (1981). Age-related changes in sleep in depressed and normal subjects. *Psychiatry Research*, *4*(1), 73-78.

Girard-Joyal, O., Faragher, A., Bradley, K., Kane, L., Hrycyk, L., & Ismail, N. (2015). Age and sex differences in c-Fos expression and serum corticosterone concentration following LPS treatment. *Neuroscience*, *305*, 293-301.

Gjerstad, J. K., Lightman, S. L., & Spiga, F. (2018). Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. *Stress*, *21*(5), 403-416.

Goel, N., Workman, J. L., Lee, T. T., Innala, L., & Viau, V. (2011). Sex differences in the HPA axis. *Comprehensive Physiology*, *4*(3), 1121-1155.

Goh, J., & Ladiges, W. (2015). Voluntary wheel running in mice. *Current Protocols in Mouse Biology*, *5*(4)283-290.

Goichot, B., Weibel, L., Chapotot, F., Gronfier, C., Piquard, F., & Brandenberger, G. (1998). Effect of the shift of the sleep-wake cycle on three robust endocrine

markers of the circadian clock. *American Journal of Physiology-Endocrinology and Metabolism*, *275*(2), E243-E248.

Goldman, L., Winget, C., Hollingshead, G., & Levine, S. (1973). Postweaning development of negative feedback in the pituitary-adrenal system of the rat. *Neuroendocrinology*, *12*(3), 199-211.

Goldney, R. D., Phillips, P. J., Fisher, L. J., & Wilson, D. H. (2004). Diabetes, depression, and quality of life: a population study. *Diabetes Care*, *27*(5), 1066-1070.

Goto, T., Horita, M., Nagai, H., Nagatomo, A., Nishida, N., Matsuura, Y., & Nagaoka, S. (2012). Tiliroside, a glycosidic flavonoid, inhibits carbohydrate digestion and glucose absorption in the gastrointestinal tract. *Molecular Nutrition & Food Research*, *56*(3), 435-445.

Green, M. R., & McCormick, C. M. (2016). Sex and stress steroids in adolescence: gonadal regulation of the hypothalamic–pituitary–adrenal axis in the rat. *General and Comparative Endocrinology*, *234*, 110-116.

Gross, B. A., Vanderheyden, W. M., Urpa, L. M., Davis, D. E., Fitzpatrick, C. J., Prabhu, K., & Poe, G. R. (2015). Stress-free automatic sleep deprivation using air puffs. *Journal of Neuroscience Methods*, *251*, 83-91.

Gruenwald, J., Graubaum, H.-J., & Harde, A. (2002). Effect of a probiotic multivitamin compound on stress and exhaustion. *Advances in Therapy*, *19*(3), 141-150.

Guidotti, G., Calabrese, F., Anacker, C., Racagni, G., Pariante, C. M., & Riva, M. A. (2013). Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: modulation by antidepressant treatment. *Neuropsychopharmacology*, *38*(4), 616-627.

Guthrie, J. P., & Jordan, F. (1972). Amine-catalyzed decarboxylation of acetoacetic acid. The rate constant for decarboxylation of a β-imino acid. *Journal of the American Chemical Society*, *94*(26), 9136-9141.

Hagenauer, M. H., Perryman, J. I., Lee, T. M., & Carskadon, M. A. (2009). Adolescent changes in the homeostatic and circadian regulation of sleep. *Developmental Neuroscience*, *31*(4), 276-284.

Hallböök, T., Lundgren, J., & Rosén, I. (2007). Ketogenic diet improves sleep quality in children with therapy-resistant epilepsy. *Epilepsia*, *48*(1), 59-65.

Hamberger, A., & Hydén, H. (1963). Inverse enzymatic changes in neurons and glia during increased function and hypoxia. *The Journal of Cell Biology*, *16*(3), 521-525.

Hammen, C., Davila, J., Brown, G., Ellicott, A., & Gitlin, M. (1992). Psychiatric history and stress: predictors of severity of unipolar depression. *Journal of Abnormal Psychology*, *101*(1), 45.

Hankin, B. L., & Abramson, L. Y. (1999). Development of gender differences in depression: Description and possible explanations. *Annals of Medicine*, *31*(6), 372-379.

Hankin, B. L., Abramson, L. Y., Moffitt, T. E., Silva, P. A., McGee, R., & Angell, K. E. (1998). Development of depression from preadolescence to young adulthood: emerging gender differences in a 10-year longitudinal study. *Journal of Abnormal Psychology*, *107*(1), 128. doi:https://doi.org/10.1037/0021-843x.107.1.128

Harbuz, M., & Lightman, S. (1989). Glucocorticoid inhibition of stress-induced changes in hypothalamic corticotrophin-releasing factor messenger RNA and proenkephalin A messenger RNA. *Neuropeptides*, *14*(1), 17-20.

Hare, T. A., Tottenham, N., Galvan, A., Voss, H. U., Glover, G. H., & Casey, B. (2008). Biological substrates of emotional reactivity and regulation in adolescence during an emotional go-nogo task. *Biological Psychiatry*, *63*(10), 927-934.

Harkness, J. H., Bushana, P. N., Todd, R. P., Clegern, W. C., Sorg, B. A., & Wisor, J. P. (2019). Sleep disruption elevates oxidative stress in parvalbumin-positive cells of the rat cerebral cortex. *Sleep*, *42*(1), zsy201.

Harri, M., Lindblom, J., Malinen, H., Hyttinen, M., Lapveteläinen, T., Eskola, S., & Helminen, H. J. (1999). Effect of access to a running wheel on behavior of C57BL/6J mice. *Comparative Medicine*, *49*(4), 401-405.

Hauri, P., Chernik, D., Hawkins, D., & Mendels, J. (1974). Sleep of depressed patients in remission. *Archives of General Psychiatry*, *31*(3), 386-391.

Hellmann, J., Vannucci, R. C., & Nardis, E. E. (1982). Blood-brain barrier permeability to lactic acid in the newborn dog: lactate as a cerebral metabolic fuel. *Pediatric Research, 16*(1), 40-44.

Henderson, S. T., Vogel, J. L., Barr, L. J., Garvin, F., Jones, J. J., & Costantini, L. C. (2009). Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutrition & Metabolism, 6*(1), 31.

Heninger, G., Delgado, P., & Charney, D. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry, 29*(01), 2-11. doi:https://doi.org/10.1055/s-2007-979535

Herbison, C. E., Allen, K., Robinson, M., Newnham, J., & Pennell, C. (2017). The impact of life stress on adult depression and anxiety is dependent on gender and timing of exposure. *Development and Psychopathology, 29*(4), 1443-1454.

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., . . . Myers, B. (2011). Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Comprehensive Physiology, 6*(2), 603-621.

Hertz, L., Dringen, R., Schousboe, A., & Robinson, S. R. (1999). Astrocytes: glutamate producers for neurons. *Journal of Neuroscience Research, 57*(4), 417-428.

Hesen, W., & Joels, M. (1996). Modulation of 5HT1A responsiveness in CA1 pyramidal neurons by in vivo activation of corticosteroid receptors. *Journal of Neuroendocrinology, 8*(6), 433-438.

Hilakivi-Clarke, L. (1991). Effects of tryptophan on depression and aggression in STZ-D mice. *Diabetes, 40*(12), 1598-1602.

Hill, A., & Lupton, H. (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Quarterly Journal of Medicine: An International Journal of Medicine, 1*(62), 135-171.

Hilton, A., & Armstrong, R. (2006). Statnote 6: post-hoc ANOVA tests.

Hirshkowitz, M., Whiton, K., Albert, S. M., Alessi, C., Bruni, O., DonCarlos, L., . . . Kheirandish-Gozal, L. (2015). National Sleep Foundation’s sleep time duration recommendations: methodology and results summary. *Sleep health, 1*(1), 40-43.

Holbrook, J. J., Liljas, A., Steindel, S. J., & Rossmann, M. G. (1975). 4 lactate dehydrogenase. In *The enzymes* (Vol. 11, pp. 191-292). Academic Press.

Holder, M. K., & Blaustein, J. D. (2014). Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. *Frontiers in Neuroendocrinology*, *35*(1), 89-110.

Holsboer, F., Von Bardeleben, U., Gerken, A., Stalla, G., & Müller, O. (1984). Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. *The New England Journal of Medicine*, *311*(17), 1127-1127.

Hong, S.-M., Chung, E.-C., & Kim, C.-H. (2015). Anti-obesity effect of fermented whey beverage using lactic acid bacteria in diet-induced obese rats. *Korean Journal for Food Science of Animal Resources*, *35*(5), 653.

Hopman, W. P., Jansen, J. B., Rosenbusch, G., & Lamers, C. B. (1984). Effect of equimolar amounts of long-chain triglycerides and medium-chain triglycerides on plasma cholecystokinin and gallbladder contraction. *The American Journal of Clinical Nutrition*, *39*(3), 356-359.

Horn, C. C., Kimball, B. A., Wang, H., Kaus, J., Dienel, S., Nagy, A., . . . Andrews, P. L. (2013). Why can't rodents vomit? A comparative behavioral, anatomical, and physiological study. *PloS One*, *8*(4), e60537.

Hydén, H., & Lange, P. W. (1962). A kinetic study of the neuron-glia relationship. *The Journal of Cell Biology*, *13*(2), 233-237.

Ikeda, Y., Okamura-Ikeda, K., & Tanaka, K. (1985). Purification and characterization of short-chain, medium-chain, and long-chain acyl-CoA dehydrogenases from rat liver mitochondria. Isolation of the holo-and apoenzymes and conversion of the apoenzyme to the holoenzyme. *Journal of Biological Chemistry*, *260*(2), 1311-1325.

Imeri, L., Mancia, M., Bianchi, S., & Opp, M. (1999). 5-Hydroxytryptophan, but not L-tryptophan, alters sleep and brain temperature in rats. *Neuroscience*, *95*(2), 445-452.

Irwin, C., McCartney, D., Desbrow, B., & Khalesi, S. (2020). Effects of probiotics and paraprobiotics on subjective and objective sleep metrics: a systematic review and meta-analysis. *European Journal of Clinical Nutrition*, *74*(11), 1536-1549.

Ismail, N., & Blaustein, J. D. (2013). Pubertal immune challenge blocks the ability of estradiol to enhance performance on cognitive tasks in adult female mice. *Psychoneuroendocrinology*, *38*(7), 1170-1177.

Ismail, N., Garas, P., & Blaustein, J. D. (2011). Long-term effects of pubertal stressors on female sexual receptivity and estrogen receptor-α expression in CD-1 female mice. *Hormones and Behavior*, *59*(4), 565-571.

Ismail, N., Kumlin, A. M., & Blaustein, J. D. (2013). A pubertal immune challenge alters the antidepressant-like effects of chronic estradiol treatment in inbred and outbred adult female mice. *Neuroscience*, *249*, 43-52.

Iwanaga, T., Takebe, K., Kato, I., Karaki, S.-I., & Kuwahara, A. (2006). Cellular expression of monocarboxylate transporters (MCT) in the digestive tract of the mouse, rat, and humans, with special reference to slc5a8. *Biomedical research*, *27*(5), 243-254.

Izawa, S., Sugaya, N., Ogawa, N., Nagano, Y., Nakano, M., Nakase, E., . . . Kodama, M. (2007). Episodic stress associated with writing a graduation thesis and free cortisol secretion after awakening. *International Journal of Psychophysiology*, *64*(2), 141-145.

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., . . . Abdelalim, A. (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), 1789-1858.

Janzer, R. C., & Raff, M. C. (1987). Astrocytes induce blood–brain barrier properties in endothelial cells. *Nature*, *325*(6101), 253-257.

Jennes, L., & Conn, P. M. (2002). Gonadotropin-releasing hormone. *Hormones, Brain and Behavior* (pp. 51-XXV): Elsevier.

Jenni, O. G., Achermann, P., & Carskadon, M. A. (2005). Homeostatic sleep regulation in adolescents. *Sleep*, *28*(11), 1446-1454.

Jenni, O. G., & Carskadon, M. A. (2004). Spectral analysis of the sleep electroencephalogram during adolescence. *Sleep*, *27*(4), 774-783.

Jeon, B., Hwang, Y., Lee, S.-Y., Kim, D., Chung, C., & Han, J.-S. (2012). The role of basolateral amygdala in the regulation of stress-induced phosphorylated extracellular signal-regulated kinase expression in the hippocampus. *Neuroscience*, *224*, 191-201. doi:https://doi.org/10.1016/j.neuroscience.2012.08.035

Jeukendrup, A. E., & Aldred, S. (2004). Fat supplementation, health, and endurance performance. *Nutrition*, *20*(7), 678-688.

Jirkof, P. (2014). Burrowing and nest building behavior as indicators of well-being in mice. *Journal of Neuroscience Methods*, *234*, 139-146.

Joels, M. (2001). Corticosteroid actions in the hippocampus. *Journal of Neuroendocrinology*, *13*(8), 657-669.

Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature Reviews Neuroscience*, *10*(6), 459-466.

Jones, D., Gershon, S., Sitaram, N., & Keshavan, M. (1987). Sleep and depression. *Psychopathology*, *20*(Suppl. 1), 20-31.

Joslowski, G., Goletzke, J., Cheng, G., Günther, A., Bao, J., Brand-Miller, J., & Buyken, A. (2012). Prospective associations of dietary insulin demand, glycemic index, and glycemic load during puberty with body composition in young adulthood. *International Journal of Obesity*, *36*(11), 1463-1471.

Kadooka, Y., Sato, M., Imaizumi, K., Ogawa, A., Ikuyama, K., Akai, Y., . . . Tsuchida, T. (2010). Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. *European Journal of Clinical Nutrition*, *64*(6), 636-643.

Kaestner, F., Hettich, M., Peters, M., Sibrowski, W., Hetzel, G., Ponath, G., . . . Rothermundt, M. (2005). Different activation patterns of proinflammatory cytokines in melancholic and non-melancholic major depression are associated with HPA axis activity. *Journal of Affective Disorders*, *87*(2-3), 305-311.

Kalil, B., Leite, C., Carvalho-Lima, M., & Anselmo-Franci, J. (2013). Role of sex steroids in progesterone and corticosterone response to acute restraint stress in rats: sex differences. *Stress*, *16*(4), 452-460.

Kane, L., & Ismail, N. (2017). Puberty as a vulnerable period to the effects of immune challenges: Focus on sex differences. *Behavioural Brain Research, 320,* 374-382.

Kashiwaya, Y., Pawlosky, R., Markis, W., King, M. T., Bergman, C., Srivastava, S., . . . Veech, R. L. (2010). A ketone ester diet increased brain malonyl CoA and uncoupling protein 4 and 5 while decreasing food intake in the normal Wistar rat. *Journal of Biological Chemistry, 285*(34), 25950-25956.

Kato-Kataoka, A., Nishida, K., Takada, M., Suda, K., Kawai, M., Shimizu, K., . . . Igarashi, T. (2016). Fermented milk containing Lactobacillus casei strain Shirota prevents the onset of physical symptoms in medical students under academic examination stress. *Beneficial microbes, 7*(2), 153-156.

Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., & Djafarian, K. (2019). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clinical trial. *Clinical Nutrition, 38*(2), 522-528.

Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., . . . Cryan, J. F. (2017). Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, Behavior, and Immunity, 61,* 50-59.

Kennedy, S. H., Evans, K. R., Krüger, S., Mayberg, H. S., Meyer, J. H., McCann, S., . . . Vaccarino, F. J. (2001). Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. *American Journal of Psychiatry, 158*(6), 899-905.

Kesl, S. L., Poff, A. M., Ward, N. P., Fiorelli, T. N., Ari, C., Van Putten, A. J., . . . D'Agostino, D. P. (2016). Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague-Dawley rats. *Nutrition & Metabolism, 13*(1), 1-15. doi:10.1186/s12986-016-0069-y

Kessler, R. C., Avenevoli, S., & Merikangas, K. R. (2001). Mood disorders in children and adolescents: an epidemiologic perspective. *Biological Psychiatry, 49*(12), 1002-1014.

Kessler, R. C., McGonagle, K. A., Zhao, S., Nelson, C. B., Hughes, M., Eshleman, S., . . . Kendler, K. S. (1994). Lifetime and 12-month prevalence of DSM-III-R

psychiatric disorders in the United States: results from the National Comorbidity Survey. *Archives of General Psychiatry*, *51*(1), 8-19.

Klaenhammer, T., & De Vos, W. (2011). An incredible scientific journey. The evolutionary tale of the lactic acid bacteria. In *The 10th LAB symposium. Thirty years of research on lactic acid bacteria* (Vol. 24, pp. 1-11). 24 Media Labs.

Knowles, J., Southmayd, S., Delva, N., MacClean, A., Cairns, J., & Letemendia, F. (1979). Five variations of sleep deprivation in a depressed woman. *The British Journal of Psychiatry*, *135*(5), 403-410.

Knudsen, G. M., Paulson, O. B., & Hertz, M. M. (1991). Kinetic analysis of the human blood-brain barrier transport of lactate and its influence by hypercapnia. *Journal of Cerebral Blood Flow and Metabolism*, *11*(4), 581-586.

Koban, M., Le, W. W., & Hoffman, G. E. (2006). Changes in hypothalamic corticotropin-releasing hormone, neuropeptide Y, and proopiomelanocortin gene expression during chronic rapid eye movement sleep deprivation of rats. *Endocrinology*, *147*(1), 421-431.

Köhler, O., Benros, M. E., Nordentoft, M., Farkouh, M. E., Iyengar, R. L., Mors, O., & Krogh, J. (2014). Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. *Journal of the American Medical Association-Psychiatry*, *71*(12), 1381-1391.

Koponen, H., Kautiainen, H., Leppänen, E., Mäntyselkä, P., & Vanhala, M. (2015). Association between suicidal behaviour and impaired glucose metabolism in depressive disorders. *BioMed Central Psychiatry*, *15*(1), 1-8.

Korenbrot, C., Huhtaniemi, I., & Weiner, R. (1977). Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction*, *17*(2), 298-303.

Kraemer, G. W., & McKinney, W. T. (1979). Interactions of pharmacological agents which alter biogenic amine metabolism and depression: An analysis of contributing factors within a primate model of depression. *Journal of Affective Disorders*, *1*(1), 33-54.

Kraus, T., Haack, M., Schuld, A., Hinze-Selch, D., & Pollmächer, T. (2001). Low leptin levels but Norma body mass indices in patients with depression or schizophrenia. *Neuroendocrinology*, *73*(4), 243-247.

Kršiak, M. (1975). Timid singly-housed mice: Their value in prediction of psychotropic activity of drugs. *British Journal of Pharmacology*, *55*(1), 141-150.

Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: a review. *Biological Psychology*, *69*(1), 113-132. doi:https://doi.org/10.1016/j.biopsycho.2004.11.009

Kupfer, D. (1976). REM latency: a psychobiologic marker for primary depressive disease. *Biological Psychiatry*, *11*(2), 159-174.

Kupfer, D., Harrow, M., & Detre, T. (1969). Sleep patterns and psychopathology. *Acta Psychiatrica Scandinavica*, *45*(1), 75-89.

Ladouceur, C. D. (2012). Neural systems supporting cognitive-affective interactions in adolescence: the role of puberty and implications for affective disorders. *Frontiers in Integrative Neuroscience*, *6*, 65.

Ladouceur, C. D., Dahl, R. E., Williamson, D. E., Birmaher, B., Ryan, N. D., & Casey, B. (2005). Altered emotional processing in pediatric anxiety, depression, and comorbid anxiety-depression. *Journal of Abnormal Child Psychology*, *33*(2), 165-177.

Lagos, P., Urbanavicius, J., Scorza, M. C., Miraballes, R., & Torterolo, P. (2011). Depressive-like profile induced by MCH microinjections into the dorsal raphe nucleus evaluated in the forced swim test. *Behavioural Brain Research*, *218*(2), 259-266.

Laroche, J., Gasbarro, L., Herman, J. P., & Blaustein, J. D. (2009a). Enduring influences of peripubertal/adolescent stressors on behavioral response to estradiol and progesterone in adult female mice. *Endocrinology*, *150*(8), 3717-3725.

Laroche, J., Gasbarro, L., Herman, J. P., & Blaustein, J. D. (2009b). Reduced behavioral response to gonadal hormones in mice shipped during the peripubertal/adolescent period. *Endocrinology*, *150*(5), 2351-2358.

Larrabee, M. G. (1995). Lactate metabolism and its effects on glucose metabolism in an excised neural tissue. *Journal of Neurochemistry*, *64*(4), 1734-1741.

Larrabee, M. G. (1996). Partitioning of CO2 production between glucose and lactate in excised sympathetic ganglia, with implications for brain. *Journal of Neurochemistry, 67*(4), 1726-1734.

Laryea, G., Schütz, G., & Muglia, L. J. (2013). Disrupting hypothalamic glucocorticoid receptors causes HPA axis hyperactivity and excess adiposity. *Molecular Endocrinology, 27*(10), 1655-1665.

Ledoux, L., Sastre, J.-P., Buda, C., Luppi, P.-H., & Jouvet, M. (1996). Alterations in c-fos expression after different experimental procedures of sleep deprivation in the cat. *Brain Research, 735*(1), 108-118. doi:https://doi.org/10.1016/0006-8993(96)00599-9

Lee, B.-H., Lo, Y.-H., & Pan, T.-M. (2013). Anti-obesity activity of Lactobacillus fermented soy milk products. *Journal of Functional Foods, 5*(2), 905-913.

Lee, J.-H., Miele, M. E., Hicks, D. J., Phillips, K. K., Trent, J. M., Weissman, B. E., & Welch, D. R. (1996). KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *Journal of the National Cancer Institute, 88*(23), 1731-1737.

Lehner, M., Wisłowska-Stanek, A., Taracha, E., Maciejak, P., Szyndler, J., Skórzewska, A., . . . Bidziński, A. (2009). The expression of c-Fos and colocalisation of c-Fos and glucocorticoid receptors in brain structures of low and high anxiety rats subjected to extinction trials and re-learning of a conditioned fear response. *Neurobiology of Learning and Memory, 92*(4), 535-543.

Leistner, C., & Menke, A. (2018). How to measure glucocorticoid receptor's sensitivity in patients with stress-related psychiatric disorders. *Psychoneuroendocrinology, 91*, 235-260. doi:https://doi.org/10.1016/j.psyneuen.2018.01.023

Leonard, B. (1996). The comparative pharmacological properties of selective serotonin re-uptake inhibitors in animals. *Selective Serotonin Reuptake Inhibitors*, 37-70.

Leproult, R., Copinschi, G., Buxton, O., & Van Cauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening. *Sleep, 20*(10), 865-870.

Leproult, R., Holmbäck, U., & Van Cauter, E. (2014). Circadian misalignment augments markers of insulin resistance and inflammation, independently of sleep loss. *Diabetes, 63*(6), 1860-1869.

Levitan, R. D., Vaccarino, F. J., Brown, G. M., & Kennedy, S. H. (2002). Low-dose dexamethasone challenge in women with atypical major depression: pilot study. *Journal of Psychiatry and Neuroscience*, *27*(1), 47.

Liang, L., Liu, P., Deng, Y., Li, J., & Zhao, S. (2022). L-lactate inhibits lipopolysaccharide-induced inflammation of microglia in the hippocampus. *International Journal of Neuroscience*, *23*, 1-8.

Lightfoot, J. T., Turner, M. J., Daves, M., Vordermark, A., & Kleeberger, S. R. (2004). Genetic influence on daily wheel running activity level. *Physiological Genomics*, *19*(3), 270-276.

Lin, A., Shih, C.-T., Chu, H.-F., Chen, C.-W., Cheng, Y.-T., Wu, C.-C., . . . Tsai, Y.-C. (2021). Lactobacillus fermentum PS150 promotes non-rapid eye movement sleep in the first night effect of mice. *Scientific Reports, 11*(1), 16313.

Lira, A., Zhou, M., Castanon, N., Ansorge, M. S., Gordon, J. A., Francis, J. H., . . . Arango, V. (2003). Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. *Biological Psychiatry*, *54*(10), 960-971.

Liu, J., Garza, J. C., Bronner, J., Kim, C. S., Zhang, W., & Lu, X.-Y. (2010). Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. *Psychopharmacology*, *207*(4), 535-545.

Liu, Q. F., Kim, H.-M., Lim, S., Chung, M.-J., Lim, C.-Y., Koo, B.-S., & Kang, S.-S. (2020). Effect of probiotic administration on gut microbiota and depressive behaviors in mice. *DARU Journal of Pharmaceutical Sciences, 28*, 181-189.

Logan, A. C., & Katzman, M. (2005). Major depressive disorder: probiotics may be an adjuvant therapy. *Medical Hypotheses*, *64*(3), 533-538.

Lohmiller, J. J., Swing, S. P., & Hanson, M. M. (2020). Reproduction and breeding. In *The laboratory rat* (pp. 157-179). Academic Press.

López-Muñoz, F., & Álamo, C. (2016). History of the discovery of antidepressant drugs. *Melatonin, Neuroprotective Agents and Antidepressant Therapy,17*, 365-383.

Lopez, J., Chalmers, D., Little, K., & Watson, S. (1998). Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus:

implications for the neurobiology of depression. *Biological Psychiatry*, *43*(8), 547-573. doi:https://doi.org/10.1016/s0006-3223(97)00484-8

Lowenbach, H., Greenhill, M. H., & Durham, N. (1947). The effect of oral administration of lactic acid upon the clinical course of depressive states. *The Journal of Nervous and Mental Disease*, *105*(4), 343-358.

Lowy, M. T., Reder, A. T., Gormley, G. J., & Meltzer, H. Y. (1988). Comparison of in vivo and in vitro glucocorticoid sensitivity in depression: relationship to the dexamethasone suppression test. *Biological Psychiatry*, *24*(6), 619-630.

Lu, Q., Mouri, A., Yang, Y., Kunisawa, K., Teshigawara, T., Hirakawa, M., . . . Nabeshima, T. (2019). Chronic unpredictable mild stress-induced behavioral changes are coupled with dopaminergic hyperfunction and serotonergic hypofunction in mouse models of depression. *Behavioural Brain Research, 372*, 112053.

Lu, X.-Y. (2007). The leptin hypothesis of depression: a potential link between mood disorders and obesity? *Current Opinion in Pharmacology*, *7*(6), 648-652.

Lu, X.-Y., Kim, C. S., Frazer, A., & Zhang, W. (2006). Leptin: a potential novel antidepressant. *Proceedings of the National Academy of Sciences*, *103*(5), 1593-1598.

Lundgaard, I., Lu, M. L., Yang, E., Peng, W., Mestre, H., Hitomi, E., . . . Nedergaard, M. (2017). Glymphatic clearance controls state-dependent changes in brain lactate concentration. *Journal of Cerebral Blood Flow and Metabolism*, *37*(6), 2112-2124.

Lustberg, L., & Reynolds III, C. F. (2000). Depression and insomnia: questions of cause and effect. *Sleep Medicine Reviews*, *4*(3), 253-262. doi:https://doi.org/10.1053/smrv.1999.0075

Ma, W., Song, J., Wang, H., Shi, F., Zhou, N., Jiang, J., . . . Zhou, M. (2019). Chronic paradoxical sleep deprivation-induced depressionlike behavior, energy metabolism and microbial changes in rats. *Life Sciences, 225*, 88-97.

Mac Giollabhui, N., Ng, T. H., Ellman, L. M., & Alloy, L. B. (2021). The longitudinal associations of inflammatory biomarkers and depression revisited: systematic

review, meta-analysis, and meta-regression. *Molecular Psychiatry, 26*(7), 3302-3314.

Mächler, P., Wyss, M. T., Elsayed, M., Stobart, J., Gutierrez, R., von Faber-Castell, A., . . . Romero-Gómez, I. (2016). In vivo evidence for a lactate gradient from astrocytes to neurons. *Cell Metabolism, 23*(1), 94-102.

Madalena, K. M., & Lerch, J. K. (2017). The effect of glucocorticoid and glucocorticoid receptor interactions on brain, spinal cord, and glial cell plasticity. *Neural Plasticity, 2017*.

Magistretti, P. J. (2009). Role of glutamate in neuron-glia metabolic coupling. *The American journal of clinical nutrition, 90*(3), 875S-880S.

Magistretti, P. J., & Allaman, I. (2015). A cellular perspective on brain energy metabolism and functional imaging. *Neuron, 86*(4), 883-901.

Magistretti, P. J., & Allaman, I. (2018). Lactate in the brain: from metabolic end-product to signalling molecule. *Nature Reviews Neuroscience, 19*(4), 235-249.

Magistretti, P. J., & Pellerin, L. (1996). Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a prominent role of astrocytes. *Cerebral Cortex, 6*(1), 50-61.

Magistretti, P. J., & Pellerin, L. (1999). Astrocytes couple synaptic activity to glucose utilization in the brain. *Physiology, 14*(5), 177-182.

Makino, S., Smith, M. A., & Gold, P. W. (1995). Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. *Endocrinology, 136*(8), 3299-3309.

Manfredi-Lozano, M., Roa, J., & Tena-Sempere, M. (2018). Connecting metabolism and gonadal function: novel central neuropeptide pathways involved in the metabolic control of puberty and fertility. *Frontiers in Neuroendocrinology, 48*, 37-49.

Mang, G. M., & Franken, P. (2012). Sleep and EEG phenotyping in mice. *Current Protocols in Mouse Biology, 2*(1), 55-74.

Mangia, S., Simpson, I. A., Vannucci, S. J., & Carruthers, A. (2009). The in vivo neuron-to-astrocyte lactate shuttle in human brain: Evidence from modeling of measured lactate levels during visual stimulation. *Journal of Neurochemistry*, *109*, 55-62.

Margaria, R., Edwards, H., & Dill, D. B. (1933). The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *American Journal of Physiology-Legacy Content*, *106*(3), 689-715.

Marotta, A., Sarno, E., Del Casale, A., Pane, M., Mogna, L., Amoruso, A., . . . Fiorio, M. (2019). Effects of probiotics on cognitive reactivity, mood, and sleep quality. *Frontiers in psychiatry*, *10*, 164.

Marquez, C., Nadal, R., & Armario, A. (2004). The hypothalamic–pituitary–adrenal and glucose responses to daily repeated immobilisation stress in rats: individual differences. *Neuroscience*, *123*(3), 601-612.

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood*, *44*(235), 291.

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood*, *45*(239), 13-23.

Marten, B., Pfeuffer, M., & Schrezenmeir, J. (2006). Medium-chain triglycerides. *International Dairy Journal*, *16*(11), 1374-1382.

Martins, P. J. F., Marques, M. S., Tufik, S., & D'Almeida, V. (2010). Orexin activation precedes increased NPY expression, hyperphagia, and metabolic changes in response to sleep deprivation. *American Journal of Physiology-Endocrinology and Metabolism*, *298*(3), E726-E734.

Marx, W., McGuinness, A. J., Rocks, T., Ruusunen, A., Cleminson, J., Walker, A. J., . . . Diaz, A. P. (2021). The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. *Molecular Psychiatry*, *26*(8), 4158-4178.

Matsumoto, M., Kibe, R., Ooga, T., Aiba, Y., Kurihara, S., Sawaki, E., . . . Benno, Y. (2012). Impact of intestinal microbiota on intestinal luminal metabolome. *Scientific Reports*, *2*(1), 1-10.

Mauvais-Jarvis, F. (2015). Sex differences in metabolic homeostasis, diabetes, and obesity. *Biology of Sex Differences*, *6*(1), 1-9.

Mavanji, V., Teske, J. A., Billington, C. J., & Kotz, C. M. (2013). Partial sleep deprivation by environmental noise increases food intake and body weight in obesity-resistant rats. *Obesity*, *21*(7), 1396-1405.

McAllister-Williams, R., Ferrier, I., & Young, A. (1998). Mood and neuropsychological function in depression: the role of corticosteroids and serotonin. *Psychological Medicine*, *28*(3), 573-584.

McCormick, C. M., & Hodges, T. (2017). Stress, glucocorticoids, and brain development in rodent models.

McCormick, C. M., & Mathews, I. Z. (2007). HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacology, Biochemistry and Behavior*, *86*(2), 220-233. doi:https://doi.org/10.1016/j.pbb.2006.07.012

McCormick, C. M., Mongillo, D., & Simone, J. (2013). Age and adolescent social stress effects on fear extinction in female rats. *Stress*, *16*(6), 678-688.

McCormick, C. M., Smythe, J. W., Sharma, S., & Meaney, M. J. (1995). Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Developmental Brain Research*, *84*(1), 55-61. doi:https://doi.org/10.1016/0165-3806(94)00153-q

McEwen, B. S. (2005). Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism: Clinical and Experimental*, *54*(5), 20-23. doi:https://doi.org/10.1016/j.metabol.2005.01.008

McGill, B. E., Bundle, S. F., Yaylaoglu, M. B., Carson, J. P., Thaller, C., & Zoghbi, H. Y. (2006). Enhanced anxiety and stress-induced corticosterone release are associated with increased Crh expression in a mouse model of Rett syndrome. *Proceedings of the National Academy of Sciences*, *103*(48), 18267-18272.

McGinnis, M. Y., Lumia, A. R., Tetel, M. J., Molenda-Figueira, H. A., & Possidente, B. (2007). Effects of anabolic androgenic steroids on the development and expression of running wheel activity and circadian rhythms in male rats. *Physiology and Behavior*, *92*(5), 1010-1018.

McIsaac, W. M., & Page, I. H. (1959). The metabolism of serotonin (5-hydroxytryptamine). *Journal of Biological Chemistry*, *234*(4), 858-864.

Meerlo, P., Koehl, M., Van der Borght, K., & Turek, F. W. (2002). Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. *Journal of Neuroendocrinology*, *14*(5), 397-402.

Meijer, O., Williamson, A., Dallman, M., & Pearce, D. (2000). Transcriptional repression of the 5-HT1A receptor promoter by corticosterone via mineralocorticoid receptors depends on the cellular context. *Journal of Neuroendocrinology, 12*(3), 245-254. doi:https://doi.org/10.1046/j.1365-2826.2000.00445.x

Melancon, M. O., Lorrain, D., & Dionne, I. J. (2014). Changes in markers of brain serotonin activity in response to chronic exercise in senior men. *Applied Physiology, Nutrition, and Metabolism, 39*(11), 1250-1256.

Menke, A., Arloth, J., Gerber, M., Rex-Haffner, M., Uhr, M., Holsboer, F., . . . Beck, J. (2014). Dexamethasone stimulated gene expression in peripheral blood indicates glucocorticoid-receptor hypersensitivity in job-related exhaustion. *Psychoneuroendocrinology, 44*, 35-46.

Metges, C. C., & Wolfram, G. (1991). Medium and long chain triglycerides labeled with 13C: a comparison of oxidation after oral or parenteral administration in humans. *The Journal of Nutrition, 121*(1), 31-36.

Michelsen, K. A., Schmitz, C., & Steinbusch, H. W. (2007). The dorsal raphe nucleus—from silver stainings to a role in depression. *Brain Research Reviews*, *55*(2), 329-342.

Miles, J. M., Haymond, M. W., & Gerich, J. E. (1981). Suppression of glucose production and stimulation of insulin secretion by physiological concentrations of ketone bodies in man. *The Journal of Clinical Endocrinology & Metabolism*, *52*(1), 34-37.

Miller, G. E., Rohleder, N., Stetler, C., & Kirschbaum, C. (2005). Clinical depression and regulation of the inflammatory response during acute stress. *Psychosomatic Medicine, 67*(5), 679-687.

Mineur, Y. S., Belzung, C., & Crusio, W. E. (2006). Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behavioural Brain Research, 175*(1), 43-50.

Minkel, J., Moreta, M., Muto, J., Htaik, O., Jones, C., Basner, M., & Dinges, D. (2014). Sleep deprivation potentiates HPA axis stress reactivity in healthy adults. *Health Psychology, 33*(11), 1430. doi:https://doi.org/10.1037/a0034219

Mitchell, G., Kassovska-Bratinova, S., Boukaftane, Y., Robert, M., Wang, S., Ashmarina, L., . . . Potier, E. (1995). Medical aspects of ketone body metabolism. Clinical and Investigative Medicine. *Medecine Clinique et Experimentale, 18*(3), 193-216.

Miura, H., Ozaki, N., Sawada, M., Isobe, K., Ohta, T., & Nagatsu, T. (2008). A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. *Stress, 11*(3), 198-209.

Mizoguchi, K., Shoji, H., Ikeda, R., Tanaka, Y., & Tabira, T. (2008). Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *Pharmacology Biochemistry and Behavior, 91*(1), 170-175.

Mizoguchi, K., Yuzurihara, M., Ishige, A., Sasaki, H., Chui, D.-H., & Tabira, T. (2001). Chronic stress differentially regulates glucocorticoid negative feedback response in rats. *Psychoneuroendocrinology, 26*(5), 443-459.

Mocking, R., Harmsen, I., Assies, J., Koeter, M., Ruhé, H., & Schene, A. (2017). Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. *Translational psychiatry, 6*(3), e756.

Mohammad-Zadeh, L., Moses, L., & Gwaltney-Brant, S. (2008). Serotonin: a review. *Journal of Veterinary Pharmacology and Therapeutics, 31*(3), 187-199.

Monoi, N., Matsuno, A., Nagamori, Y., Kimura, E., Nakamura, Y., Oka, K., . . . Murakoshi, M. (2016). Japanese sake yeast supplementation improves the quality of sleep: a double-blind randomised controlled clinical trial. *Journal of Sleep Research, 25*(1), 116-123.

Monroe, S. M., Slavich, G. M., & Gotlib, I. H. (2014). Life stress and family history for depression: The moderating role of past depressive episodes. *Journal of Psychiatric Research, 49*, 90-95.

Moodie, J. L., Campisi, S. C., Salena, K., Wheatley, M., Vandermorris, A., & Bhutta, Z. A. (2020). Timing of pubertal milestones in low-and middle-income countries: a systematic review and meta-analysis. *Advances in Nutrition*, *11*(4), 951-959.

Moore, R. (1983). Organization and function of a central nervous system circadian oscillator: the suprachiasmatic hypothalamic nucleus. In *Federation proceedings* (Vol. 42, No. 11, pp. 2783-2789).

Morgello, S., Uson, R. R., Schwartz, E. J., & Haber, R. S. (1995). The human blood-brain barrier glucose transporter (GLUT1) is a glucose transporter of gray matter astrocytes. *Glia*, *14*(1), 43-54.

Mueller, P. S., Heninger, G. R., & McDonald, R. K. (1969). Intravenous glucose tolerance test in depression. *Archives of General Psychiatry*, *21*(4), 470-477.

Murack, M., Chandrasegaram, R., Smith, K. B., Ah-Yen, E. G., Rheaume, É., Malette-Guyon, É., . . . Messier, C. (2021). Chronic sleep disruption induces depression-like behavior in adolescent male and female mice and sensitization of the hypothalamic-pituitary-adrenal axis in adolescent female mice. *Behavioural Brain Research*, *399*, 113001.

Murack, M., & Messier, C. (2019). The impact of lactic acid and medium chain triglyceride on blood glucose, lactate and diurnal motor activity: A re-examination of a treatment of major depression using lactic acid. *Physiology and Behavior*, *208*, 112569.

Murphy, P., Likhodii, S., Nylen, K., & Burnham, W. (2004). The antidepressant properties of the ketogenic diet. *Biological Psychiatry*, *56*(12), 981-983.

Murphy, T., Han, L., Allore, H., Peduzzi, P., Gill, T., & Lin, H. (2010). Treatment of death in the analysis of longitudinal studies of gerontological outcomes. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *66*(1), 109-114.

Murray, E., Sharma, R., Smith, K. B., Mar, K. D., Barve, R., Lukasik, M., . . . Thomas, B. J. (2019). Probiotic consumption during puberty mitigates LPS-induced immune responses and protects against stress-induced depression-and anxiety-like behaviors in adulthood in a sex-specific manner. *Brain, Behavior, and Immunity*, *81*, 198-212.

Murray, E., Smith, K. B., Stoby, K. S., Thomas, B. J., Swenson, M. J., Arber, L. A., . . . Ismail, N. (2020). Pubertal probiotic blocks LPS-induced anxiety and the associated neurochemical and microbial outcomes, in a sex dependent manner. *Psychoneuroendocrinology*, *112*, 104481.

Nakagawa, M., Yamamoto, H., Kawaji, M., Miura, N., Wakame, K., & Endo, T. (2018). Effects of lactic acid bacteria-containing foods on the quality of sleep: a placebo-controlled, double-blinded, randomized crossover study. *Functional Foods in Health and Disease*, *8*(12), 579-596.

Nakakita, Y., Tsuchimoto, N., Takata, Y., & Nakamura, T. (2016). Effect of dietary heat-killed Lactobacillus brevis SBC8803 (SBL88™) on sleep: A non-randomised, double blind, placebo-controlled, and crossover pilot study. *Beneficial microbes*, *7*(4), 501-509.

Nakrani, M. N., Wineland, R. H., & Anjum, F. (2020). Physiology, Glucose Metabolism. *StatPearls [Internet]*.

Natelson, B. H., Ottenweller, J. E., Cook, J. A., Pitman, D., McCarty, R., & Tapp, W. N. (1988). Effect of stressor intensity on habituation of the adrenocortical stress response. *Physiology and Behavior*, *43*(1), 41-46.

Naylor, E., Aillon, D. V., Barrett, B. S., Wilson, G. S., Johnson, D. A., Johnson, D. A., . . . Petillo, P. A. (2012). Lactate as a biomarker for sleep. *Sleep*, *35*(9), 1209-1222.

Nehlig, A., Wittendorp-Rechenmann, E., & Dao Lam, C. (2004). Selective uptake of [14C] 2-deoxyglucose by neurons and astrocytes: high-resolution microautoradiographic imaging by cellular 14C-trajectography combined with immunohistochemistry. *Journal of Cerebral Blood Flow and Metabolism*, *24*(9), 1004-1014.

Nelson, A. B., Faraguna, U., Zoltan, J. T., Tononi, G., & Cirelli, C. (2013). Sleep patterns and homeostatic mechanisms in adolescent mice. *Brain sciences*, *3*(1), 318-343.

Nelson, J. C., & Davis, J. M. (1997). DST studies in psychotic depression: a meta-analysis. *American Journal of Psychiatry*, *154*(11), 1497-1503.

Nikbakht, E., Khalesi, S., Singh, I., Williams, L. T., West, N. P., & Colson, N. (2018). Effect of probiotics and synbiotics on blood glucose: a systematic review and meta-analysis of controlled trials. *European Journal of Nutrition*, *57*(1), 95-106.

Nishida, K., Sawada, D., Kuwano, Y., Tanaka, H., Sugawara, T., Aoki, Y., . . . Rokutan, K. (2017). Daily administration of paraprobiotic Lactobacillus gasseri CP2305 ameliorates chronic stress-associated symptoms in Japanese medical students. *Journal of Functional Foods, 36*, 112-121.

Nouwen, A., Nefs, G., Caramlau, I., Connock, M., Winkley, K., Lloyd, C. E., . . . European Depression in Diabetes (EDID) Research Consortium. (2011). Prevalence of depression in individuals with impaired glucose metabolism or undiagnosed diabetes: a systematic review and meta-analysis of the European Depression in Diabetes (EDID) Research Consortium. *Diabetes Care, 34*(3), 752-762.

Novati, A., Hulshof, H. J., Koolhaas, J. M., Lucassen, P. J., & Meerlo, P. (2011). Chronic sleep restriction causes a decrease in hippocampal volume in adolescent rats, which is not explained by changes in glucocorticoid levels or neurogenesis. *Neuroscience, 190*, 145-155.

Nyholm, P. G., & Pascher, I. (1993). Orientation of the saccharide chains of glycolipids at the membrane surface: conformational analysis of the glucose-ceramide and the glucose-glyceride linkages using molecular mechanics (MM3). *Biochemistry, 32*(5), 1225-1234.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research, 277*, 32-48. doi:10.1016/j.bbr.2014.07.027

Offord, D. R., Boyle, M. H., Szatmari, P., Rae-Grant, N. I., Links, P. S., Cadman, D. T., . . . Woodward, C. A. (1987). Ontario Child Health Study: II. Six-month prevalence of disorder and rates of service utilization. *Archives of General Psychiatry, 44*(9), 832-836.

Ogawa, Y., Miyoshi, C., Obana, N., Yajima, K., Hotta-Hirashima, N., Ikkyu, A., . . . Yanagisawa, M. (2020). Gut microbiota depletion by chronic antibiotic treatment alters the sleep/wake architecture and sleep EEG power spectra in mice. *Scientific Reports, 10*(1), 19554.

Ogilvie, R. P., & Patel, S. R. (2017). The epidemiology of sleep and obesity. *Sleep Health, 3*(5), 383-388.

Ojeda, S., Urbanski, H., Knobil, E., & Neill, J. (1994). Physiology of reproduction. In *The laboratory rat* (pp. 145-176). Academic Press.

Okita, T. W., Rodriguez, R. L., & Preiss, J. (1981). Biosynthesis of bacterial glycogen. Cloning of the glycogen biosynthetic enzyme structural genes of Escherichia coli. *Journal of Biological Chemistry, 256*(13), 6944-6952.

Oliverio, A., & Malorni, W. (1979). Wheel running and sleep in two strains of mice: plasticity and rigidity in the expression of circadian rhythmicity. *Brain Research, 163*(1), 121-133.

Ono, T., Shimizu, T., & Yoshida, M. (1991). Characteristics of wheel-running activity in nine inbred strains of male mice under 12L-12D cycle. *Journal of the Mammalogical Society of Japan, 16*(2), 47-58.

Onozato, M., Umino, M., Shoji, A., Ichiba, H., Tsujino, N., Funatogawa, T., . . . Fukushima, T. (2020). Serum d-and l-lactate, pyruvate and glucose levels in individuals with at-risk mental state and correlations with clinical symptoms. *Early Intervention in Psychiatry, 14*(4), 410-417.

Organization, W. H. (2008). *The global burden of disease: 2004 update*. World Health Organization.

Osei, E. (2020). Disturbed Glucose Metabolism in Patients with a TIA or Ischemic Stroke: Prognosis and Long-term Treatment.

Osimo, E. F., Pillinger, T., Rodriguez, I. M., Khandaker, G. M., Pariante, C. M., & Howes, O. D. (2020). Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. *Brain, Behavior, and Immunity, 87*, 901-909.

Ostrander, M. M., Richtand, N. M., & Herman, J. P. (2003). Stress and amphetamine induce Fos expression in medial prefrontal cortex neurons containing glucocorticoid receptors. *Brain Research, 990*(1-2), 209-214. doi:https://doi.org/10.1016/j.brainres.2003.07.001

Ou, X.-M., Storring, J. M., Kushwaha, N., & Albert, P. R. (2001). Heterodimerization of mineralocorticoid and glucocorticoid receptors at a novel negative response element of the 5-HT1A receptor gene. *Journal of Biological Chemistry, 276*(17), 14299-14307.

Owen, O., Morgan, A., Kemp, H., Sullivan, J., Herrera, M., & Cahill, G. J. (1967). Brain metabolism during fasting. *The Journal of Clinical Investigation*, *46*(10), 1589-1595.

Pan, J. W., Rothman, D. L., Behar, K. L., Stein, D. T., & Hetherington, H. P. (2000). Human brain β-hydroxybutyrate and lactate increase in fasting-induced ketosis. *Journal of Cerebral Blood Flow and Metabolism*, *20*(10), 1502-1507.

Pan, L., & Gilbert, F. (1992). Activation of 5-HT1A receptor subtype in the paraventricular nuclei of the hypothalamus induces CRH and ACTH release in the rat. *Neuroendocrinology*, *56*(6), 797-802. doi:https://doi.org/10.1159/000126332

Pardridge, W. M. (2007). Blood–brain barrier delivery. *Drug Discovery Today*, *12*(1-2), 54-61.

Park, S. J., Smith, C. P., Wilbur, R. R., Cain, C. P., Kallu, S. R., Valasapalli, S., . . . Velpula, K. K. (2018). An overview of MCT1 and MCT4 in GBM: small molecule transporters with large implications. *American Journal of Cancer Research*, *8*(10), 1967.

Parker Jr, C. R., & Mahesh, V. (1976). Hormonal events surrounding the natural onset of puberty in female rats. *Biology of Reproduction*, *14*(3), 347-353.

Partrick, K. A., Rosenhauer, A. M., Auger, J., Arnold, A. R., Ronczkowski, N. M., Jackson, L. M., . . . Huhman, K. L. (2021). Ingestion of probiotic (Lactobacillus helveticus and Bifidobacterium longum) alters intestinal microbial structure and behavioral expression following social defeat stress. *Scientific Reports*, *11*(1), 1-12.

Patricia, B., Raymond, J., Milot, M. R., Merali, Z., & Plamondon, H. (2014). Evidence of lasting dysregulation of neuroendocrine and HPA axis function following global cerebral ischemia in male rats and the effect of Antalarmin on plasma corticosterone level. *Hormones and Behavior*, *65*(3), 273-284.

Paxinos, G., & Franklin, K. B. (2019). *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*. Academic press.

Paykel, E. S. (2022). Basic concepts of depression. *Dialogues in Clinical Neuroscience*.

Pearson, N. J., Johnson, L. L., & Nahin, R. L. (2006). Insomnia, trouble sleeping, and complementary and alternative medicine: analysis of the 2002 national health interview survey data. Archives of Internal Medicine, 166(16), 1775-1782.

Pellerin, L. (2010). Food for thought: the importance of glucose and other energy substrates for sustaining brain function under varying levels of activity. *Diabetes and Metabolism*, *36*, S59-S63.

Pellerin, L., & Magistretti, P. J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proceedings of the National Academy of Sciences*, *91*(22), 10625-10629.

Pho, H., Berger, S., Freire, C., Kim, L. J., Shin, M.-K., Streeter, S. R., . . . Dergacheva, O. (2021). Leptin receptor expression in the dorsomedial hypothalamus stimulates breathing during NREM sleep in db/db mice. *Sleep*, *44*(6), zsab046.

Picchioni, D., Fukunaga, M., Carr, W. S., Braun, A. R., Balkin, T. J., Duyn, J. H., & Horovitz, S. G. (2008). fMRI differences between early and late stage-1 sleep. *Neuroscience Letters*, *441*(1), 81-85.

Pignatelli, D., Xiao, F., Gouveia, A. M., Ferreira, J. G., & Vinson, G. P. (2006). Adrenarche in the rat. *Journal of Endocrinology*, *191*(1), 301-308.

Pine, D. S., Cohen, E., Cohen, P., & Brook, J. (1999). Adolescent depressive symptoms as predictors of adult depression: moodiness or mood disorder? *American Journal of Psychiatry*, *156*(1), 133-135.

Piñeyro, G., & Blier, P. (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacological Reviews*, *51*(3), 533-591.

Pinto-Sanchez, M. I., Hall, G. B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J. T., . . . Rieder, A. (2017). Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. *Gastroenterology*, *153*(2), 448-459. e448.

Poggiogalle, E., Jamshed, H., & Peterson, C. M. (2018). Circadian regulation of glucose, lipid, and energy metabolism in humans. *Metabolism*, *84*, 11-27.

Poroyko, V. A., Carreras, A., Khalyfa, A., Khalyfa, A. A., Leone, V., Peris, E., . . . Hubert, N. (2016). Chronic sleep disruption alters gut microbiota, induces

systemic and adipose tissue inflammation and insulin resistance in mice. *Scientific Reports, 6*(1), 35405.

Posner, J. B., Plum, F., & Van Poznak, A. (1969). Cerebral metabolism during electrically induced seizures in man. *Archives of Neurology, 20*(4), 388-395.

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine, 61*(2), 197-204.

Puttonen, S., Härmä, M., & Hublin, C. (2010). Shift work and cardiovascular disease—pathways from circadian stress to morbidity. *Scandinavian Journal of Work, Environment and Health*, 96-108.

Radulovacki, M., Virus, R., Djuricic-Nedelson, M., & Green, R. (1984). Adenosine analogs and sleep in rats. *Journal of Pharmacology and Experimental Therapeutics, 228*(2), 268-274.

Rao, S. S., Rehman, A., Yu, S., & De Andino, N. M. (2018). Brain fogginess, gas and bloating: a link between SIBO, probiotics and metabolic acidosis. *Clinical and Translational Gastroenterology, 9*(6), 162.

Reinherz, H. Z., Stewart-Berghauer, G., Pakiz, B., Frost, A. K., Moeykens, B. A., & Holmes, W. M. (1989). The relationship of early risk and current mediators to depressive symptomatology in adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry, 28*(6), 942-947.

Renn, C. L., Carozzi, V. A., Rhee, P., Gallop, D., Dorsey, S. G., & Cavaletti, G. (2011). Multimodal assessment of painful peripheral neuropathy induced by chronic oxaliplatin-based chemotherapy in mice. *Molecular Pain, 7*(1), 29.

Rhodes, J., Hosack, G., Girard, I., Kelley, A., Mitchell, G., & Garland, T. (2001). Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology, 158*(2), 120-131.

Richardson-Jones, J. W., Craige, C. P., Guiard, B. P., Stephen, A., Metzger, K. L., Kung, H. F., . . . Beck, S. G. (2010). 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron, 65*(1), 40-52.

Riddell, M. C. (2008). The endocrine response and substrate utilization during exercise in children and adolescents. *Journal of Applied Physiology*, *105*(2), 725-733.

Roberts, R. A., Soames, A. R., James, N. H., Gill, J. H., & Wheeldon, E. B. (1995). Dosing-induced stress causes hepatocyte apoptosis in rats primed by the rodent nongenotoxic hepatocarcinogen cyproterone acetate. *Toxicology and Applied Pharmacology*, *135*(2), 192-199.

Robillard, R., Dion, K., Pennestri, M. H., Solomonova, E., Lee, E., Saad, M., . . . Quilty, L. (2021). Profiles of sleep changes during the COVID-19 pandemic: Demographic, behavioural and psychological factors. *Journal of Sleep Research*, *30*(1), e13231.

Robillard, R., Hermens, D. F., Naismith, S. L., White, D., Rogers, N. L., Ip, T. K., . . . Smith, K. L. (2015). Ambulatory sleep-wake patterns and variability in young people with emerging mental disorders. *Journal of Psychiatry & Neuroscience*, *40*(1), 28.

Robillard, R., Naismith, S., Rogers, N., Scott, E., Ip, T., Hermens, D. F., & Hickie, I. (2013). Sleep-wake cycle and melatonin rhythms in adolescents and young adults with mood disorders: comparison of unipolar and bipolar phenotypes. *European Psychiatry*, *28*(7), 412-416.

Robillard, R., Naismith, S. L., Rogers, N. L., Ip, T. K., Hermens, D. F., Scott, E. M., & Hickie, I. B. (2013). Delayed sleep phase in young people with unipolar or bipolar affective disorders. *Journal of Affective Disorders*, *145*(2), 260-263.

Robillard, R., Naismith, S. L., Smith, K. L., Rogers, N. L., White, D., Terpening, Z., . . . Scott, E. M. (2014). Sleep-wake cycle in young and older persons with a lifetime history of mood disorders. *PloS One*, *9*(2), e87763.

Roebuck, A., Monasterio, V., Gederi, E., Osipov, M., Behar, J., Malhotra, A., . . . Clifford, G. (2013). A review of signals used in sleep analysis. *Physiological Measurement*, *35*(1), R1.

Roenneberg, T., Wirz-Justice, A., & Merrow, M. (2003). Life between clocks: daily temporal patterns of human chronotypes. *Journal of Biological Rhythms*, *18*(1), 80-90.

Romeo, R. D. (2010). Adolescence: a central event in shaping stress reactivity. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *52*(3), 244-253. doi:https://doi.org/10.1002/dev.20437

Romeo, R. D., Bellani, R., Karatsoreos, I. N., Chhua, N., Vernov, M., Conrad, C. D., & McEwen, B. S. (2006). Stress history and pubertal development interact to shape hypothalamic-pituitary-adrenal axis plasticity. *Endocrinology*, *147*(4), 1664-1674. doi:https://doi.org/10.1210/en.2005-1432

Romeo, R. D., Lee, S. J., Chhua, N., McPherson, C. R., & McEwen, B. S. (2004). Testosterone cannot activate an adult-like stress response in prepubertal male rats. *Neuroendocrinology*, *79*(3), 125-132.

Romeo, R. D., Lee, S. J., & McEwen, B. S. (2004). Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. *Neuroendocrinology*, *80*(6), 387-393.

Romeo, R. D., & McEwen, B. S. (2006). Stress and the adolescent brain. *Annals of the New York Academy of Sciences*, *1094*(1), 202-214.

Romijn, A. R., Rucklidge, J. J., Kuijer, R. G., & Frampton, C. (2017). A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. *Australian and New Zealand Journal of Psychiatry*, *51*(8), 810-821.

Ross, R. E., VanDerwerker, C. J., Saladin, M. E., & Gregory, C. M. (2023). The role of exercise in the treatment of depression: biological underpinnings and clinical outcomes. *Molecular Psychiatry*, *28*(1), 298-328.

Rothman, D. L., Behar, K. L., Hyder, F., & Shulman, R. G. (2003). In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: implications for brain function. *Annual Review of Physiology*, *65*(1), 401-427.

Rowe, R. C., Sheskey, P. J., & Owen, S. C. (2006). Handbook of pharmaceutical excipients. *American Journal of Pharmaceutical Education*, *68*(1-5), BF1.

Rubin, R. T., Heist, E. K., McGeoy, S. S., Hanada, K., & Lesser, I. M. (1992). Neuroendocrine aspects of primary endogenous depression: XI. Serum melatonin

measures in patients and matched control subjects. *Archives of General Psychiatry*, *49*(7), 558-567.

Rudolf, G., & Tölle, R. (1978). Sleep deprivation and circadian rhythm in depression. *Psychopathology*, *11*(4), 198-212.

Ruscica, M., Macchi, C., Gandini, S., Morlotti, B., Erzegovesi, S., Bellodi, L., & Magni, P. (2016). Free and bound plasma leptin in anorexia nervosa patients during a refeeding program. *Endocrine*, *51*(2), 380-383.

Rutter, M., Izard, C. E., & Read, P. B. (1986). *Depression in young people: Developmental and clinical perspectives*: Guilford Press.

Sada, N., Lee, S., Katsu, T., Otsuki, T., & Inoue, T. (2015). Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science*, *347*(6228), 1362-1367.

Sandler, M., Reveley, M., & Glover, V. (1981). Human platelet monoamine oxidase activity in health and disease: a review. *Journal of Clinical Pathology*, *34*(3), 292-302.

Saré, R. M., Song, A., Levine, M., Lemons, A., Loutaev, I., Sheeler, C., . . . Smith, C. B. (2019). Chronic sleep restriction in developing male mice results in long lasting behavior impairments. *Frontiers in Behavioral Neuroscience*, *13*, 90.

Schaap, J., & Meijer, J. H. (2001). Opposing effects of behavioural activity and light on neurons of the suprachiasmatic nucleus. *European Journal of Neuroscience*, *13*(10), 1955-1962.

Schmid, S. M., Hallschmid, M., Jauch-Chara, K., Born, J., & Schultes, B. (2008). A single night of sleep deprivation increases ghrelin levels and feelings of hunger in normal-weight healthy men. *Journal of Sleep Research*, *17*(3), 331-334.

Schmidt, M., Enthoven, L., Van Der Mark, M., Levine, S., De Kloet, E., & Oitzl, M. (2003). The postnatal development of the hypothalamic–pituitary–adrenal axis in the mouse. *International Journal of Developmental Neuroscience*, *21*(3), 125-132.

Schneider, J. E. (2004). Energy balance and reproduction. *Physiology and Behavior*, *81*(2), 289-317.

Schneider, J. E., & Watts, A. G. (2002). Energy balance, ingestive behavior, and reproductive success. In *Hormones, brain and behavior* (pp. 435-IV). Academic Press.

Schroeder, B. O., & Backhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nature Medicine*, *22*(10), 1079-1089. doi:10.1038/nm.4185

Schurr, A. (2014). Cerebral glycolysis: a century of persistent misunderstanding and misconception. *Frontiers in Neuroscience*, *8*, 360.

Schurr, A., Payne, R. S., Miller, J. J., & Rigor, B. M. (1997). Brain lactate, not glucose, fuels the recovery of synaptic function from hypoxia upon reoxygenation: an in vitro study. *Brain Research*, *744*(1), 105-111.

Schwartz, R. M., Boyes, S., & Aynsley-Green, A. (1989). Metabolic effects of three ketogenic diets in the treatment of severe epilepsy. *Developmental Medicine and Child Neurology*, *31*(2), 152-160.

Seaton, T. B., Welle, S. L., Warenko, M. K., & Campbell, R. G. (1986). Thermic effect of medium-chain and long-chain triglycerides in man. *The American Journal of Clinical Nutrition*, *44*(5), 630-634.

Sekeres, M. J., Moscovitch, M., Winocur, G., Pishdadian, S., Nichol, D., & Grady, C. L. (2021). Reminders activate the prefrontal-medial temporal cortex and attenuate forgetting of event memory. *Hippocampus*, *31*(1), 28-45.

Senior, B., & Loridan, L. (1968). Direct regulatory effect of ketones on lipolysis and on glucose concentrations in man. *Nature*, *219*(5149), 83.

Shahanoor, Z., Sultana, R., Baker, M. R., & Romeo, R. D. (2017). Neuroendocrine stress reactivity of male C57BL/6N mice following chronic oral corticosterone exposure during adulthood or adolescence. *Psychoneuroendocrinology*, *86*, 218-224.

Shanahan, T. L., & Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *The Journal of Clinical Endocrinology & Metabolism*, *73*(2), 227-235.

Shapero, B. G., Curley, E. E., Black, C. L., & Alloy, L. B. (2019). The interactive association of proximal life stress and cumulative HPA axis functioning with depressive symptoms. *Depression and Anxiety*, *36*(11), 1089-1101.

Shivavedi, N., Chatterjee, S. S., & Kumar, V. (2014). Stress response modulating effects of lactic acid in mice. *Therapeutic Targets for Neurological Diseases*, 1.

Silva, R. H., Abilio, V. C., Takatsu, A. L., Kameda, S. R., Grassl, C., Chehin, A. B., . . . Frussa-Filho, R. (2004). Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology*, *46*(6), 895-903. doi:10.1016/j.neuropharm.2003.11.032

Silverman, A. (1988). The gonadotropin-releasing hormone (GnRH) neuronal systems: immunocytochemistry. *The Physiology of Reproduction*.

Sisk, C. L., & Foster, D. L. (2004). The neural basis of puberty and adolescence. *Nature Neuroscience, 7*(10), 1040-1047.

Smajkic, A. (2009). Depression in adolescents: current treatments, suicidality and evaluation of novel treatment strategies. *Bosnian Journal of Basic Medical Sciences*, *9*(Suppl 1), S83.

Smith, D., Pernet, A., Hallett, W. A., Bingham, E., Marsden, P. K., & Amiel, S. A. (2003). Lactate: a preferred fuel for human brain metabolism in vivo. *Journal of Cerebral Blood Flow and Metabolism*, *23*(6), 658-664.

Smith, J. T., & Clarke, I. J. (2007). Kisspeptin expression in the brain: catalyst for the initiation of puberty. *Reviews in Endocrine and Metabolic Disorders*, *8*(1), 1-9.

Smith, K. B., Murray, E., Chandrasegaram, R., Liang, J., Mallet, J.-F., Matar, C., . . . Ismail, N. (2021). Pubertal immune challenge suppresses the hypothalamic-pituitary-gonadal axis in male and female mice. *Brain Research Bulletin, 170*, 90-97.

Smith, K. S., Greene, M. W., Babu, J. R., & Frugé, A. D. (2019). Psychobiotics as treatment for anxiety, depression, and related symptoms: a systematic review. *Nutritional Neuroscience*, *24*(12), 963-977.

Snyder, D. K., Clemmons, D. R., & Underwood, L. E. (1988). Treatment of obese, diet-restricted subjects with growth hormone for 11 weeks: effects on anabolism, lipolysis, and body composition. *The Journal of Clinical Endocrinology & Metabolism*, *67*(1), 54-61.

Sollars, P. J., Weiser, M. J., Kudwa, A. E., Bramley, J. R., Ogilvie, M. D., Spencer, R. L., . . . Pickard, G. E. (2014). Altered entrainment to the day/night cycle attenuates the daily rise in circulating corticosterone in the mouse. *PloS One, 9*(11), e111944.

Sonnenschein, M., Mommersteeg, P. M., Houtveen, J. H., Sorbi, M. J., Schaufeli, W. B., & van Doornen, L. J. (2007). Exhaustion and endocrine functioning in clinical burnout: an in-depth study using the experience sampling method. *Biological Psychology, 75*(2), 176-184.

Sousa, N., Lukoyanov, N., Madeira, M., Almeida, O., & Paula-Barbosa, M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience, 97*(2), 253-266.

Späni, D., Arras, M., König, B., & Rülicke, T. (2003). Higher heart rate of laboratory mice housed individually vs in pairs. *Laboratory Animals, 37*(1), 54-62.

Spiegal, K., Tasali, E., Penev, P., & Van Cauter, E. (2004). Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels and increased hunger and appetite. *Annals of International Medicine, 141*(11), 846-850.

Spiegel, K., Leproult, R., L'Hermite-Balériaux, M., Copinschi, G., Penev, P. D., & Van Cauter, E. (2004). Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *The Journal of Clinical Endocrinology & Metabolism, 89*(11), 5762-5771.

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *The Lancet, 354*(9188), 1435-1439.

Spiegel, K., Tasali, E., Leproult, R., & Van Cauter, E. (2009). Effects of poor and short sleep on glucose metabolism and obesity risk. *Nature Reviews Endocrinology, 5*(5), 253-261.

Spiess, J., Rivier, J., Rivier, C., & Vale, W. (1981). Primary structure of corticotropin-releasing factor from ovine hypothalamus. *Proceedings of the National Academy of Sciences, 78*(10), 6517-6521.

Spinedi, E., Chisari, A., Pralong, F., & Gaillard, R. C. (1997). Sexual dimorphism in the mouse hypothalamic-pituitary-adrenal axis function after endotoxin and insulin stresses during development. *Neuroimmunomodulation, 4*(2), 77-83.

Stamatakis, K. A., & Punjabi, N. M. (2010). Effects of sleep fragmentation on glucose metabolism in normal subjects. *Chest, 137*(1), 95-101.

Stapel, B., Fraccarollo, D., Westhoff-Bleck, M., Bauersachs, J., Lichtinghagen, R., Jahn, K., . . . Frieling, H. (2022). Impact of fasting on stress systems and depressive symptoms in patients with major depressive disorder: a cross-sectional study. *Scientific Reports, 12*(1), 7642.

Swallow, J. G., Carter, P. A., & Garland, T. (1998). Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics*, *28*(3), 227-237.

Szabo, S. T., & Blier, P. (2001). Effects of the selective norepinephrine reuptake inhibitor reboxetine on norepinephrine and serotonin transmission in the rat hippocampus. *Neuropsychopharmacology*, *25*(6), 845-857.

Tadavarty, R., Kaan, T., & Sastry, B. (2009). Long-term depression of excitatory synaptic transmission in rat hippocampal CA1 neurons following sleep-deprivation. *Experimental Neurology*, *216*(1), 239-242. doi:https://doi.org/10.1016/j.expneurol.2008.11.012

Tagaki, W., & Westheimer, F. H. (1968). Acetoacetate decarboxylase. Catalysis of hydrogendeuterium exchange in acetone. *Biochemistry, 7*(3), 901-905.

Takemura, N., Okubo, T., & Sonoyama, K. (2010). Lactobacillus plantarum strain No. 14 reduces adipocyte size in mice fed high-fat diet. *Experimental Biology and Medicine*, *235*(7), 849-856.

Tamtaji, O. R., Taghizadeh, M., Kakhaki, R. D., Kouchaki, E., Bahmani, F., Borzabadi, S., . . . Asemi, Z. (2019). Clinical and metabolic response to probiotic administration in people with Parkinson's disease: a randomized, double-blind, placebo-controlled trial. *Clinical Nutrition*, *38*(3), 1031-1035.

Tang, F., Lane, S., Korsak, A., Paton, J., Gourine, A., Kasparov, S., & Teschemacher, A. (2014). Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nature communications*, *5*(1), 3284.

Tang, Y., Preuss, F., Turek, F. W., Jakate, S., & Keshavarzian, A. (2009). Sleep deprivation worsens inflammation and delays recovery in a mouse model of colitis. *Sleep Medicine*, *10*(6), 597-603. doi:10.1016/j.sleep.2008.12.009

Tanner, J. M. (1962). Growth at adolescence.

Tao, Y.-W., Gu, Y.-L., Mao, X.-Q., Zhang, L., & Pei, Y.-F. (2020). Effects of probiotics on type II diabetes mellitus: a meta-analysis. *Journal of Translational Medicine*, *18*(1), 1-11.

Tarokh, L., Carskadon, M. A., & Achermann, P. (2012). Dissipation of sleep pressure is stable across adolescence. *Neuroscience*, *216*, 167-177.

Thevenet, J., De Marchi, U., Santo Domingo, J., Christinat, N., Bultot, L., Lefebvre, G., . . . Wiederkehr, A. (2016). Medium-chain fatty acids inhibit mitochondrial metabolism in astrocytes promoting astrocyte–neuron lactate and ketone body shuttle systems. *The Federation of American Societies for Experimental Biology Journal*, *30*(5), 1913-1926.

Thompson, R. S., Roller, R., Mika, A., Greenwood, B. N., Knight, R., Chichlowski, M., . . . Fleshner, M. (2017). Dietary prebiotics and bioactive milk fractions improve NREM sleep, enhance REM sleep rebound and attenuate the stress-induced decrease in diurnal temperature and gut microbial alpha diversity. *Frontiers in Behavioral Neuroscience, 10*, 240.

Thon, M., Hosoi, T., Yoshii, M., & Ozawa, K. (2014). Leptin induced GRP78 expression through the PI3K-mTOR pathway in neuronal cells. *Scientific Reports*, *4*(1), 1-8.

Tillmann, S., Awwad, H. M., Eskelund, A. R., Treccani, G., Geisel, J., Wegener, G., & Obeid, R. (2018). Probiotics Affect One-Carbon Metabolites and Catecholamines in a Genetic Rat Model of Depression. *Molecular Nutrition & Food Research*, *62*(7), 1701070.

Traul, K., Driedger, A., Ingle, D., & Nakhasi, D. (2000). Review of the toxicologic properties of medium-chain triglycerides. *Food and Chemical Toxicology*, *38*(1), 79-98.

Tsai, Y.-T., Cheng, P.-C., & Pan, T.-M. (2014). Anti-obesity effects of gut microbiota are associated with lactic acid bacteria. *Applied Microbiology and Biotechnology*, 98(1), 1-10.

Tubbs, A. S., Dollish, H. K., Fernandez, F., & Grandner, M. A. (2019). The basics of sleep physiology and behavior. In *Sleep and health* (pp. 3-10). Academic Press.

Turner, M. J., Kleeberger, S. R., & Lightfoot, J. T. (2005). Influence of genetic background on daily running-wheel activity differs with aging. *Physiological Genomics*, *22*(1), 76-85.

Tzadok, M., Nissenkorn, A., Porper, K., Matot, I., Marcu, S., Anikster, Y., . . . Zeev, B. B. (2014). The many faces of Glut1 deficiency syndrome. *Journal of Child Neurology*, *29*(3), 349-359.

Uchida, H., Yamamoto, H., Kisaki, Y., Fujino, J., Ishimaru, Y., & Ikeda, H. (2004). D-lactic acidosis in short-bowel syndrome managed with antibiotics and probiotics. *Journal of Pediatric Surgery*, *39*(4), 634-636.

Uehara, T., Sakado, K., Sakado, M., Sato, T., & Someya, T. (1999). Relationship between stress coping and personality in patients with major depressive disorder. *Psychotherapy and Psychosomatics*, *68*(1), 26-30.

Vahl, T. P., Ulrich-Lai, Y. M., Ostrander, M. M., Dolgas, C. M., Elfers, E. E., Seeley, R. J., . . . Herman, J. P. (2005). Comparative analysis of ACTH and corticosterone sampling methods in rats. *American Journal of Physiology-Endocrinology and Metabolism*, *289*(5), E823-E828.

Valentinuzzi, V. S., Scarbrough, K., Takahashi, J. S., & Turek, F. W. (1997). Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *273*(6), R1957-R1964.

Van Cauter, E., Van Coevorden, A., & Blackman, J. (1990). Modulation of neuroendocrine release by sleep and circadian rhythmicity. *Advances in neuroendocrine regulation of reproduction*, *113*, 122.

Van den Bulck, J. (2004). Television viewing, computer game playing, and Internet use and self-reported time to bed and time out of bed in secondary-school children. *Sleep*, *27*(1), 101-104.

van der Schuur, W. A., Baumgartner, S. E., Sumter, S. R., & Valkenburg, P. M. (2018). Media multitasking and sleep problems: A longitudinal study among adolescents. *Computers in Human Behavior*, *81*, 316-324.

Van Loo, P., Everse, L., Bernsen, M., Baumans, V., Hellebrekers, L., Kruitwagen, C., & Den Otter, W. (1997). Analgesics in mice used in cancer research: reduction of discomfort? *Laboratory Animals*, *31*(4), 318-325.

Van Reeth, O., Weibel, L., Spiegel, K., Leproult, R., Dugovic, C., & Maccari, S. (2000). Physiology of sleep (review)–interactions between stress and sleep: from basic research to clinical situations. *Sleep Medicine Reviews*, *4*(2), 201-219.

Vandenbergh, J. G. (1967). Effect of the presence of a male on the sexual maturation of female mice. *Endocrinology*, *81*(2), 345-349.

Vázquez, D. M., & Akil, H. (1993). Pituitary-adrenal response to ether vapor in the weanling animal: characterization of the inhibitory effect of glucocorticoids on adrenocorticotropin secretion. *Pediatric Research*, *34*(5), 646-653.

Vázquez, M. J., Romero-Ruiz, A., & Tena-Sempere, M. (2015). Roles of leptin in reproduction, pregnancy and polycystic ovary syndrome: consensus knowledge and recent developments. *Metabolism: Clinical and Experimental*, *64*(1), 79-91.

Verbeke, G. (1997). Linear mixed models for longitudinal data. In *Linear mixed models in practice* (pp. 63-153): Springer, New York, NY.

Vetrivelan, R., Fuller, P. M., Yokota, S., Lu, J., & Saper, C. B. (2012). Metabolic effects of chronic sleep restriction in rats. *Sleep*, *35*(11), 1511-1520.

Voutsinos-Porche, B., Bonvento, G., Tanaka, K., Steiner, P., Welker, E., Chatton, J.-Y., . . . Pellerin, L. (2003). Glial glutamate transporters mediate a functional metabolic crosstalk between neurons and astrocytes in the mouse developing cortex. *Neuron*, *37*(2), 275-286.

Walker, E. F., Walder, D. J., & Reynolds, F. (2001). Developmental changes in cortisol secretion in normal and at-risk youth. *Development and Psychopathology*, *13*(3), 721-732.

Wallace, C. J., & Milev, R. V. (2021). The efficacy, safety, and tolerability of probiotics on depression: clinical results from an open-label pilot study. *Frontiers in psychiatry*, *12*, 132.

Wang, J., Peng, X., Li, M., & Pan, Y. (2013). Construction and application of dynamic protein interaction network based on time course gene expression data. *Proteomics*, *13*(2), 301-312.

Wang, L., Hermens, D. F., Hickie, I. B., & Lagopoulos, J. (2012). A systematic review of resting-state functional-MRI studies in major depression. *Journal of Affective Disorders*, *142*(1-3), 6-12.

Wang, X., Cai, Z.-d., Jiang, W.-t., Fang, Y.-y., Sun, W.-x., & Wang, X. (2022). Systematic review and meta-analysis of the effects of exercise on depression in adolescents. *Child and Adolescent Psychiatry and Mental Health, 16*(1), 16.

Wang, X., Zhang, D., & Lu, X.-Y. (2015). Dentate gyrus-CA3 glutamate release/NMDA transmission mediates behavioral despair and antidepressant-like responses to leptin. *Molecular Psychiatry*, *20*(4), 509. doi:https://doi.org/10.1038/mp.2014.75

Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research, 588*(2), 341-345.

Watanabe, Y., Sakai, R. R., McEwen, B. S., & Mendelson, S. (1993). Stress and antidepressant effects on hippocampal and cortical 5-HT1A and 5-HT2 receptors and transport sites for serotonin. *Brain Research, 615*(1), 87-94.

Weber, B., & Barros, L. F. (2015). The astrocyte: powerhouse and recycling center. *Cold Spring Harbor Perspectives in Biology*, *7*(12), a020396.

Weibel, L., & Brandenberger, G. (1998). Disturbances in hormonal profiles of night workers during their usual sleep and work times. *Journal of Biological Rhythms, 13*(3), 202-208.

Weibel, L., Follenius, M., Spiegel, K., Ehrhart, J., & Brandenberger, G. (1995). Comparative effect of night and daytime sleep on the 24-hour cortisol secretory profile. *Sleep, 18*(7), 549-556.

Weinert, D., Eimert, H., Erkert, H. G., & Schneyer, U. (1994). Resynchronization of the circadian corticosterone rhythm after a light/dark shift in juvenile and adult mice. *Chronobiology International, 11*(4), 222-231.

Weiser, M., & Handa, R. (2009). Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic–pituitary–adrenal axis via estrogen receptor alpha within the hypothalamus. *Neuroscience, 159*(2), 883-895.

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*(2-3), 77-84. doi:https://doi.org/10.1016/0165-0327(93)90025-f

Weissman, M. M., & Klerman, G. L. (1977). Sex differences and the epidemiology of depression. *Archives of General Psychiatry*, *34*(1), 98-111.

Weitzman, E. D., Zimmerman, J. C., Czeisler, C. A., & Ronda, J. (1983). Cortisol secretion is inhibited during sleep in normal man. *The Journal of Clinical Endocrinology & Metabolism*, *56*(2), 352-358.

Welch, J. E., Farrar, G. E., Dunn, A. J., & Saphier, D. (1993). Central 5-HT1A receptors inhibit adrenocortical secretion. *Neuroendocrinology*, *57*(2), 272-281. doi:https://doi.org/10.1159/000126369

Westerblad, H., Allen, D. G., & Lannergren, J. (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *Physiology*, *17*(1), 17-21.

Wilson, G. S., & Gifford, R. (2005). Biosensors for real-time in vivo measurements. *Biosensors and Bioelectronics*, *20*(12), 2388-2403.

Wilson, M. E. (1992). Factors determining the onset of puberty. In *Sexual differentiation* (pp. 275-312). Springer, Boston, MA.

Winokur, A., Maislin, G., Phillips, J. L., & Amsterdam, J. D. (1988). Insulin resistance after oral glucose tolerance testing in patients with major depression. *American Journal of Psychiatry*, *145*(3), 325-330.

Wittmann, M., Dinich, J., Merrow, M., & Roenneberg, T. (2006). Social jetlag: misalignment of biological and social time. *Chronobiology International*, *23*(1-2), 497-509.

Wolf, N. J., & Hopko, D. R. (2008). Psychosocial and pharmacological interventions for depressed adults in primary care: a critical review. *Clinical Psychology Review*, *28*(1), 131-161.

Wolfson, A. R., Carskadon, M. A., Acebo, C., Seifer, R., Fallone, G., Labyak, S. E., & Martin, J. L. (2003). Evidence for the validity of a sleep habits survey for adolescents. *Sleep*, *26*(2), 213-216.

Wright Jr, K. P., Badia, P., & Wauquier, A. (1995). Topographical and temporal patterns of brain activity during the transition from wakefulness to sleep. *Sleep*, *18*(10), 880-889.

Wright, K. P., Hughes, R. J., Kronauer, R. E., Dijk, D.-J., & Czeisler, C. A. (2001). Intrinsic near-24-h pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. *Proceedings of the National Academy of Sciences*, *98*(24), 14027-14032.

Wulsin, A. C., Wick-Carlson, D., Packard, B. A., Morano, R., & Herman, J. P. (2016). Adolescent chronic stress causes hypothalamo–pituitary–adrenocortical hypo-responsiveness and depression-like behavior in adult female rats. *Psychoneuroendocrinology*, *65*, 109-117.

Yamada, N., Katsuura, G., Ochi, Y., Ebihara, K., Kusakabe, T., Hosoda, K., & Nakao, K. (2011). Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinology*, *152*(7), 2634-2643.

Yang, J., Ruchti, E., Petit, J.-M., Jourdain, P., Grenningloh, G., Allaman, I., & Magistretti, P. J. (2014). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proceedings of the National Academy of Sciences*, *111*(33), 12228-12233.

Yang, S.-R., Sun, H., Huang, Z.-L., Yao, M.-H., & Qu, W.-M. (2012). Repeated sleep restriction in adolescent rats altered sleep patterns and impaired spatial learning/memory ability. *Sleep*, *35*(6), 849-859.

Yellen, G. (2018). Fueling thought: Management of glycolysis and oxidative phosphorylation in neuronal metabolism. *Journal of Cell Biology*, *217*(7), 2235-2246. doi:10.1083/jcb.201803152

Zhang, J. Q., & Ji, L. L. (2016). Gender differences in peak blood lactate concentration and lactate removal. *Annals of Sports Medicine and Research*, *3*(7), 1088.

Zimmer, E. R., Parent, M. J., Souza, D. G., Leuzy, A., Lecrux, C., Kim, H.-I., . . . Rosa-Neto, P. (2017). [18 F] FDG PET signal is driven by astroglial glutamate transport. *Nature Neuroscience*, *20*(3), 393-395.