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Transcriptomic Prediction of Early-Life Stress on Antidepressant Treatment Outcomes and Sex Differences

Parel, Sero Toriano

Princeton, NJ : Princeton University

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TRANSCRIPTOMIC PREDICTION OF EARLY-LIFE STRESS ON ANTIDEPRESSANT TREATMENT OUTCOMES AND SEX DIFFERENCES

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A DISSERTATION
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Abstract

Early-life stress (ELS) is estimated to affect more than one billion children worldwide every year. ELS includes abuse and neglect, as well as poverty, environmental destruction, and war. ELS poses a major risk for an individual's future development of depression and other neuropsychiatric disorders, and this early experience may decrease the efficacy of antidepressant drug treatment. Importantly, women are twice as likely to be diagnosed with major depressive disorder compared to men, although less is known about the variation in the efficacy of antidepressant treatment by sex and gender. Understanding the link between ELS and antidepressant outcomes is therefore crucial for improving therapeutic interventions. Here I investigated the predictive role of ELS in altering the efficacy of different classes of antidepressants, focusing on the brain's nucleus accumbens and sex differences. To emphasize sex differences in ELS, I review in detail the current literature on the role of sex in moderating genome-wide transcriptional patterns in the brain. Further, I conducted translational studies in which I leveraged and performed computational analyses of cross-species genome-wide data from humans and mice treated with different classes of antidepressants. I describe the experiments I conducted of *in vivo* pharmacological approaches to understand how molecular changes in the brain following ELS may causally alter antidepressant response. Combining computational analyses and experimentation, I found that gene expression patterns in the nucleus accumbens—a brain center of reward and mood—correspond with transcriptional patterns of antidepressant treatment failure. Additionally, transcriptional patterns predicting treatment failure were strongest among female subjects. By profiling global gene expression changes in the brain that contribute to antidepressant treatment outcomes, this research aims to inform translational, clinical decision-making and enhance possible sex-specific efficacy of therapeutic interventions for individuals with depression and a history of ELS.

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Mga kapwang siyentista at iskolar ng bayan—bukod sa ating pag-aaral sa laboratoryo at paaralan, iunalad natin ang agham at teknolohiya sa obhektibong pagsuri ng lipunan, pagsulong ng pambansang demokrasya, at pagpapalaya ng sambayanan mula sa mga salot na inaapi at pinagsasamantalahan sa masa.

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Chapter 1: Introduction

Early-life stress (ELS) affects nearly one billion children across the world in a single year (UNICEF, 2020). In such forms as child abuse and neglect, ELS is well known to be a critical risk factor for anxiety, depression, addiction, and other neuropsychiatric disorders (Copeland et al., 2018; Jonson-Reid et al., 2012). A history of ELS may also affect future outcomes of the medical treatment of depression, such that first-line or commonly prescribed drugs may fail to mitigate symptoms for patients. Here, I propose to conduct research on the question: Does ELS predict future outcomes of therapeutic drugs? That is, will the later efficacy of antidepressant therapy be conditioned by ELS? The following original, main hypothesis was developed: ELS alters gene expression patterns in the brain that render non-response to standard antidepressant treatment in mice that are susceptible to adult stress. To test this central hypothesis, the following research objectives were developed for a two-pronged approach: (1) from a computational perspective, generation of predictions of how ELS may alter antidepressant treatment outcomes using existing gene expression datasets; and (2) from an experimental perspective, development of a behavioral paradigm that fully spans early life to post-antidepressant treatment in an animal model.

To address a significant gap in knowledge, I sought to understand how ELS alters brain development at the level of transcription and, in turn, how ELS influences susceptibility to future stress, depression-like behavior, and antidepressant treatment response (Parel et al., 2023). We implement behavioral and molecular approaches to the NAc, a nexus of the reward and mood circuitry. The central hypothesis of this proposal posits that ELS may alter transcriptional patterning in the NAc that, in turn, will increase non-response to standard antidepressant treatment in mice that are susceptible to adult stress. To test this hypothesis, I originally

presented the following specific aims that have the potential translational significance to identify gene expression signatures characteristic of ELS in patients with depression who may require non-traditional treatment to more directly target genome-wide changes that contribute to the lifelong impacts of childhood trauma:

In Aim 1, I determined whether transcriptional response to ELS in the NAc predicts antidepressant treatment response. To address the specific hypothesis that changes in gene expression patterns in the NAc are similar between the experience of ELS and non-response to antidepressant treatment, the research design leveraged three existing RNA-sequencing (RNA-seq) gene expression datasets and applied bioinformatic and computational analyses. The first RNA-seq dataset was from the NAc in a mouse model for ELS. In this model, ELS was found to increase lifelong risk for depression-like behavior, but only after additional stress in adulthood. The second RNA-seq dataset was from a study that examined imipramine and ketamine antidepressant treatment response or non-response in the NAc of mice susceptible to adult stress. Lastly, the third dataset was from peripheral blood monocytes of human patients with major depression assessed for antidepressant treatment response or non-response. The anticipated outcome was that the transcriptional patterns in the NAc of ELS mice in the first dataset would correspond to patterns of treatment non-response among mice in the second data set and humans in the third data set. Given current clinical evidence suggesting that depressed patients with a history of ELS may respond better to atypical treatment, I took advantage of the second dataset to test the prediction that ELS predicts favorable response to ketamine in a mouse model.

In Aim 2, I tested how ELS may contribute to antidepressant treatment non-response in behavior and in transcriptional patterns in the NAc. To address the specific hypothesis that mice

subjected to ELS will have a reduced behavioral response to a typical antidepressant treatment but more favorable behavioral response to atypical treatment, the experimental strategy applied basic pharmacological testing to a validated mouse model for ELS. First, randomly assigned ELS or standard reared mice that were susceptible to adult stress were treated with the traditional antidepressant escitalopram, the rapid-acting drug ketamine, or saline as a vehicle control. Further, I analyzed behavioral and transcriptional responses to assess the outcomes of the different treatments. The expected results were that ELS reduces response rates to the standard medication escitalopram, while ELS mice susceptible to adult stress exhibit improved response to the atypical antidepressant ketamine.

Overview of early-life stress

Early-life stress (ELS) and adversity refers to an expansive range of challenging stimuli and experiences that are faced between birth and adulthood. Between these landmarks in age, the brain undergoes immense developments at multiple levels: from the epigenetic and systems level of the brain, as well as to the behavioral levels—for both bottom-up and top-down processes. Development in the brain and behavior can be especially reactive to stressors, in the most general sense, any challenge posed to the body. Behavioral response to stress and adversity manifests in real time as well as shapes future stress responses. Stress and adversity during early life and their lifelong consequences are thus of particular concern from both scientific and societal perspectives.

Stress and adversity during childhood is far from uncommon. Globally, nearly one billion children between the ages of 2–17 years experience physical, sexual, or emotional violence or neglect annually every year, according to the World Health Organization in 2014 when the global population was 7.3 billion (Hillis et al., 2016; Scheuplein et al., 2023). The United Nations

reports that 300 million children between the ages of 2–4 are subjected to physical discipline from caregivers and the 1 in 3 students aged 13–15 who experience bullying (UNICEF, 2020). The United Nations International Children's Emergency Fund (UNICEF) reported that over 300,000 children were exposed to the devastating effects and aftermath of war and other armed conflict between 2005 and 2022 (Alhattab & English, 2022; English, 2023). Moreover, violence against children disproportionately affects girls: 15 million adolescent girls aged 15–19 years are coerced into sex in their lifetime (UNICEF, 2020). Further, girls may be more likely to be exposed to sexual abuse, whereas boys may be more likely to experience physical abuse (Danielsson et al., 2009; Eriksen et al., 2015; Lippus et al., 2020; Tourigny et al., 2008). The impact of ELS is uneven across communities and is exacerbated by recent and ongoing global issues such as war, climate change (Self-Brown et al., 2013), and the coronavirus COVID-19 pandemic (Bryant et al., 2020). In the United States, more than 60% of children are exposed to potentially traumatic events by the age of 16 years, and more than 30% are exposed to multiple traumatic experiences (Copeland et al., 2007; McLaughlin et al., 2013). Abuse and neglect disproportionately affects girls (8.7 per 1,000) than boys (7.5 per 1,000 boys) in the United States (U.S. Department of Health & Human Services, Administration for Children and Families, Administration on Children, Youth and Families, Children’s Bureau, 2023). Immigrant children are exposed to particular migration-related stress and trauma including family separation, detention, and deportation, which may render lifelong risk of developing neuropsychiatric disorders (Sidamon-Eristoff et al., 2022; Teicher, 2018; von Werthern et al., 2018).

Within the childhood stage, ELS is commonly associated with emotional and behavioral problems in children that manifest in family, school, and other social settings and relationships. Behavioral problems may further develop into behavior disorders including

attention-deficit/hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder (CD) (McLaughlin et al., 2013). In turn, these behavior disorders are associated with such behaviors as impulsivity and risk-taking into adolescence and later (Burke et al., 2002; Schaeffer et al., 2003). ELS is but one possible factor for these behavioral disorders, which also are also affected by genetic and developmental factors.

Whereas ELS may be framed in terms of stressor stimuli that are introduced to an individual, it is important to consider more recent, complementary perspectives that distinguish some forms of ELS as functions of deprivation or threat of stressor stimuli (McLaughlin et al., 2014, 2019; Sheridan & McLaughlin, 2014). In this framework, experiences of deprivation entail the absence of expected or enriching stimuli, whether environmental, social, or otherwise. Neglect and poverty are examples of deprivation. Experiences of threat involve actual or potential (threatening) death or physical harm. Abuse and other physical violence are examples of threat. Moreover, this perspective argues that a given experience of ELS can have, on one hand, high or low levels of deprivation; and, on the other hand, high or low levels of threat. In the brain, regions that have been shown to be involved in ELS (as threat and deprivation) are also in regions and circuits that are well known in fear conditioning and learning, e.g., amygdala, medial prefrontal cortex, and hippocampus in neuroimaging studies (McLaughlin et al., 2019). This framework complements and parallels a previous review focusing on defensive behavior and circuitry involved in ELS (Murthy & Gould, 2020).

Early-life stress and lifetime risk of depressive disorders

ELS is strongly linked to a lifetime risk of depression and other mood, anxiety, and substance use disorders (Chapman et al., 2004; McLaughlin et al., 2010; Scott et al., 2012). Abuse and neglect in particular can contribute to the development of depression, a devastating yet prevalent mood

disorder. Patients with depressive disorders often feel sad, empty, or irritable moods. A major depressive episode is marked by depressed mood or the loss of interest or pleasure in all or nearly all activities for most of the day nearly every day, which last at least 2 weeks (American Psychiatric Association, 2022). In the United States, depression affects approximately 7% of adults in a given year, and the lifetime rate of depression is approximately 17% of adults. Children who are exposed to multiple occurrences and different forms of stressors and adversity are particularly at risk for major depressive disorder (Chapman et al., 2004). Women are twice as likely to be diagnosed with depression than men, and transgender women in particular have a nearly 4-fold risk for depression (Hoffman, 2014; Pellicane & Ciesla, 2022; Witcomb et al., 2018). Other striking sex and gender differences are discussed in full in Chapter 2.

In these studies on humans and animal models of neurological and psychiatric disorders, ELS increases sensitivity to future stress that is demonstrated in both the brain and behavior. However, there is a lack of translational studies between human and animal models of ELS prior to depressive behavior and molecular changes in the brain. For example, although not in the setting of early life, adult chronic stress in mice and major depressive disorder (MDD) in humans have been shown to share similar patterns in gene expression changes (Scarpa et al., 2020). The few studies such as this example are valuable for providing precedence for our study linking ELS in mice and antidepressant outcomes in humans, demonstrating that transcriptional patterns can be reproducible between human and mouse studies. Our study on ELS in mice and antidepressant outcomes in humans, integrating behavior with transcriptomics, is described in Chapter 3.

Antidepressant treatment outcomes with a history of early-life stress

The successful or failed treatment outcomes for an individual may be related to a history of ELS, in addition to their already higher risk for developing depression (Bernet & Stein, 1999; Dube et al., 2005; Hammen et al., 2000; Kendler et al., 1995; Kessler et al., 1997; Nemeroff & Binder, 2014). While the link between ELS and antidepressant treatment outcomes continues to be studied, a slowly growing body of literature continues to reveal that a history of ELS predicts worse rates and course of depression, in addition to poorer outcomes after standard antidepressant treatment. For example, many patients with depression who experienced childhood trauma find that selective serotonin reuptake inhibitors (SSRIs, e.g., escitalopram and sertraline) and serotonin-norepinephrine reuptake inhibitors (SNRIs, e.g., desvenlafaxine) either do not improve their symptoms or have symptoms return at the same rate (Williams et al., 2016). Recent clinical studies demonstrate that such patients with both depression and a history of ELS may be more likely to respond to atypical antidepressant drugs such as ketamine (O'Brien et al., 2019, 2021).

The few existing studies on this connection, however, conflict or provide unsatisfying conclusions. In one clinical study, adolescents with MDD but without a history of ELS were shown to respond to the antidepressant drug fluoxetine. For adolescents with MDD and a history of physical abuse, no antidepressant treatment was found to be more effective than placebo; and for those with MDD and a history of sexual abuse, placebo was more effective than psychotherapy (Lewis et al., 2010). In another study, adults with MDD and a history of ELS responded better to psychotherapy than drug treatment (Nemeroff et al., 2003). In a meta-analysis, ELS predicted poor response to antidepressant drug treatment alone, as well as poor response even to the combination of drug treatment and psychotherapy (Nanni et al., 2012).

Much of depression research and antidepressant drug developments are predicated on the foundational hypothesis that the underlying basis of depression requires monoamines and their actions in the synapse, a hypothesis that was formulated as late as the 1960s (Bunney & Davis, 1965; Heninger et al., 1996; Schildkraut, 1995). The monoamine hypothesis has since driven much of drug development and research on mood as well as anxiety disorders. In our research in ELS discussed in Chapter 3, we investigated specifically monoamine-based pharmacotherapies with the SSRI escitalopram, the SNRI desvenlafaxine, and the tricyclic imipramine. Additionally, the rapid-acting drug ketamine is in a different class of antidepressants, as discussed below.

Escitalopram

Escitalopram is used principally in the treatment of major depressive disorders and secondarily of other neuropsychiatric disorders including generalized anxiety and obsessive-compulsive disorders (Bræstrup & Sanchez, 2004). Introduced in the early 2000s belonging to the selective serotonin reuptake inhibitor (SSRI) class of antidepressants, the mechanism of action of escitalopram entails blocking the reabsorption of serotonin, or 5-hydroxytryptamine (5-HT), in the synaptic cleft (Burke et al., 2002; Culpepper, 2002). Serotonin is implicated in many behaviors including stress, mood, fear, aggression, and addiction. Among SSRIs, escitalopram is the most selective in its mechanism of action. Escitalopram binds to and inactivates the serotonin transporter (SERT) of the presynaptic cell, and consequently serotonin levels and signaling are elevated in the synapse (Kasper et al., 2009; Landy et al., 2023). These effects mediated by escitalopram and other SSRIs on brain regions such as the nucleus accumbens affect behavior by regulating mood, mediating response to reward stimuli, and alleviating symptoms of depression.

As described in Chapter 3, I utilized escitalopram for antidepressant treatment in a mouse model for ELS and adult stress.

Desvenlafaxine

Desvenlafaxine is a medication used to treat major depressive disorder (Lourenco & Kennedy, 2009; Naseeruddin et al., 2023). Introduced in 2008, desvenlafaxine is classified as a serotonin-norepinephrine inhibitor (SNRI) and is also a monoamine-based drug similar to the SSRI class of antidepressants (Liebowitz et al., 2008). Norepinephrine is involved in stress, as well as attention and arousal, all of which are important for symptoms of depression. As an SNRI, desvenlafaxine's mechanism of action entails inhibiting the reuptake of serotonin and norepinephrine, with 10 times greater affinity for serotonin than norepinephrine (Sansone & Sansone, 2014). Desvenlafaxine has yet to be studied specifically in the nucleus accumbens.

Imipramine

Imipramine is used for treatment of depression as well as anxiety. Approved by the Food and Drug Administration (FDA) in the 1950s, imipramine is a relatively old drug treatment for depression. As a second-line antidepressant, imipramine is prescribed particularly for severe depression and other atypical features of depression. As a tertiary amine tricyclic antidepressant (TCA), imipramine blocks the reuptake of the neurotransmitters serotonin and norepinephrine, with a greater affinity for serotonin (Fayez & Gupta, 2023; Gillman, 2007). Consequently, serotonin and norepinephrine levels are increased in the synaptic cleft, mediating an antidepressant effect.

Ketamine

Introduced in the 1960s originally as an anesthetic agent, ketamine has been used as an antidepressant therapeutic more recently than the other drugs above (Coyle & Laws, 2015; Khorramzadeh & Lotfy, 1973; Krystal et al., 2019; Rosenbaum et al., 2023). Whereas SSRI, SNRI, and TCA drugs act on monoamine neurotransmitter-dependent processes, ketamine has a different mechanism in that it is a non-selective, non-competitive antagonist of N-methyl-D-aspartate (NMDA) and glutamate receptors (Moghaddam et al., 1997). At anesthetic doses, downstream effects include a dissociative anesthetic state, analgesia, and normal muscular, cardiovascular, and respiratory functions (Duman, 2018; Nichols & Paciullo, 2019). At subanesthetic doses as an antidepressant therapeutic, ketamine is rapid-acting and may provide relief to depressed patients within hours or days (Autry et al., 2011; Berman et al., 2000; Kim et al., 2023). In contrast, SSRIs, SNRIs, and TCAs may take weeks or months until patients with depression notice relief of their symptoms.

The brain in early-life stress and antidepressants: Significance of the nucleus accumbens

The nucleus accumbens (NAc) is a brain region that is importantly involved in mood and reward. Moreover, the NAc has been increasingly implicated in response to early-life and adult stress, depressive disorders, and antidepressant treatment response (Bagot et al., 2017; Hanson et al., 2021; Hodes et al., 2015; Ordoñes Sanchez et al., 2021; Peña, Smith, et al., 2019; Russo & Nestler, 2013). As part of the ventral striatum and larger limbic system, the NAc integrates multiple signals from the amygdala, hippocampus, prefrontal cortex, and the ventral tegmental area. From these signals, the NAc outputs regulation of behavioral response to positive and negative valence stimuli (Polter & Kauer, 2014; Russo & Nestler, 2013). The developing brain

during and beyond early life involves changes in gene expression and epigenetic regulation of transcription, and these molecular effects impact brain circuit formation, maturation, and function and mediate susceptibility or resilience to neuropsychiatric disorders throughout life.

The NAc has been increasingly implicated in response to early-life and adult stress, depressive disorders, and antidepressant treatment response (Bagot et al., 2017; Hanson et al., 2021; Hodes et al., 2015; Ordoñes Sanchez et al., 2021; Peña, Smith, et al., 2019; Russo & Nestler, 2013). ELS also heightens sensitivity of neurons in NAc to experience of future stress, which may contribute to lifelong stress hypersensitivity (Balouek et al., 2022). ELS alters gene expression and epigenetic regulation within NAc of both male and female mice, effects which endure into adulthood (Kronman et al., 2021; Peña, Smith, et al., 2019). The NAc and its activity can be blunted by ELS in humans, and ELS reduces associated responses to rewards, mediating the impact of ELS on depressive symptoms (Hanson et al., 2015).

However, it is not yet known whether enduring molecular changes in NAc following ELS are related to altered antidepressant treatment efficacy, and how these vary with sex and gender (Parel & Peña, 2022). Compared to other key brain regions such as prefrontal cortex and hippocampus, the NAc can generate transcriptional profiles that are highly associated with effective response or failure of antidepressant treatment with either the tricyclic imipramine or to ketamine (Bagot et al., 2017). Imipramine was shown to have a greater effect on transcriptional patterns in the nucleus accumbens, as well as the amygdala, compared to other regions (Bagot et al., 2017).With escitalopram treatment, the nucleus accumbens has been shown to recover decreased levels of dopamine associated with depression-like behavior (Furuse et al., 2019; Hovelsø et al., 2011; Minami et al., 2017; Shalaby & Kamal, 2009). At the transcriptional level

in the NAc, escitalopram can reverse gene expression changes associated with stress and susceptibility (Song et al., 2019).

In the broader circuitry of the NAc, ELS and consequent behavioral changes and disorders are also associated with regions such as the neuroendocrine-related hypothalamus-pituitary-adrenal (HPA) axis and the mood- and reward-related limbic system, including the hippocampus, prefrontal cortex, and amygdala. Imbalances that may occur in the reward circuit are strongly associated with mood and anxiety disorders (Hanson et al., 2021; Herzberg & Gunnar, 2020). Neuroimaging studies have found that ELS prior to depression is associated with morphological and circuit changes, such as decreased representation in the cortex of sensorimotor processes and reduced volume of the hippocampus (Heim et al., 2013; Smith, 2005; Vythilingam et al., 2002). At the molecular level, ELS and depression share similar gene expression changes such as those related to glucocorticoid receptor signaling, trophic factors, and neurotransmission in the HPA axis, hippocampus, and prefrontal cortex (Agorastos et al., 2019; Anacker et al., 2014).

The relation between ELS and antidepressant non-response, however, has yet to be studied directly in the brain. Of particular, the NAc of the brain is highly implicated in depression- and anxiety-like behaviors resulting from ELS in humans and non-human animal models alike. Lasting changes in transcriptional, or gene expression, patterns in the NAc of a mouse model have recently been observed to reprogram susceptibility to future stress after ELS, and further investigating these molecular-level changes may be important for understanding how ELS leads to altered antidepressant response. Moreover, understanding sex-related molecular profiles within NAc may also be key in linking a life-history of stress with antidepressant treatment response.

Previous publications and presentations

Chapter 2 is adapted from the review: Parel, S. T., & Peña, C. J. (2022). Genome-wide Signatures of Early-Life Stress: Influence of Sex. *Biological Psychiatry*, 91(1), 36–42. https://doi.org/10.1016/j.biopsych.2020.12.010.

Chapter 3 is adapted from the research article: Parel, S. T., Bennett, S. N., Cheng, C. J., Timmermans, O. C., Fiori, L. M., Turecki, G., & Peña, C. J. (2023). Transcriptional signatures of early-life stress and antidepressant treatment efficacy. *Proceedings of the National Academy of Sciences of the United States of America*, 120(49), e2305776120. https://doi.org/10.1073/pnas.2305776120. The authors contributed the following: S.T.P., S.N.B., and C.J.P. designed research; S.T.P., S.N.B., C.J.C., and C.J.P. performed research; S.T.P., C.J.C., O.C.T., and C.J.P. analyzed data; L.M.F. and G.T. contributed sequencing data; and S.T.P. and C.J.P. wrote the paper. Components of chapter 3 were presented at the following meetings:

- Parel ST, Turecki G, & Peña CJ (2022). Transcriptomic prediction of antidepressant treatment after early-life stress. Stress Neurobiology, University of South Carolina, Columbia, SC. Poster presentation.
- Parel ST, Bennett SN, Turecki G, & Peña CJ (2022). Transcriptomic prediction of antidepressant treatment after early-life stress. Society for Neuroscience, San Diego, CA. Poster presentation.
- Parel ST, Turecki G, & Peña CJ (2023). Transcriptomic networks of early-life stress and antidepressant treatment efficacy. Society for Neuroscience, Washington, DC. Abstract only.

Chapter 2: Genome-wide signatures of early-life stress

Chapter abstract

Here, we expand our review of emerging literature on genome-wide transcriptional and epigenetic signatures of ELS and the potential moderating influence of sex. The complexity of how ELS interacts with brain development and sex to impart risk for multifaceted neuropsychiatric disorders is unlikely to be understood by examining changes in single genes. We discuss evidence both that there are latent sex differences revealed by ELS and that ELS itself produces latent transcriptomic changes revealed by adult stress. In instances where there are broad similarities in global signatures of ELS among females and males, genes that contribute to these patterns are largely distinct based on sex. As this area of investigation grows, an effort should be made to better understand the sex-specific impact of ELS within the human brain, specific contributions of chromosomal versus hormonal sex, how ELS alters the time course of normal transcriptional development, and the cell-type specificity of transcriptomic and epigenomic changes in the brain. A better understanding of how ELS interacts with sex to alter transcriptomic and epigenomic signatures in the brain will inform individualized therapeutic strategies to prevent or ameliorate depression and other psychiatric disorders in this vulnerable population.

Introduction

Early life stress (ELS) contributes to an individual's lifetime risk of developing neuropsychiatric disorders, and these conditions differentially affect individuals by gender and assigned sex. In humans, non-human primates, and rodent models alike, ELS alters development of the brain

from the systems to the molecular levels. Developmental changes in gene expression and epigenetic regulation of transcription impact brain circuit formation, maturation, and function, and mediate susceptibility or resilience to neuropsychiatric disorders throughout life. Following the introduction of ELS and sex differences in the first chapter, here I provide a deeper review of the current literature at the intersection of ELS, sex differences, and transcriptomics.

There are well-documented contributions of sex (including genetic, hormonal, and gender aspects) to the experience of ELS and mental health outcomes following ELS. Female and transgender children (not mutually exclusive) have higher rates of adverse childhood experiences and abuse (Chapman et al., 2004; Giano et al., 2020; Giovanardi et al., 2018; Schnarrs et al., 2019), which in turn may influence rates of depression across the lifespan. While in childhood the incidence of depression is equal among self-identified girls and boys, after puberty the prevalence of major depressive disorder is nearly twice as high among self-identified women as men. Sex hormones likely play a mediating role for at least some of this disparity, as higher incidence of depression is observed throughout the reproductive period, and the risk for females peaks during periods associated with large hormonal changes, including during puberty, peripartum periods, and perimenopause (Steiner et al., 2003). Historically, however, studies of ELS and depression either included only male subjects or did not examine/report interactions of ELS experience with sex.

Here, we review the literature to date on genome-wide signatures of ELS and the potential moderating influence of sex. We focus on studies of early adversity in children or neonatal stress in animal models. Although adolescent stress is often considered distinct from ELS, we include discussion on two studies of sex differences in transcriptomic response to adolescent stress. For further discussion of genome-wide sex differences in adult depression, we

refer readers to current reviews and other articles in this special issue (Brivio et al., 2020). For a detailed discussion of sex differences in gene expression in the brain more broadly, we direct readers to a recent review by Gegenhuber and Tollkuhn (Gegenhuber & Tollkuhn, 2020).

Definitions of Sex and Strategies to Study Sex Differences in Transcriptomics

"Sex" refers to biological and physiological characteristics, including X/Y chromosome complement, gonadal anatomy, hormone levels and function, or a combination thereof. While sex is usually categorized as male or female, there can be variation in each of these characteristics. In the studies reviewed here, we presume that "sex" is self-identified in human subjects and is assigned according to external genitalia in animal models. "Gender," on the other hand, is a constructed perception of one's sex as a function of social and cultural expectations. Human studies that use the term "gender" often do so erroneously to mean sex, and model organisms do not have gender. This review specifically discusses transcriptomic differences related to sex, not gender.

It is useful to consider different categories of sex differences (Becker & Tollkuhn, 2018; McCarthy et al., 2012) that may be reflected within transcriptomic or epigenomic patterns (Figure 1). In most stress research, studies measure the same behavioral or transcriptional end points, which exist on a continuum, and quantitative sex differences are observed if female and male averages are different. For example, there are well-described sex differences in steroid hormone receptor expression in the brain (Gegenhuber & Tollkuhn, 2020). A trait may be sexually dimorphic if the end point has multiple forms that are largely distinct between females and males, such as with mating behaviors. In transcriptional studies, sexual dimorphism may

occur if females and males engage distinct networks of genes [as illustrated by (Walker et al., 2020)

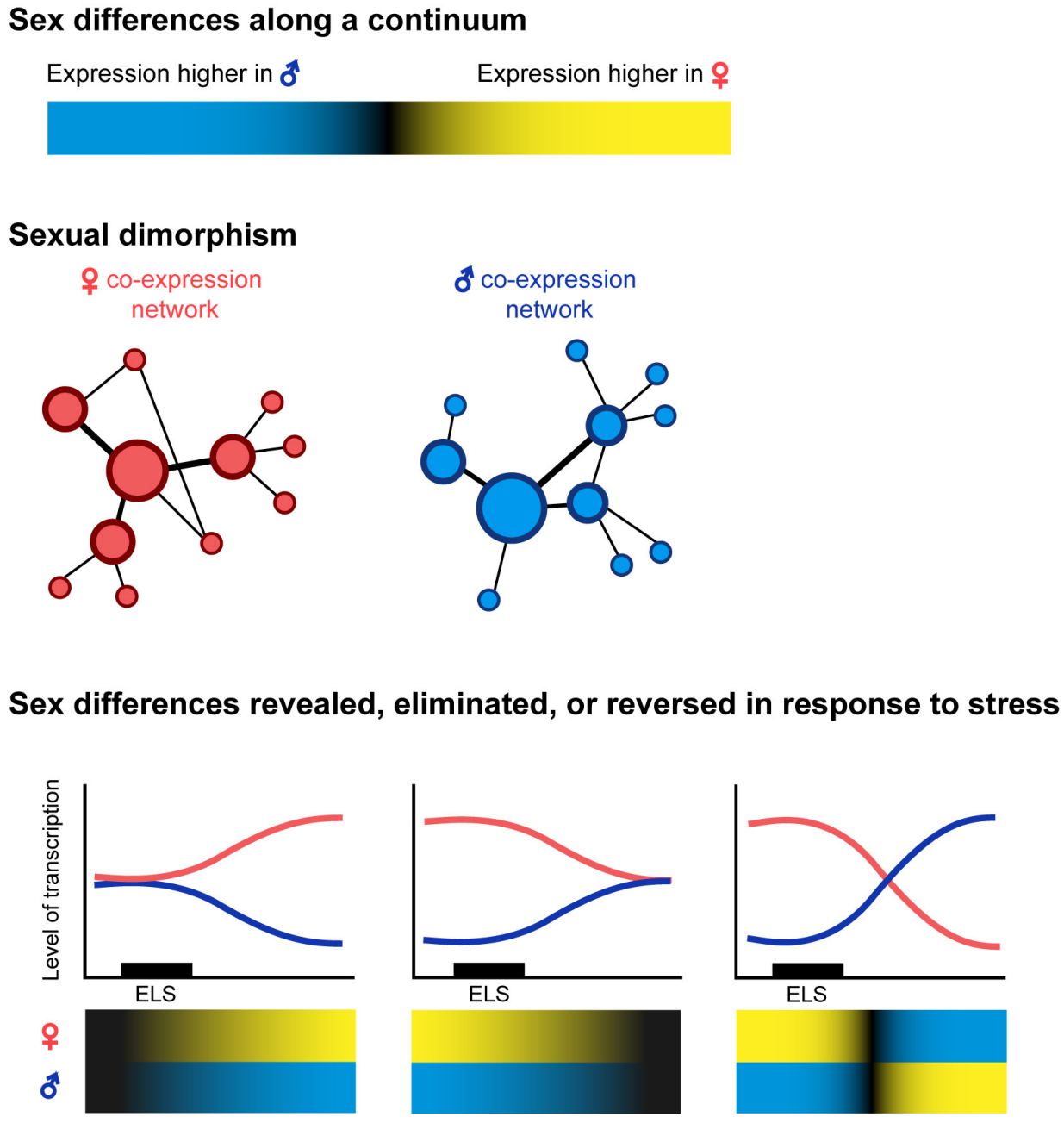


Figure 1. Example types of sex differences in transcriptomic patterns and potential response to ELS.

(Walker et al., 2020)]. It is worth noting that sex differences in expression or sexually dimorphic gene networks may serve to promote sex differences in behavior, or distinct transcriptional trajectories may converge on the same functional cellular or behavioral end point, an example of compensation (De Vries, 2004) or latent sex differences (Oberlander & Woolley, 2017). There may also be latent sex differences in which expression is similar at baseline, but undetected sex differences elsewhere in the system (e.g., in epigenetic regulation) cause expression to diverge in response to a challenge such as ELS. Alternatively, baseline sex differences may converge or reverse after stress or other challenges. For example, adolescent social isolation stress eliminates sex differences in some defensive behaviors and leads to feminization, masculinization, or sometimes a reversal of sex-specific gene expression in different groups of genes (Figure 1) (Walker et al., 2020).

Sex Differences in Neuropsychiatric and Behavioral Response to ELS

The moderating effect of sex on consequences of ELS in humans and animals has been comprehensively reviewed in a recent paper (White & Kaffman, 2019). Clinical research suggests that outcomes are dependent on the type (dimensions of threat vs. deprivation) and timing of ELS and the timing of data collection. Studies of Romanian children orphaned at birth found cognitive delays and behavioral and emotional problems in toddlers, but no sex differences (Smyke et al., 2007). Behavioral problems emerged more predominantly in boys by about 5 years, and increased sensitivity to internalizing disorders emerged among females by

adolescence (Humphreys et al., 2015). Despite sex-linked differences in depression and other neuropsychiatric disorders, only about half of properly powered clinical studies found sex differences in outcomes associated with ELS (White & Kaffman, 2019). Effects of ELS and sex in rodent studies are also mixed and may likewise depend on type and timing of ELS. Different studies using a limited bedding and nesting ELS paradigm have reported anxiety-like and depression-like behaviors and impaired cognitive behavior among both female and male rodents, although some studies directly examining sex differences report female-specific effects (Bath et al., 2017; Goodwill et al., 2019; Molet et al., 2014, 2016). The impact of ELS on rodent behavior may also emerge earlier in females but may last longer across the life span in males. Maternal separation stress in rodents is associated with increased anxiety-like behavior more consistently among male rodents (Demaestri et al., 2020; Romeo et al., 2003), although other studies found similar effects on anxiety-like behavior in females interacting with hormonal state (Jaric, Rocks, Cham, et al., 2019). Our research uses a combination of maternal separation and reduced nesting material in mice and finds that ELS by itself minimally impacts depression-like behavior but increases susceptibility to a second hit of stress in adulthood similarly among females and males (Peña et al., 2017; Peña, Nestler, et al., 2019; Peña, Smith, et al., 2019).

Genome-wide Signatures of Sex or ELS

Sex Differences in Transcriptomics Before Stress

Despite similarities in neuropsychiatric and behavioral outcomes, there are significant sex differences in the transcriptional control of neural mechanisms serving these behaviors even at baseline without stress. Some sex differences in transcription are hormonally linked and can be observed at different stages of the female menstrual cycle (women) or estrous cycle (rodents and other animals). The rodent estrous cycle is typically divided into 4 stages that can be determined

by vaginal cytology and that correspond to differing levels of circulating hormones: proestrus, estrus, metestrus, and diestrus. Estradiol levels are highest during proestrus in rodents (Becker & Tollkuhn, 2018). Hormonally linked differences in transcription were identified in the medial prefrontal cortex (PFC) of male mice and female mice in diestrus or proestrus. In fact, there were 20-fold more differentially expressed genes in PFC between proestrus and diestrus females than between females in either cycle state and males (Duclot & Kabbaj, 2015). Cycle state–dependent gene expression was related to neuronal signaling, cellular metabolism, and synaptic function, among other pathways. Four core genotypes mice can further be used to specifically distinguish between chromosomal and gonadal hormone effects of sex (McCarthy et al., 2012). In genetically modified four core genotypes mice, the testis-determining gene *Sry* has been moved to an autosome, which enables expression of *Sry* (and differentiation of the bipotential gonad into testes) independent from the Y chromosome and thus separates genetic (XX or XY) from gonadal (*Sry*+ with testes or *Sry*+ with ovaries) sex. While four core genotypes mice have not been used to study transcriptomic effects of ELS as of yet, baseline differences in transcription were recently described between chromosomally XX females and XY males in three key brain regions including the basolateral amygdala, PFC, and nucleus accumbens (NAc) (Breen et al., 2019). Genetic sex was found to drive approximately 25% of sex differences in gene expression, and gonadal sex (presence or absence of *Sry* and gonad-dependent developmental hormone exposure) between 23% and 31%, particularly genes involved in immune function (Breen et al., 2019). Activity-dependent immediate early genes were also driven by gonadal hormones and more highly expressed among mice with ovaries than testes. Moreover, chromatin organization is dynamically regulated by estrous cycle state in gonadal females (Jaric, Rocks, Greally, et al., 2019). Together, these studies indicate widespread basal influence of sex chromosomes and

hormones on the transcriptome even before stress experience. These basal differences need to be carefully considered when assessing the additional effect and interaction of ELS on the transcriptome.

Genome-wide Signatures of Human Child Abuse and Maltreatment

Several important human studies have examined the transcriptomic and epigenomic consequences of ELS and risk for depression within both brain and peripheral tissue. These studies have included female and male subjects but statistically controlled for sex rather than examined sex as a mediating factor. A history of child abuse is associated with altered DNA methylation at hundreds of CpG sites across all 23 chromosomes within postmortem hippocampus (Labonté et al., 2012), postmortem anterior cingulate cortex (Lutz et al., 2017), saliva of children (Kaufman et al., 2018; Yang et al., 2013), and blood of adults (Houtepen et al., 2016). Both hypo- and hypermethylation were detected in all tissue types, indicating specificity of changes rather than global gain or loss. Neuron-specific differential methylation in the hippocampus occurred at a cluster of genes involved in plasticity (Labonté et al., 2012). In the anterior cingulate, child abuse before age 14 most significantly altered DNA methylation and transcription of oligodendrocyte- and myelin-related genes associated with myelin thickness in this brain region (Lutz et al., 2017). Differential methylation sampled from peripheral tissues predicted depression and altered functional brain connectivity in maltreated children (Kaufman et al., 2018) and altered cortisol stress reactivity in adults with prior history of childhood trauma (Houtepen et al., 2016), advocating for the utility of peripheral methylome signatures as biomarkers. While it is important that these studies included both female and male subjects, future studies should be powered sufficiently to detect sex differences rather than controlling for sex as a covariate.

Putting It Together: Genome-wide Signatures of ELS and Sex

Sex Differences in the DNA Methylome in Response to ELS

Sex differences in peripheral T-cell 5mC DNA methylation patterns were examined in a nonhuman primate model of maternal deprivation (Massart et al., 2016). T-cell samples were taken across juvenile development to understand the development and persistence of peripheral DNA methylation changes. Sex-specific developmental changes in DNA methylation increased with age and were predicted to be downstream of the steroid hormone estradiol, although the majority of age-related DNA methylation changes were common between females and males. Maternal deprivation altered DNA methylation in opposite directions among females and males (latent sex differences), with the most pronounced sex-specific differences early in development (day 14), fading with age, and regulated by inflammatory pathways (Massart et al., 2016).

Sex Differences in the Transcriptomic Response to ELS

Few studies to date have compared the transcriptomic response to ELS in females and males, although more have included either males or females in different contexts (Bordner et al., 2011; Marrocco et al., 2019; Morrison et al., 2017, 2020). An important question for ELS studies is the time course through which molecular and behavioral changes emerge and either persist or are returned to control levels. One time-course study examined hypothalamic gene expression changes among female and male chickens after ELS (brief daily social isolation of chicks from postnatal days [P] 4 to 26) at both juvenile and adult time points using a microarray. Changes in transcription, hormones, and exploratory and anxiety-like behavior persisted into adulthood in males but not females (Elfwing et al., 2015). Gene ontology analysis of differentially expressed genes in male chickens revealed changes predominately in neuronal differentiation,

morphogenesis, and axon guidance, suggesting that ELS interacts directly with developmental rather than steady-state processes in the hypothalamus.

We examined the long-lasting transcriptomic response to ELS (a combination of maternal separation and limited nesting material from P10 to P17) in adult female and male mice, across three different brain regions [ventral tegmental area (VTA), NAc, and PFC (Peña et al., 2017; Peña, Smith, et al., 2019)]. Our study sought to understand how ELS might alter lasting transcriptomic patterns both on its own and in response to a second stressor in adulthood. However, it is important to note that direct comparisons between female and male mice were not made owing to differences in cohort and type of adult stress between female and male mice. In this study, there was not a significant relationship between female cycle state and behavior, and tissue was taken when most female mice were in diestrus (Peña, Smith, et al., 2019).

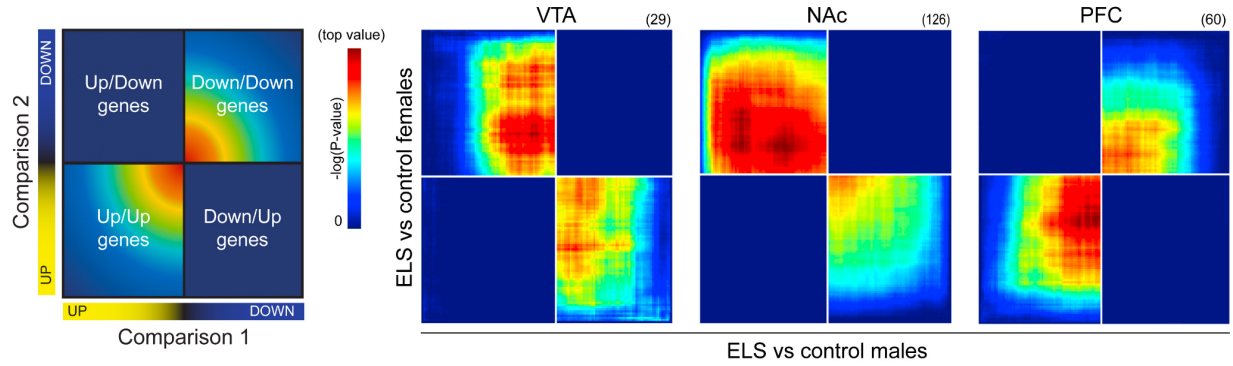


Figure 2. Opposite transcriptomic signatures of ELS in male and female mouse brains

New rank-rank hypergeometric overlap analyses of genome-wide expression data from (Peña, Smith, et al., 2019) indicate opposite signatures of ELS in male and female NAc and, to a lesser extent, VTA, while signatures of ELS in the PFC are similar among males and females. Analysis methods were identical to those previously reported (Peña, Smith, et al., 2019). Pixels represent the overlap between the transcriptome of each comparison as noted, with the significance of overlap (−log10(p-value) of a hypergeometric test) color coded. Maximum significance of overlap was allowed to be different in each brain region and is noted in parentheses at the top right of each image. A two-sided version of rank-rank hypergeometric overlap was used to test for coincident and opposite enrichment such that the lower left quadrants include co-upregulated genes, upper right quadrants include co-downregulated genes, and upper left and lower right quadrants include oppositely regulated genes (up-down and

down-up, respectively). Genes along each axis are sorted from most to least significantly regulated from the middle to outer corners. ELS, early-life stress; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.

Several broad patterns emerged from this transcriptomic analysis. First, new threshold-free rank-rank hypergeometric overlap analyses of these datasets (Gene Expression Omnibus accession GSE89692) reveal opposite genome-wide signatures of response to ELS among females and males in VTA and NAc but similar responses to ELS in PFC (Figure 2). This is consistent with the opposing transcriptional signatures of depression previously identified in postmortem brain tissue from women and men (Labonté et al., 2017; Seney et al., 2018) and in response to adolescent stress in rats (Rowson et al., 2019), confirming divergent responses to stress across the life span. This rank-rank hypergeometric overlap analysis does not, however, reflect whether there are baseline sex differences in expression or whether different networks of genes are used to reach the same cellular end point. Second, when rank-rank hypergeometric overlap analyses were performed within each sex separately, ELS alone (before depression-like behavior) still programmed depression-like gene expression patterns similar to those from adult stressed mice that did show behavioral changes (Peña, Smith, et al., 2019). This depression-like pattern was observed in each brain region similarly among females and males. Together, these studies consistently show that stress across the life span (ELS, adolescent stress, adult stress, and depression) induces latent, opposing transcriptomic signatures in females and males but that within a sex, response to stress is similar at each age. Finally, exposure to a second stress in adulthood did not simply exaggerate expression changes induced by ELS or adult stress alone but induced a modified transcriptional response. In other words, in both males and females, ELS induces latent transcriptomic changes—possibly through changes in the epigenome—that are later revealed by adult stress.

A final pattern to emerge was that the combination of ELS and adult stress enriches gene expression changes in the PFC associated with critical period plasticity. Plasticity-associated genes were also enriched among differentially methylated genes in the human hippocampus following child abuse (Labonté et al., 2012). In mice, transcriptional signatures of plasticity were enriched among female mice and depleted among male mice after ELS. This suggests that females and males arrive at similar behavioral stress sensitivity through divergent transcriptional/plasticity mechanisms. Given the timing of ELS during a sensitive window (Peña et al., 2017), it is possible that ELS interferes with natural developmental plasticity to either inappropriately preserve plasticity beyond the normal window and render mice hyperreactive to future stimuli, or prematurely or otherwise inappropriately reduce plasticity such that mice cannot actively cope with future stress.

Although some broad patterns (such as induction of a depression-like transcriptomic state and latent response to adult stress) were similar among female and male mice, the genes contributing to and regulating these transcriptional states were largely sex specific. These differences may reflect different baseline gene expression levels, as demonstrated in other studies (Breen et al., 2019; Hodes et al., 2015), or sex-specific gene network correlations. Nevertheless, a male-specific predicted upstream regulator in VTA from this preclinical mouse model of ELS (the transcription factor OTX2) was ultimately useful to predict clinical outcomes following ELS in children. Genome-wide analysis of salivary DNA methylation from female and male children aged 8–15 years found that child maltreatment and OTX2 methylation predicted depression in the children, and increased OTX2 methylation was associated with altered functional connectivity within the brain (Kaufman et al., 2018). This demonstrates the translational

potential of preclinical animal models to understanding molecular correlates of child maltreatment in humans.

Sex Differences in Transcriptomic Response to Adolescent Stress

Adolescence is marked by a surge of gonadal steroid hormones, heightened plasticity in some brain regions, increasing independence, and changes in motivated behavior (Walker et al., 2017). While adolescence precedes adulthood, it is considered separately here from animal models of ELS earlier in the neonatal/juvenile period (although human studies typically lump stress occurring before age 18).

Similar to ELS and adult depression, adolescent stress from P38 to P49 (mixed social defeat and restraint) in rats led to opposing transcriptional signatures between females and males in the hippocampus. Adolescent stress likewise modified the transcriptional response to adult stress, similar to ELS in mice, indicating latent regulatory mechanisms (Rowson et al., 2019). Adolescent social isolation from P22 to P42 also diminished baseline sex differences in transcription in the medial amygdala (a sexually dimorphic brain region) such that a large portion of sexually dimorphic genes were feminized, masculinized, or reversed (Walker et al., 2020). Unfortunately, similar analyses focusing on sexually dimorphic gene expression response to stress have not yet been applied to datasets of neonatal ELS or adult stress, so it is not yet known whether stress at different ages can similarly disrupt basal sex differences or whether this effect is specific to stress occurring during the onset of puberty.

Common to both ELS- and adolescent stress–induced transcriptomic changes is a regulatory role for hormone-responsive transcription factors. The top predicted upstream regulator of adolescent stress response in the female and male hippocampus was estrogen receptor alpha (ESR1) (Rowson et al., 2019), which was also the only predicted upstream

regulator common to females and males after ELS in NAc (Peña, Smith, et al., 2019). In the amygdala, a key driver of sex-specific transcriptomic response to adolescent stress is CRYM, a thyroid hormone–binding protein (Walker et al., 2020). Both ESR1 and CRYM are transcription factors simultaneously responsive to hormonal environment and capable of directly regulating transcription of thousands of genes. Finally, these studies show that manipulation of sex-specific predicted transcriptional regulators leads to sex-specific functional impact on behavior. Overexpression of Crym in the amygdala led to distinct transcriptional alterations in females versus males and abolished sex differences in exploratory and anxiety-like behavior (Walker et al., 2020). This demonstrates the predictive validity of bioinformatic analyses of complex genome-wide data.

Conclusions

Sex and ELS interact to impact transcription across the genome. There are latent sex differences revealed by ELS, and ELS itself produces latent transcriptomic changes revealed by adult stress. In instances where there are broad similarities in global signatures of ELS among females and males, the individual genes differentially regulated by ELS, and the predicted upstream regulators of these genes, are largely non-overlapping. These important transcriptional and epigenetic patterns—above and beyond individual gene changes—could only be revealed through sequencing, highlighting the unique power of such genome-wide approaches.

Sex differences in response to ELS have major implications for response to neuropsychiatric disorder treatments. Posttraumatic stress disorder treatment response is predicted by an interaction between history of child abuse and DNA methylation profiles specifically in female patients (Pape et al., 2018). Antidepressant treatment response or nonresponse is also influenced by gonadal hormones (LeGates et al., 2019). Rich genome-wide

datasets of sex-specific response to ELS may be mapped to drug signatures available in different cell lines and tissues to identify novel and potentially sex-specific treatments for this vulnerable population (Glicksberg et al., 2019). Additional human studies, a focus on hormonal contributions to sex differences, and increased cellular specificity of transcriptomic and epigenomic changes across development will lead to a better understanding of how ELS interacts with sex to increase risk for depression and other neuropsychiatric disorders as well as facilitate development of individualized treatment.

Chapter 3: Transcriptome-wide signatures of early-life stress and antidepressant treatment efficacy

Chapter abstract

Individuals with a history of early-life stress tend to have altered course of depression and lower treatment response rates. Research suggests that early-life stress alters brain development, but the molecular changes in the brain following early-life stress that may mediate altered antidepressant response have not been systematically studied. Sex and gender also impact risk of depression and treatment response. Here, we leveraged existing RNA-sequencing datasets from: 1) blood samples from depressed female- and male-identifying patients treated with escitalopram or desvenlafaxine and assessed for treatment response or failure; 2) the nucleus accumbens (NAc) of female and male mice exposed to early-life stress with or without adult stress; and 3) the NAc of mice after adult stress, antidepressant treatment with imipramine or ketamine, and assessed for treatment response or failure. We find that transcriptomic signatures of adult stress after a history of early-life stress correspond with transcriptomic signatures of treatment non-response, across species and multiple classes of antidepressants. Transcriptomic correspondence with treatment outcome was stronger among females and weaker among males. We next pharmacologically tested these predictions in our mouse model of early-life and adult social defeat stress and treatment with either chronic escitalopram or acute ketamine. Among female mice, the strongest predictor of behavior was an interaction between early-life stress and ketamine treatment. Among males, however, early experience and treatment were poor predictors of behavior, mirroring our bioinformatic predictions. These studies provide neurobiological evidence for

molecular adaptations in the brain related to sex and early-life stress that contribute to antidepressant treatment response (Parel et al., 2023).

Introduction

Early-life stress (ELS) increases sensitivity to stress (Peña et al., 2017) and risk for developing major depression and other mood and drug disorders by 2 to 4-fold (Jonson-Reid et al., 2012). Stress early in life may alter developmental brain trajectories, and not just steady-state processes, which has the potential to augment the long-term impact of stress. Depressed patients with a history of ELS have worse forms of depression and may constitute a unique subtype of depression. Childhood adversity predicts earlier onset of depression, more frequent episodes, and increased recurrence of depression (Bernet & Stein, 1999). Moreover, while fewer than 50% of depressive patients achieve full remission, efficacy of treatment with traditional antidepressant medications is even lower among those with a history of early life stress (Nanni et al., 2012). Interestingly, some research has suggested that depressed patients with a history of ELS may achieve a more efficacious response to sub-anesthetic ketamine treatment—increasingly used as a rapid-acting antidepressant—compared to traditional monoamine-targeting antidepressants (O'Brien et al., 2019). However, the ways in which ELS alters brain development to change the risk and course of depression and response to treatment are poorly understood.

In the brain, the nucleus accumbens is involved in stress, mood disorders, and, although less understood, antidepressant treatment outcomes. Given that the NAc integrates expansive and diverse signaling from the amygdala, hippocampus, prefrontal cortex, and the ventral tegmental area, the disruption of these systems can strongly affect the development and course of mood and anxiety disorders (Bagot et al., 2017; Hanson et al., 2021). ELS blunts development of the ventral striatum, a structure that contains the NAc, in humans wherein ELS reduces responses to

rewarding stimuli, in turn mediating the impact of ELS on depressive symptoms (Hanson et al., 2015). ELS also heightens sensitivity of NAc neurons in the experience of future stress, and this priming may contribute to lifelong stress hypersensitivity (20). On the molecular scale, ELS alters gene expression and epigenetic regulation within NAc of both male and female mice, effects which endure into adulthood (Kronman et al., 2021; Peña, Smith, et al., 2019). Moreover, transcriptional profiles of the NAc—more so than other key brain regions such as prefrontal cortex and hippocampus—are highly associated with effective response or failure of antidepressant treatment with either the tricyclic imipramine or to ketamine (Bagot et al., 2017). However, it is not yet known whether enduring molecular changes in the NAc following ELS are related to altered antidepressant treatment efficacy, and how these changes vary with sex and gender (Parel & Peña, 2022). Understanding sex-related molecular profiles within NAc may therefore be key in linking a life-history of stress with antidepressant treatment response.

We hypothesized that ELS alters the NAc at a molecular level, which contributes to reduced antidepressant treatment efficacy. Recent studies have examined genome-wide transcriptional changes associated with ELS in mice, and with antidepressant treatment response or failure in humans and mice across a variety of antidepressant classes including tricyclics, serotonin and norepinephrine reuptake inhibitors, and ketamine (Bagot et al., 2017; Fuh et al., 2023; Peña et al., 2017; Peña, Smith, et al., 2019). In lieu of any human studies that have directly examined both antidepressant treatment efficacy and history of adversity, we leveraged the combination of these datasets to test whether transcriptomic patterns associated with ELS correspond with those associated with antidepressant response or non-response, across species and sexes. We then tested our predictions in vivo in our mouse model of ELS among both assigned-male and assigned-female mice treated with either ketmaine or escitalopram. Our

highly translational approach integrates both bioinformatic comparisons and behavioral pharmacology to show that female sex, lifecourse stress, and type of antidepressant treatment are the strongest predictors of behavior.

Materials and methods

Transcriptomic datasets and analysis

Three independent RNA-seq datasets (see Supporting Information: Methods) were analyzed for predicting the effects of early-life stress (ELS) on antidepressant response. The first dataset includes the nucleus accumbens (NAc) of female- and male-assigned mice that experienced ELS with or without adult stress (AS) in a 2×2 design (Peña et al., 2017; Peña, Smith, et al., 2019). The second dataset includes the NAc of adult, male-assigned mice that experienced chronic social defeat stress and were subsequently categorized as stress-naive, resilient, susceptible, and responsive/non-responsive to either of the antidepressants imipramine or ketamine (Bagot et al., 2017). The primary comparisons were between imipramine/ketamine non-responders v. responders. The third dataset (see Fig. 1A) includes blood samples from healthy controls and patients with major depressive disorder (MDD) before and after antidepressant treatment with escitalopram/desvenlafaxine. The primary comparisons were between non-responders v. responders to antidepressant treatment. RNA-seq analyses for these datasets utilized the R packages `DESeq2`, `removeBatchEffect`, and `limma` (Love et al., 2014).

RRHO analysis compared gene expression datasets in a threshold-free manner, detailed in the Supporting Information: Methods (Cahill et al., 2018; Plaisier et al., 2010). Two-sided RRHO assessed coincident and opposite enrichment (Cahill et al., 2018). Genes were ranked by -log10(p-value) multiplied by fold change sign. Genes expressed (>2 base mean) in both lists

were included for human-mouse comparisons. RRHO difference maps of pixels were calculated with respect to Z-scores converted to p-values represented by pixels. Gene ontology analysis utilized `clusterProfiler`, `org.Hs.eg.db`, and `org.Mm.eg.db` packages (Wu et al., 2021; Yu et al., 2012).

Mice

Subjects were C57BL/6J mice, bred and reared in-house (Supporting Information: Methods). Pups were assessed for sex at birth and assigned as “female” or “male” at weaning by their external genitalia on postnatal day P21. All experiments with animals were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Princeton University.

Early-life and adult stress paradigms

Litters were randomly assigned to early-life stress (ELS) or standard-rearing (Std) conditions. ELS litters had limited nesting from P10-17 and pups were separated from their home cages for 3-4 h/day at random times each day (Peña et al., 2017; Peña, Nestler, et al., 2019; Peña, Smith, et al., 2019). Adult stress (AS) involved a chronic non-discriminatory social defeat stress paradigm effective for both male and female mice (Dieterich et al., 2021; Yohn et al., 2019). Experimental female- and male-assigned mice were simultaneously exposed to attacks from a novel aggressor Swiss Webster mouse daily for 10 consecutive days. Mice then underwent behavioral testing for three consecutive days before tissue collection. More details in Supporting Information: Methods.

Behavioral testing

Mice underwent behavioral testing beginning the day after social defeat, and again after antidepressant treatment (Fig. 3A). For each round of testing, one behavioral test was conducted per day: social interaction (SI), open field, and novelty-suppressed feeding (NSF) tests, fully detailed in the Supporting Information: Methods. We tested male mice in the social interaction test and female mice primarily in the novelty-suppressed feeding test. All mice were tested on the open field test.

Antidepressant treatment

Mice susceptible to social defeat stress were randomly assigned to saline, escitalopram, or ketamine treatment after the first round of behavioral testing (Supporting Information: Methods). Saline-treated mice received 0.1 mL sterile normal saline (i.p.) daily for 21 days, and escitalopram-treated mice received escitalopram (10 mg/kg, i.p.) for 21 days. The ketamine group received 0.1 mL sterile normal saline (i.p.) daily for 20 days, followed by a single dose of ketamine (10 mg/kg, i.p.) on day 21.

Statistical analyses of behavior

A two-way ANOVA was used to determine main effects and interactions. A behavioral difference score was calculated for within-animal change in behavior with treatment. Differences in latency to eat in the NSF test were calculated by Log-rank Mantel-Cox survival analysis. Fixed effects models were generated to examine the effects of ELS, treatment, and their interactions on latency to eat in the NSF test for females and SI ratio for males. Further details of statistical analyses are in the Supporting Information: Methods.

Results

Similarities in transcriptional patterns between ELS in mice and antidepressant treatment non-response in human MDD patients.

We first sought to test whether gene expression patterns—or "signatures"— in the brain resulting from ELS with or without adult stress (AS) corresponded to gene expression changes associated with treatment efficacy among human MDD patients. To test this, we leveraged two existing bulk RNA-sequencing datasets. The first dataset from mouse NAc included assigned-female and assigned-male tissue after ELS alone, adult stress (AS) alone, or ELS+AS which was associated with the greatest rates of depression-like behavior (Peña et al., 2017; Peña, Smith, et al., 2019). ELS consisted of daily maternal separation and limited nesting from P10-17; AS consisted of chronic social defeat stress or sub-threshold variable stress for male and female mice, respectively. The second dataset included white blood cell-derived RNA-seq profiles from self-identified female and male MDD patients both before and after randomly-assigned treatment with either escitalopram or desvenlafaxine for eight weeks, and assessment for treatment efficacy (Fig. 1A) (Fuh et al., 2023). Escitalopram (brand-name Lexapro) is a serotonin-selective reuptake inhibitor; desvenlafaxine (brand-name Pristiq) is a serotonin-norepinephrine reuptake inhibitor. Data across both antidepressant treatments was combined for these analyses. Only genes common to both humans and mice and expressed in both tissues were kept. To assess similarities between these datasets across the entire transcriptome in a threshold-free manner, we used rank-rank hypergeometric overlap analysis (Fig. 1B). To evaluate potential sex differences, and because we found opposite patterns of gene expression response to antidepressant treatment and stress across sexes [Fig. S1A-B; (Hodes et al., 2015; Labonté et al., 2017; Seney et al., 2018)], we analyzed female and male datasets separately.

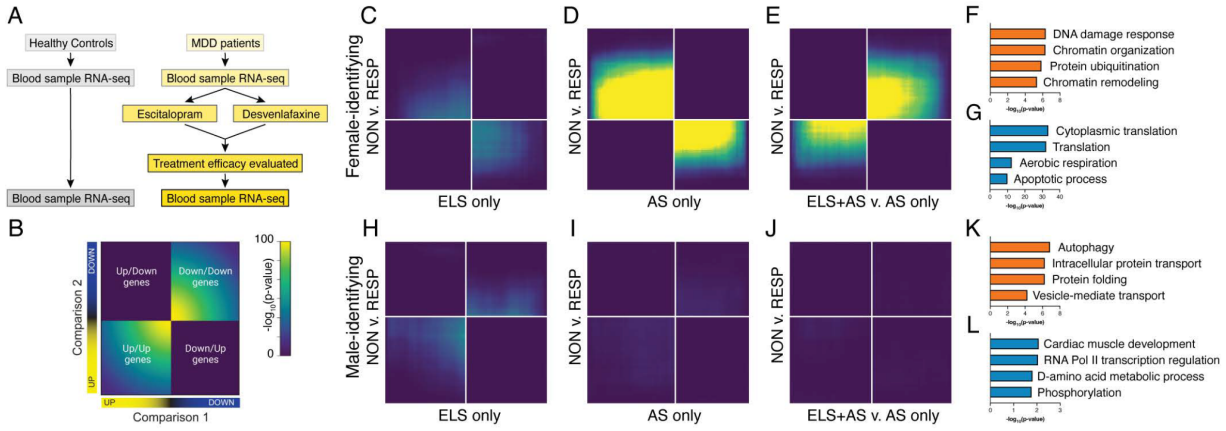


Figure 1. Comparing transcriptomic patterns of ELS or adult stress in mice with antidepressant treatment efficacy in humans

(A) Study design for white blood cell (WBC)-derived RNA-seq from healthy control subjects or patients with major depressive disorder (MDD), taken before and after antidepressant treatment with either escitalopram or desvenlafaxine and assessed for treatment response or non-response.

(B) Key for threshold-free comparisons of differentially expressed genes (DEGs) by two-sided rank-rank hypergeometric overlap (RRHO) analysis: genes may be co-regulated or oppositely regulated between two comparisons. Pixels represent the overlap between DEGs in each comparison. The significance of overlap (-log10(p-value)) of a hypergeometric test is color-coded, with a fixed maximum of 100 across all comparisons (C-E, H-J). Genes along each axis are sorted from most to least significantly regulated from the middle to outer corners.

(C-E) RRHO's comparing DEGs related to treatment efficacy from female WBC with DEGs related to ELS with or without AS from female mouse NAc.

Gene ontology analysis of genes co-upregulated (orange, F) or co-downregulated (teal, G) from E.

(H-J) RRHO's comparing DEGs related to treatment efficacy from male WBC with DEGs related to ELS with or without AS from male mouse NAc. Gene ontology analysis of genes co-upregulated (orange, K) or co-downregulated (teal, L) from J.

ELS-only and AS-only DEGs are vs standard-reared control; ELS+AS DEGs are v. AS-alone to assess the difference in gene expression after AS with vs without prior ELS.

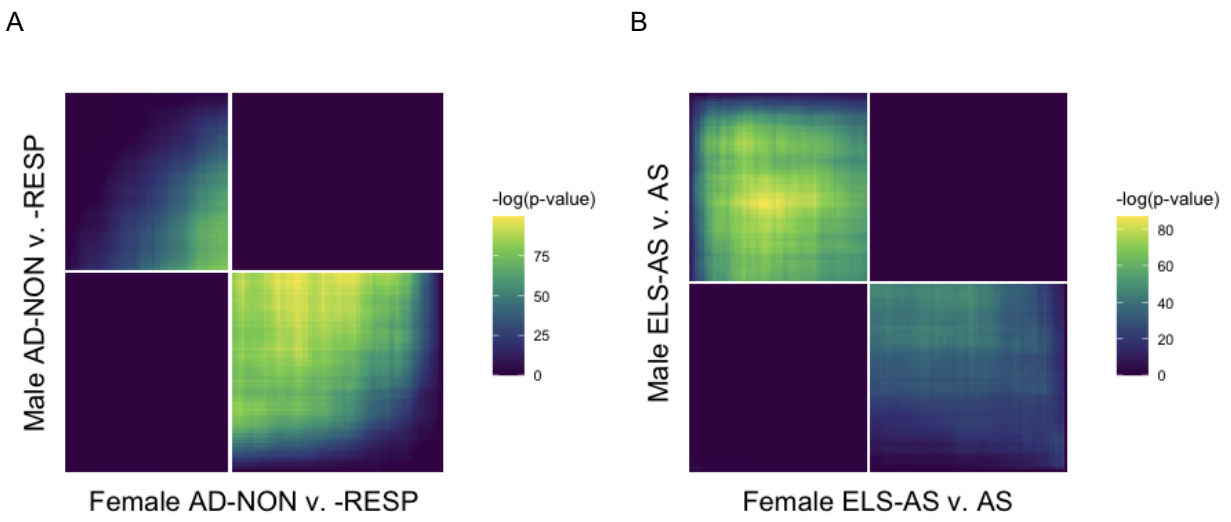


Figure S1. Sex differences in transcriptomic response to treatment or stress

Threshold-free RRHO analysis compares gene expression changes across two comparisons. Lower left quadrant includes co-upregulated genes; upper right quadrant includes co-downregulated genes, and upper left and lower right quadrants include oppositely regulated genes (up-down and down-up, respectively). Genes along each axis are sorted from most to least significantly regulated from the middle to outer corners. Pixels represent the overlap between DEGs in each comparison, with the significance of overlap (-log10(p-value)) of a hypergeometric test color-coded as indicated by the key for each RRHO map.

(A) Gene expression patterns from white blood cells of MDD patients who were non-responsive vs responsive to antidepressant treatment are opposite between female-identifying and male-identifying patients.

(B) Gene expression patterns from NAc of mice exposed to ELS+AS vs only-AS are opposite between assigned-females and assigned-males.

It is important to note, however, that mouse NAc RNA-seq data used in B was generated from different cohorts, and males and females experienced different adult stressors, making it impossible to distinguish between sex and cohort effects.

Despite differences in species and tissue type, we found correspondence among transcriptional signatures of treatment response that varied by sex and lifecourse history of stress. Among females, gene expression patterns in NAc after ELS alone (Fig. 1C) and AS alone (Fig. 1D) were most similar to gene expression patterns in blood of antidepressant responders, while

gene expression patterns following ELS+AS were most similar to antidepressant non-responders (Fig. 1E). Genes co-upregulated by ELS+AS and antidepressant non-response among females were enriched for functions related to responses to DNA damage, chromatin organization, and protein ubiquitination (Fig. 1F), while genes co-downregulated were enriched for functions related to translation and apoptosis (Fig. 1G). Among males, gene expression patterns in NAc after ELS alone (Fig. 1H) and AS alone (Fig. 1I) were weakly similar to gene expression patterns in blood of antidepressant non-responders, while gene expression patterns following ELS+AS were not strongly associated with response to antidepressant treatment in either direction (Fig. 1J). Nevertheless, genes co-upregulated by ELS+AS and antidepressant non-response among males were enriched for functions related to autophagy, intracellular protein transport, and protein folding (Fig. 1K-L).

Similarities in transcriptional patterns between ELS and antidepressant treatment non-response in mice.

Grounded in these patient-relevant data, we next sought to determine whether gene expression signatures of stress across the lifespan correspond with antidepressant treatment response or non-response directly within mouse NAc in order to validate and extend the previous conclusions within a single tissue type. As above, we hypothesized that transcriptomic signatures of ELS would be most similar to those of treatment non-response. To test this, we integrated the RNA-seq data from ELS with or without AS in the NAc used above with previously published RNA-seq from male NAc after AS (chronic social defeat stress) and treatment of susceptible mice with either chronic imipramine (brand-name Tofranil) or acute sub-anesthetic ketamine and behavioral categorization of non-response or response to treatment (Fig. 2A-J) (Bagot et al., 2017). Across these mouse comparisons, the strongest similarities were between female NAc and

response to imipramine: both ELS alone (Fig. 2F) and AS alone (Fig. 2G) had transcriptomic patterns most similar to imipramine responders, while ELS+AS in female mice was most similar to imipramine non-responders (Fig. 2H). Similarly, ELS+AS among female mice was most similar to transcriptional signatures of non-response to ketamine (Fig. 2C), while AS alone weakly corresponded with positive ketamine treatment response (Fig. 2B), and ELS alone was not strongly associated with response to ketamine treatment in either direction (Fig. 2A). Put another way, for both ketamine and imipramine, the difference between experience of AS with prior ELS versus AS without prior ELS predicts the difference between treatment non-response versus response. Among male mice, only the transcriptional signature of the combination of ELS+AS was associated with positive imipramine response (Fig. 2P-R). Neither ELS nor AS were strongly associated with response to ketamine treatment in either direction (Fig. 2K-M). We next applied gene-ontology analysis to investigate whether genes co-upregulated and co-downregulated by ELS+AS and treatment were enriched for specific functions. Genes co-upregulated by ELS+AS and ketamine non-response within female NAc were enriched for protein transport, DNA repair, mRNA processing, and DNA damage response (Fig. 2D). Genes co-upregulated by ELS+AS and imipramine non-response were enriched for chromatin organization, protein transport, phosphorylation, and DNA damage response (Fig. 2I). Genes downregulated by ELS+AS and upregulated by ketamine response within male NAc were enriched for transcriptional regulation, semaphorin-complex signaling, intracellular signaling, and chloride transport (Fig. 2N). Finally, genes downregulated by ELS+AS and upregulated by imipramine response were enriched for transcriptional regulation, phosphorylation, intracellular signaling, and chromatin organization (Fig. 2S).

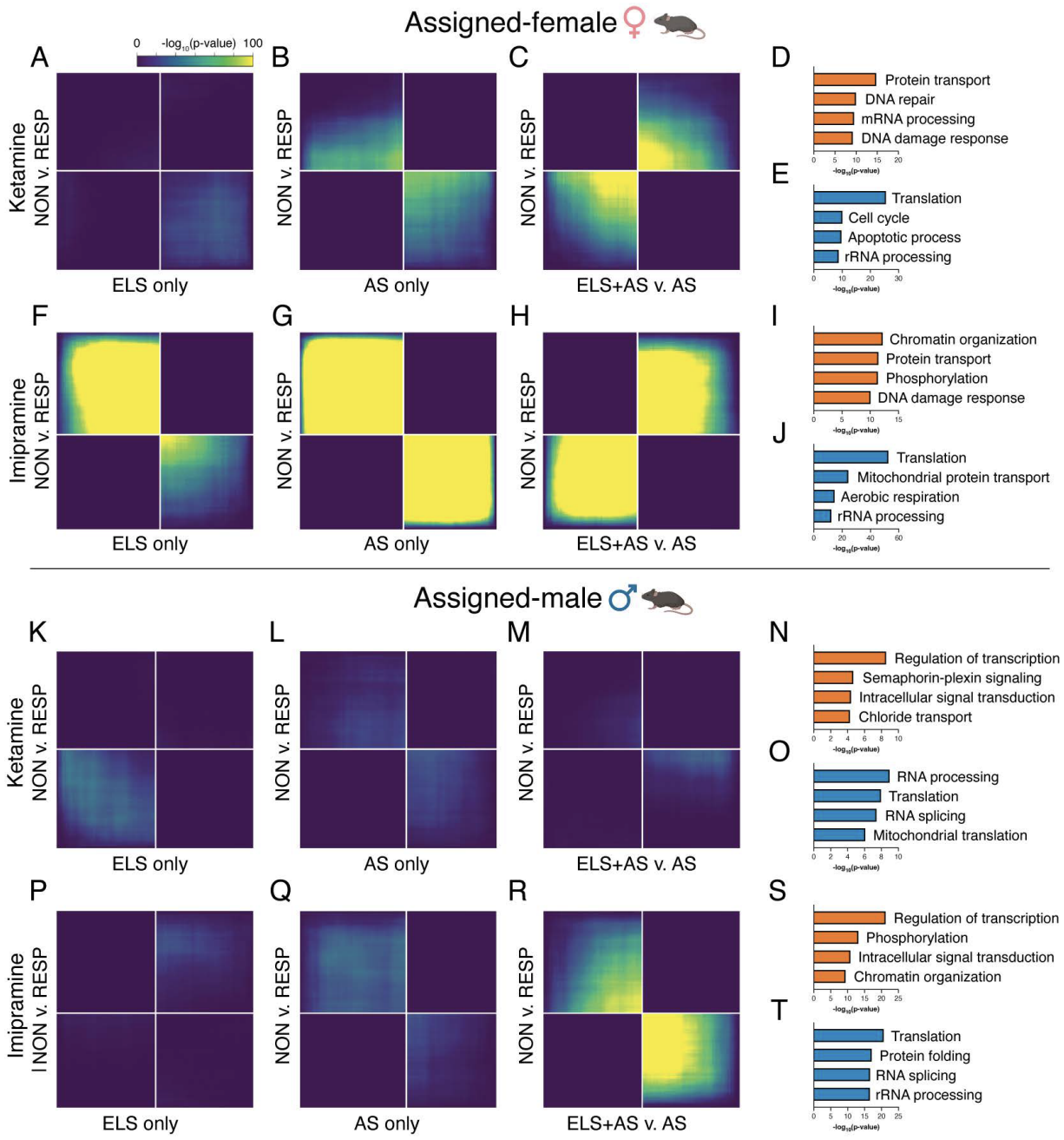


Figure 2. Comparing transcriptomic patterns of ELS or adult stress with antidepressant treatment efficacy within mouse NAc

Threshold-free RRHO analysis compares gene expression changes after ELS or AS with expression changes between antidepressant non-response vs response, all within mouse NAc. Genes may be co-regulated or oppositely regulated between two comparisons. Pixels represent the overlap between DEGs in each comparison, with the significance of overlap (-log10(p-value)) of a hypergeometric test color-coded, with a fixed maximum of 100 across all comparisons.

RRHO comparisons were made separately for assigned-female (A-J) and assigned-male (K-T) mice against datasets for both ketamine and imipramine treatment efficacy from male mouse NAc, as indicated.

Gene ontology analysis was performed for genes co-upregulated (orange) or co-downregulated (teal) from ELS+AS vs AS comparisons: D and E correspond to C; I and J correspond to H; N and O correspond to M; S and T correspond to R.

Testing antidepressant treatment response in mice

We next sought to test the bioinformatic predictions that ELS would reduce antidepressant treatment efficacy, particularly among females. We tested both escitalopram (10 mg/kg i.p. for 21 days) to match the human comparisons, and ketamine (saline for 20 days followed by a single 10 mg/kg i.p. dose) to match the mouse comparisons and as a novel and rapid antidepressant (Fig. 3A).

We first sought to validate that ELS and adult social defeat stress altered behavior among female (Fig. 3) and male (Fig. 4) mice. All mice were tested for exploratory and avoidant behavior in an open field test. Among females, there was a main effect of social defeat to decrease time spent in the center of the open field (Fig. 3B), indicating female mice are responsive to social defeat stress. However, there was no effect of ELS nor interaction on open field behavior among females (Fig. 3B-C). Decreased social interaction has been a well-validated measure of response to chronic social defeat among males, and to a lesser extent among females (Berton et al., 2006; Krishnan et al., 2007; Lopez & Bagot, 2021; Yohn et al., 2019). There was a main effect of ELS but not of defeat, nor an interaction between early life and adult stress, on social interaction (SI) ratio (Fig. 3D). Although female mice have been reported to have reduced SI ratio similar to males after social defeat stress (Harris et al., 2018; Takahashi et al., 2017; Yohn et al., 2019), females in our study remained highly social.

We previously found an effect of ELS and AS on novelty-suppressed feeding (NSF) behavior among females (Peña, Smith, et al., 2019) and thus tested female mice in this test. We confirmed a main effect of social defeat on latency to eat (Fig. 3E). Survival curve analysis of latency to eat in the NSF also differed across groups (Fig. 3F). Post-hoc analysis indicated a difference between Std-Defeat and ELS-Defeat conditions and a trend between Std-Ctrl and ELS-Ctrl. Together, the open field and NSF tests show that female mice are sensitive to social defeat stress, and ELS exacerbates behavioral differences in an NSF test. Mice categorized as susceptible were then randomly assigned to saline, escitalopram, or ketamine treatment groups.

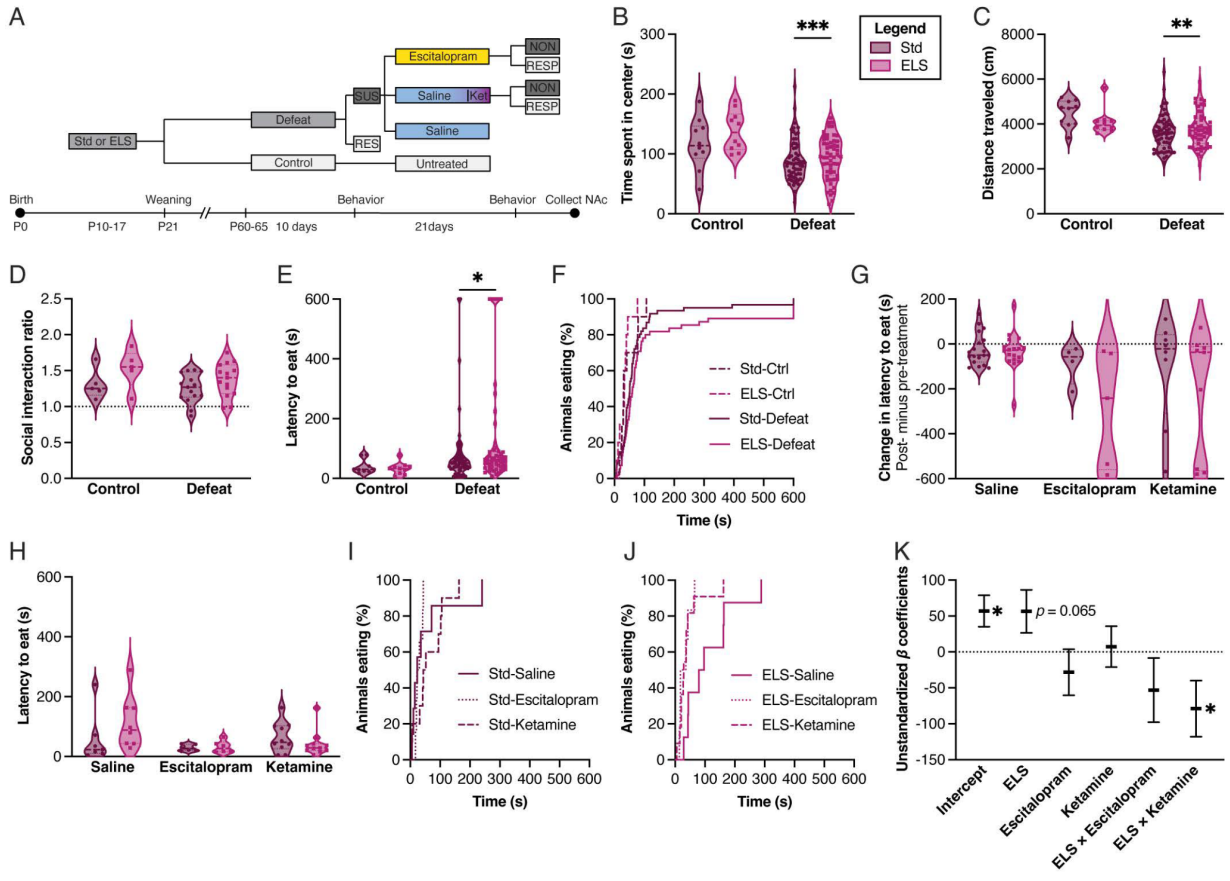


Figure 3. Behavioral testing of assigned-female mice

(A) Schematic of behavior and pharmacology timeline. Mice were first tested after social defeat stress and prior to pharmacological treatment (B-F; n=10, 10, 60, 55), and again after treatment with either saline, 21 days of escitalopram, or 1 day of ketamine (G-K; n=7, 8, 6, 6, 10, 11).

Open field test: (B) Time spent in the center of an arena [main effect of social defeat stress: two-way ANOVA, $F(1,132)=17.32$, $p<0.001$].

(C) Total distance traveled in an arena was decreased by social defeat stress [$F(1,132)=10.40$, $p=0.0016$].

Social interaction: (D) Ratio of time spent in interaction zone with v. without aggressor (main effect of ELS: $F(1,36)=4.406$, $p=0.0429$).

Novelty-suppressed feeding: (E) Pre-treatment latency to eat [main effect of social defeat: $F(1,131)=3.966$, $p=0.0485$; main effect of ELS: $F(1,30)=7.611$, $p=0.010$.

(F) Survival curve analysis of pre-treatment latency to eat [Log-rank Mantel-Cox across all groups: $X^2=17.98$, $p=0.0004$; between Std-Defeat and ELS-Defeat: $X^2=11.32$, $p=0.0008$; trend between Std-Ctrl and ELS-Ctrl: $X^2=2.846$, $p=0.0916$].

(G) Difference in latency to eat before and after treatment [escitalopram: two-way ANOVA, $F(1,30)=24.407$, $p<0.001$; ELS: $F(1,30)=7.611$, $p=0.010$; ELS × escitalopram interaction: $F(1,30)=9.375$, $p=0.005$].

(H) Post-treatment latency to eat [escitalopram v. saline: two-way ANOVA, $F(1,30)=24.407$, $p<0.001$].

(I-J) Survival curve of post-treatment latency (significant difference across all groups: Log-rank Mantel-Cox, $X^2=16.83$, $p=0.0185$).

(J) Among ELS mice, NSF latency differed between ELS-Def-Sal and ELS-Def-Esc groups ($X^2=7.932$, $p=0.0049$) and between ELS-Def-Sal and ELS-Def-Ket ($X^2=6.117$, $p=0.0134$).

(K) Fixed effects model of post-treatment latency to eat as a function of stress and treatment [trend in overall difference across all groups: $F(5,42) = 2.229$, $r2=0.2097$, $p=0.0691$; significant main effect of ELS: $B=56.48$, $t=1.892$, $p=0.0654$; ELS × ketamine interaction ($B= -78.94$, $t=2.021$, $p=0.0498$), no main effect of escitalopram ($B= -28.249$, $t = -0.880$, $p = 0.3837$), and no ELS × escitalopram interaction ($B= -53.19$, $p=0.24$)].

After treatment, female mice were tested again for behavior in the open field and NSF tasks. There was a trend for an effect of escitalopram relative to saline [$F(1,23)=2.945$, $p=0.100$] and a significant effect of ketamine [$F(1,32)=5.488$, $p=0.026$] on time in the center of the open field, but no main effect or interaction with ELS (not shown).

We next calculated behavioral difference scores for NSF latencies (post-treatment - pre-treatment behavior) to determine the efficacy of treatment relative to each animal's own initial behavior. Female mice were considered responsive to treatment if the NSF difference score was <0 (reduced latency to eat with treatment). Change in NSF latency with control saline treatment was tightly clustered around 0, indicating relatively stable NSF behavioral phenotype among both Std and ELS groups (Fig. 3G). There was a main effect of escitalopram treatment relative to saline on change in latency to eat with treatment (Fig. 3G), a main effect of ELS with ELS-mice taking longer to eat, and an interaction between ELS and escitalopram treatment . All escitalopram-treated females improved their NSF latency (difference score <0) and were considered responsive to treatment by this measure (Fig. 3G). There was also a main effect of ketamine to reduce feeding latency relative to saline (Fig. 3H) but no main effect of ELS nor an interaction between ELS and ketamine treatment. 60-85% of ketamine-treated females improved their NSF latency and were considered responsive to treatment by this measure (Fig. 3G). Survival curve analyses of latency to eat in NSF were significantly different across treatment groups overall (Fig. 3I-J). In particular among ELS mice, NSF latency differed between ELS-Def-Sal and ELS-Def-Esc groups (X^2=7.932, p=0.0049) and between ELS-Def-Sal and ELS-Def-Ket (X^2=6.117, p=0.0134; Fig. 3I-J). We also used fixed effects modeling to determine whether early experience, treatment, and their interaction predicted female NSF latency. There was an overall trend for this model,(with an interaction between ELS and ketamine treatment as the strongest predictor of ameliorated female behavior (Fig. 3K). In contrast, escitalopram did not strongly predict behavior as a main effect (B = -28.249, t = -0.880, p = 0.3837) or in interaction with ELS. In contrast, ELS was the strongest predictor of longer NSF latencies, interpreted as more depression-like behavior. Thus, in contrast to our bioinformatic predictions

but in support of prior clinical observations, this model indicates that ketamine may be an efficacious treatment among females that previously experienced ELS.

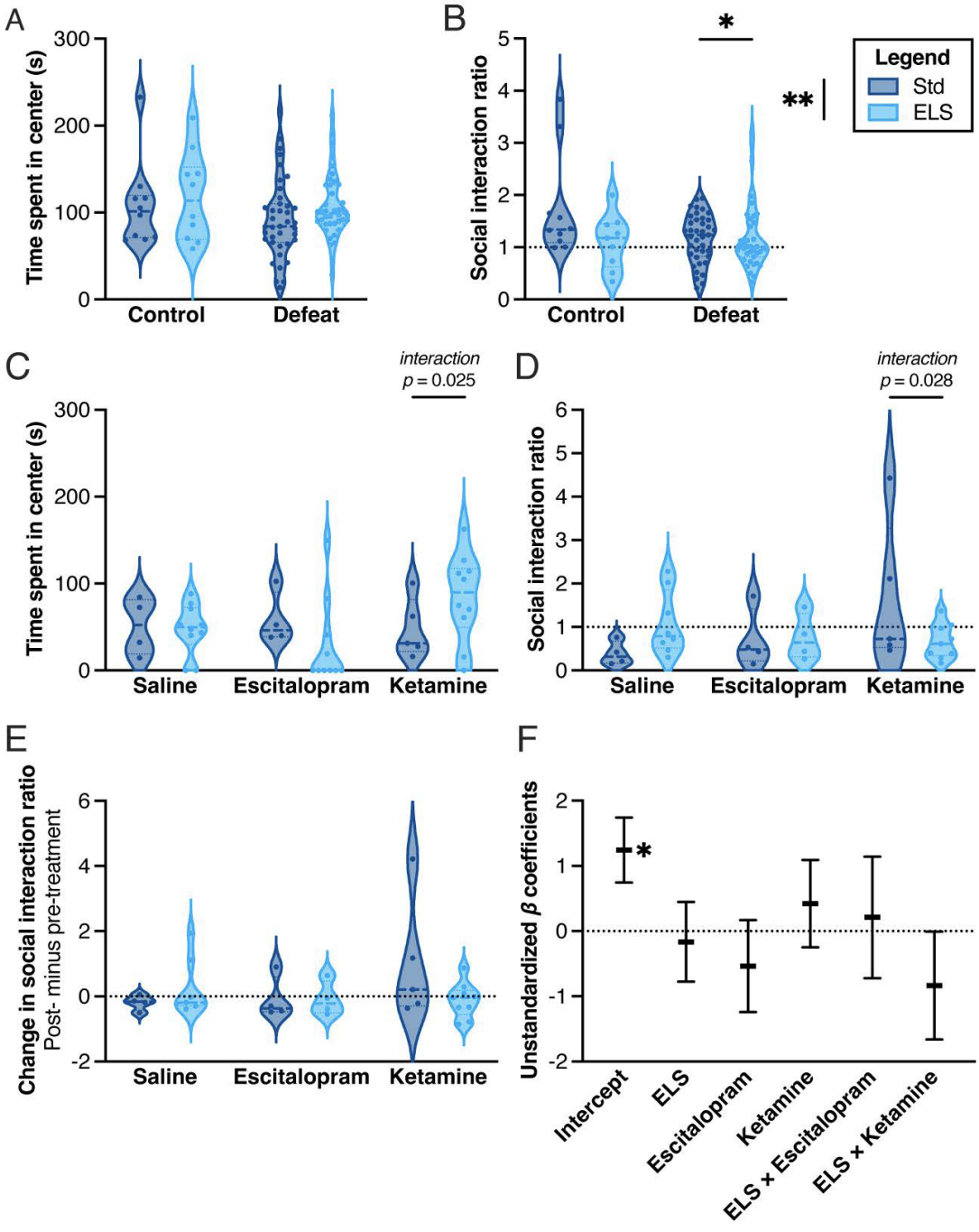


Figure 4. Behavioral testing of assigned-male mice

Mice were first tested after social defeat stress and prior to pharmacological treatment (A-B; n=10, 10, 40, 38), and again after treatment with either saline, 21 days of escitalopram, or 1 day of ketamine (C-F; n=4, 8, 4, 4, 5, 9).

Open field test: (A) Pre-treatment time spent in the center of an arena did not significantly differ by group.

Social interaction: (B) Pre-treatment ratio of time spent in interaction zone with v. without aggressor [main effect of ELS: two-way ANOVA: $F(1,94)=7.662$, $p=0.0068$; main effect of social defeat: $F(1,94)=5.620$, $p=0.0198$; interaction of ELS × social defeat: $F(1,94)=4.401$, $p=0.0386$].

(C) Post-treatment time spent in the center of an arena in the open field test [interaction of ELS × ketamine: $F(1,21)=5.841$, $p=0.025$].

(D) Post-treatment social interaction ratio [interaction of ELS × ketamine: $F(1,22)=5.546$, $p=0.028$].

(E) Difference in social interaction ratio before and after treatment [trend in interaction of ELS × ketamine: $F(1,22)=3.479$, $p=0.076$].

(F) Fixed effects model of post-treatment latency to eat as a function of stress and treatment was not significant overall.

Among males, there was no effect of either stress on open field behavior prior to treatment (Fig. 4A). Whereas females were tested in the NSF task, males were tested for social interaction. On measures of social interaction, there was a main effect of ELS, a main effect of social defeat, and an interaction between ELS and social defeat on social interaction behavior (SI ratio) (Fig. 4B), validating previous findings that ELS sensitizes male mice to adult social defeat stress (Bagot et al., 2017). Mice categorized as susceptible were then randomly assigned to treatment groups, and tested again in the open field and SI tests.There was no effect of escitalopram relative to saline on open field exploration, but there was a significant interaction between ELS and ketamine on open field center time, such that ELS mice treated with ketamine increased center exploration, indicative of recovery (Fig. 4C). We again did not find an effect of escitalopram on social interaction behaviors (Fig. 4D-E). However, with ketamine treatment, there was an interaction between ELS and ketamine on SI ratio (Fig. 4D), and a trend for an interaction on change in SI ratio (Fig. 4E). Treatment response was previously defined as an

increase in SI behavior (Bagot et al., 2017). Based on this definition, 25% of Std and ELS mice were responsive to escitalopram treatment and 60% of Std mice and 33% of ELS mice were responsive to ketamine treatment, compared to a 25% spontaneous recovery rate among saline-treated males of both groups (Fig. 4E). However, fixed effects modeling including early experience, treatment, and their interaction did not significantly predict male SI ratio (Fig. 4F). Together across sexes, these data show little effect of escitalopram and a mixed effect of

ASSIGNED-FEMALES

		Pre-treatment			Pre-treatment			
Fig.	**Behavior**	**ELS**	**Defeat**	**Interaction**	**ELS**	**Esc.**	**Ket.**	**Interaction**
3B	OFT: Time spent in arena center	–	▼	–	–	(▼)	▼	(ELS x Escitalopram)
3C	OFT: Distance traveled	–	–	–	–	–	–	–
3D	Social interaction: Ratio	▲	–	–	N/A	N/A	N/A	N/A
3E-K	NSF: Latency to eat	–	▲	Std-Defeat v. ELS-Defeat (Std-Ctrl v. ELS-Ctrl)	▼[1]	▼	▼	ELS x Escitalopram (▼ ELS x Ketamine)

ASSIGNED-MALES

		Pre-treatment			Pre-treatment			
Fig.	**Behavior**	**ELS**	**Defeat**	**Interaction**	**ELS**	**Esc.**	**Ket.**	**Interaction**
3B	OFT: Time spent in arena center	–	–	–	–	–	–	ELS x Ketamine
3D	Social interaction: Ratio	▼	▼	ELS x Defeat	–	–	–	ELS x Ketamine

Table S1. Summary of behavioral pharmacology. Direction of effects are stratified by assigned sex, early experience, adult experience, and treatments. For main effects and interactions that are statistically significant ($p < 0.05$) or trending ($0.10 > p > 0.05$; indicated by parentheses), ▲ indicates an increase, and ▼ indicates a decrease in the behavior. N/A indicates behavior was not measured.
[1]Change over time in latency was significant when subtracting post-treatment latency from pre-treatment (corresponding to Fig. 3G), however this effect was not significant in standalone post-treatment measurement.

ketamine treatment, depending on behavior test: among female mice, ketamine had a main effect on open field behavior, while the interaction of ELS and ketamine was the strongest predictor of changes in NSF; and among males, ketamine-treated mice saw improvement in behavior in open field but not in SI. While the lack of effect of escitalopram is surprising given its efficacy in previous publications (Anwar et al., 2013; Fitzgerald et al., 2019; Sánchez et al., 2003) and in females in our study (Fig. 3G-J), this finding does mirror our bioinformatic predictions. A summary of behavioral findings across tests and treatments is in Table 1.

Discussion

The inherently heterogeneous nature of depression is reflected in individual differences in antidepressant responsiveness. Depressed patients with a history of ELS have worse forms of depression and may constitute a unique subtype of major depressive disorder. However, the ways in which ELS alters brain development to change the risk and course of depression and response to treatment are poorly understood. To parse the roles that sex and life-course stress play in predicting antidepressant response, we undertook a series of translational studies that leveraged peripheral and central gene biomarkers of treatment response among humans and mice treated with different antidepressants. We found that a history of ELS prior to adult stress altered patterns of gene expression in NAc which corresponded with patterns of antidepressant treatment failure from both mice and humans, across classes of antidepressants. Consistent with a greater risk for depression among individuals identifying as women, we also found transcriptional patterns predicting treatment failure to be strongest among female subjects. Among female mice susceptible to adult chronic social defeat stress and treated with either chronic escitalopram or acute ketamine, the strongest predictor of behavior was an interaction between ELS and

ketamine treatment. Among males however, early experience and treatment were poor predictors of behavior, mirroring our bioinformatic predictions

Transcriptional signatures of early-life and adult stress predict antidepressant failure

Despite several major caveats (different species, different tissue types in brain vs periphery, a lack of information on ELS history among human patients), we found transcriptomic patterns in mouse NAc after ELS+AS that corresponded with patterns of antidepressant non-response in human blood. The correspondence across mouse and human tissue was weaker than within mouse brain, as would be expected based on larger transcriptomic differences across tissues (Yang et al., 2018), but these findings nonetheless support the conclusion that a history of ELS alters molecular profiles in the brain in a way that relates to human treatment efficacy. Importantly, our bioinformatic comparisons show that it is the difference between experience of adult stress with vs without prior ELS that corresponds to the difference between treatment responders and non-responders.

One important question that remains is whether it is a prior history of ELS specifically before additional adult stress that predicts antidepressant treatment failure at the molecular level, or whether two hits of stress at any age (such as multiple adult stressors) would similarly impact the brain and treatment response. While we cannot currently make these bioinformatic predictions, clinical research suggests that timing, type, and number of stressful life events does impact risk for MDD and treatment resistance (Amital et al., 2008; Kohn et al., 2001; McLaughlin et al., 2010).

Sex differences in depression and antidepressant treatment response

Depression disproportionately affects women wherein women are twice as likely to be diagnosed with depression than are men. However, the amount of research on depression including sex as a biological variable in clinical studies and females in pre-clinical animal models is not commensurate with this higher prevalence. The few genome-wide studies that have directly examined sex-related factors at baseline and in stress and depression have exposed not only a lack of overlap in differentially expressed genes in other corticolimbic brain regions but also, more importantly, significantly opposite directions of and sex-specific gene expression between men and women with MDD (Labonté et al., 2017; Seney et al., 2018). Further, sex differences in behaviors and transcriptional patterns associated with depression are driven differently by gonadal versus genetic sex (Paden et al., 2020). Within the NAc, which has been strongly associated with antidepressant response/failure across classes of antidepressants (Bagot et al., 2017), there are also sex differences in transcription at baseline and in response to stress (Hodes et al., 2015) indicating that sex-related molecular profiles of the brain may indeed affect treatment response. Indeed, we find clear sex differences associated with treatment response across life stress and all classes of antidepressants. However, we also find some similarities in the functions of genes altered across sexes and treatments in non-response. Dimension reduction by gene ontology analysis revealed an enrichment of co-upregulated genes associated with chromatin organization with female escitalopram/desvenlafaxine response, and female and male imipramine response. There was also an enrichment of co-downregulated genes associated with translation across female escitalopram/desvenlafaxine response and female and male ketamine and imipramine response. This indicates that similar gene pathways may be engaged across sexes in treatment response despite differences in specific gene expression changes and efficacy. Overall, our results support the call for both research and medical care providers to consider the

timing of stress experiences as well as sex during antidepressant treatment (Hodes & Epperson, 2019).

Sex differences in behavioral pharmacology

Our bioinformatic analysis of treatment response within the NAc of female mice exposed to two hits of stress strongly predicted treatment failure with escitalopram/desvenlafaxine and imipramine and weakly predicted failure with ketamine. Among males, transcriptomic profiles of ELS prior to adult stress weakly predicted escitalopram/desvenlafaxine treatment failure, did not show any correspondence with ketamine efficacy, and moderately predicted positive response to imipramine. In contrast, transcriptomic profiles derived from mice exposed to ELS alone (in which mice did not show depression-like behavioral profiles) and adult stress alone (with mixed depression-like behavior) largely predicted positive response to treatment among females, while again transcriptional signatures of life stress had little correspondence with treatment response among males.

Consistent with these predictions, we found significant sex-related differences in behavior, with females generally showing stronger response to treatment, both compared to saline and compared to males. To the extent that studies have examined the role of sex in antidepressant efficacy, there are indications that women may respond better to SSRI/SNRI's (such as escitalopram and desvenlafaxine) and men may respond better to tricyclics (such as imipramine) (Berlanga & Flores-Ramos, 2006; Khan et al., 2005; Kornstein et al., 2000; LeGates et al., 2019; Raskin, 1974). Indeed, the only transcriptomic prediction for treatment efficacy after two hits of stress was among males with imipramine treatment. We did not treat mice with imipramine in the current study, and previous pre-clinical studies have not examined sex differences, but it would be interesting for future research to test these life-stress x sex

predictions with imipramine, which may also show stronger treatment failure among ELS-females. Although all female mice treated with escitalopram universally improved their NSF latencies, the main effect of ELS indicated that improvement was modestly but significantly stronger among standard-reared than ELS female mice. Thus, the degree of escitalopram efficacy was somewhat reduced by ELS. Somewhat surprisingly, we did not find a strong effect of either treatment among male mice susceptible to social defeat, with or without ELS. Ketamine appeared to improve anxiety-like behavior in an open field among ELS but not Std males, but improved social interaction behavior among Std but not ELS mice. Our findings on the mixed effects of ketamine treatment outcomes are similar to previous studies that also observed mixed effects of ketamine depending on assigned sex, degree of stress, and the behavioral test used (Fitzgerald et al., 2019, 2021; Johnston et al., 2021). Although 237 mice were started in the study across two cohorts, given complex study design, one caveat is that treatment group size is still small. Another caveat is that only one dose of each drug was tested. Although dosages were based on literature from male mouse studies (Anwar et al., 2013; Bagot et al., 2017; Sánchez et al., 2003), it is possible that different doses are needed to separate the impact of ELS on treatment outcome, particularly for male mice receiving ketamine. While there are some indications of milder reduction in depression symptoms among women treated with ketamine than men, other research has indicated that female rodents have higher sensitivity to ketamine and that higher doses (e.g. 30 mg/kg) are needed for male mice (Carrier & Kabbaj, 2013; Coyle & Laws, 2015; Gerhard & Duman, 2018; Wright & Kabbaj, 2018).

Childhood trauma and Ketamine as an antidepressant

While ketamine is primarily used as a dissociative anesthetic, it was first described as a potential antidepressant in 1973 (Khorramzadeh & Lotfy, 1973). Ketamine acts acutely as a

non-competitive NMDA receptor antagonist (Autry et al., 2011; Moghaddam et al., 1997), is rapid-acting with relief from depression within hours to days (55), and long-lasting with an average duration of effect of days to weeks after a single treatment regime, with sustained effects described after multiple doses (Kim et al., 2023). In contrast, typical first-line antidepressants including tricyclics, SSRI's, and SNRI's (such as imipramine, escitalopram, and desvenlafaxine, respectively) act on monoamine systems, take several weeks for antidepressant effects to be felt, and must be taken daily. Given these contrasts, there has been increasing interest in ketamine as an antidepressant, particularly for treatment-resistant depression (Akil et al., 2018; Krystal et al., 2019). Recent research has suggested that while ELS may predispose a worse response to traditional antidepressant treatment (Nanni et al., 2012), ketamine may in fact be a more efficacious treatment among individuals with high childhood trauma burden (O'Brien et al., 2019). Here, transcriptional signatures of ELS with or without adult stress showed low correspondence with signatures of ketamine response or non-response in NAc with the exception of ELS+AS in females corresponding to ketamine non-response (Fig. 2C). Contrary to our bioinformatic predictions but consistent with the hypothesis that ketamine could be more effective in patients with high childhood trauma (O'Brien et al., 2019), the strongest predictor of improved female NSF latencies in our model was the interaction between ELS and ketamine treatment (Fig. 3M). Ketamine has also been described as a potential prophylactic that can promote resilience in the face of later stress (Brachman et al., 2016; Mastrodonato et al., 2022; McGowan et al., 2017, 2018). It is therefore possible that treatment of ELS-exposed mice prior to a second hit of adult stress would be more efficacious than treatment after the second hit of stress. This possibility has translational appeal, given that children who have experienced early

adversity are already identified as at-risk and would therefore be better candidates for prophylactic treatment than the average population.

In silico predictions to discover novel antidepressants

One drawback of the current study is that while analyses predicted antidepressant non-response after ELS which is consistent with the literature (Nanni et al., 2012), the ideal goal would be to identify treatments with greater potential efficacy in individuals with a history of ELS. Transcriptional signatures of many drugs and compounds have been generated *in vitro* (Hodos et al., 2016). Future research may be able screen these datasets *in silico* for compounds whose transcriptional signatures oppose that of ELS for additional testing in vivo. This would facilitate discovery of novel potential antidepressants with greater efficacy in this sub-population of depressed patients who may have unique and long-lasting transcriptional signatures in the brain and need unique treatment plans.

Conclusion

This work provides translational, genome-wide evidence for sex- and early-life stress-related molecular adaptations in the brain that may contribute to antidepressant treatment response, potentially requiring unique pharmacological treatments.

Supporting information: Materials and methods

Transcriptomic Datasets

Three independent RNA-seq datasets were used for bioinformatic analyses for predicting the effects of a history of ELS on future antidepressant response. The first dataset (GEO GSE89692) includes the NAc of a mouse model for ELS wherein assigned female and male mice experienced ELS with or without adult stress (AS) in a 2×2 design (Peña et al., 2017; Peña, Smith, et al., 2019). Adult stress in these datasets

consisted of chronic social defeat stress for assigned-male mice and sub-threshold variable stress for assigned-female mice. In these datasets, ELS sensitized mice to experience of AS to produce greater depression-like behaviors. Whole-tissue RNA-seq was performed on 4-6 subjects per group as previously described (Peña et al., 2017; Peña, Smith, et al., 2019). Differential gene expression analyses were computed via the R package `DESeq2` (Love et al., 2014). The following comparisons were used, separately for assigned-female and -male mice: ELS-Control vs Std-Control; Std-AS vs Std-Control; ELS-AS vs Std-AS. The second dataset (GEO GSE81672) includes the NAc of adult assigned-male mice that underwent chronic social defeat stress and were categorized as stress-naive, resilient to adult stress, susceptible to adult stress and responsive to either antidepressant treatment with the tricyclic imipramine or sub-anesthetic ketamine, or susceptible and non-responsive to one of the treatments (Bagot et al., 2017). The primary comparisons used were Imipramine Non-Responders vs Imipramine Responders and Ketamine Non-Responders vs Ketamine Responders. Whole-tissue RNA-seq was included on 6-10 subjects per group, as previously described (Bagot et al., 2015). Differential expression analyses were computed via the R package limma::voom (Law et al., 2014; Ritchie et al., 2015). The third RNA-seq dataset (see Fig. 1A) includes peripheral white blood cell samples from control subjects and patients with major depressive disorder (MDD) at two time points, before and 8 weeks after antidepressant treatment with either escitalopram (a selective serotonin reuptake inhibitor) or desvenlafaxine (a serotonin and norepinephrine reuptake inhibitor) and assessment for response or non-response to treatment on two depression rating scales: Montgomery-Åsberg Depression Rating Scale (MADRS) and Hamilton Depression Rating Scale (HAMD). The patient cohort, blood sample collection, processing, and sequencing as previously described (Fuh et al., 2023). This sample included 31 healthy control subjects (22 self-identified female and 9 self-identified male, with sex additionally matched by genotyping) and 186 MDD subjects (111 female, 75 male). Of the MDD patients, 91 received escitalopram and 95 received desvenlafaxine. MDD patients were classified as responders or non-responders based on a $\geq 50\%$ reduction in scores from baseline, and only patients where response to antidepressant treatment was consistent on both rating scales were included, resulting in 69 RESP (n=41 female and 28 male) and 70

NRES (n=47 female and 23 male) subjects. The primary comparisons used were antidepressant (escitalopram + desvenlafaxine) Non-Responders vs Responders, separately for female- and male-identifying subjects. RNA-seq normalization for these subjects was conducted using `DESeq2` and were log2-transformed and corrected for age, gender, and RIN using the removeBatchEffect function of limma. For comparison between mouse and human datasets, only homologous genes with baseMean expression >2 in both datasets were included.

Bioinformatic comparisons

Bioinformatic analyses were performed using R and Python. Genes included in differential analyses had the following parameters for each comparison: base mean > 2, significance as uncorrected $p < 0.05$, and threshold for fold-change > 30% (log2 FC, LFC > |0.3875|). DEGs included in Venn diagrams were limited to protein-coding genes. Gene lists for heatmaps included a union of all DEGs from any one comparison with matched LFC values regardless of significance in other comparisons.

Bioinformatic analyses were performed using R and Python. Rank-rank hypergeometric overlap (RRHO) analysis was implemented to compare pairs of independent gene expression datasets in a threshold-free manner as previously described (Cahill et al., 2018; Plaisier et al., 2010). A two-sided version of RRHO analysis was used to evaluate coincident and opposite enrichment (Cahill et al., 2018). In each pairwise comparison of datasets, genes are ranked by the -log10(p-value) multiplied by the sign of the fold change from the differential expression analysis (Love et al., 2014). For each set of comparisons, gene lists were filtered for genes expressed (>2 base mean) in both lists, such that human-mouse comparisons only included orthologous genes. RRHO difference maps were produced for each pair of match comparisons by calculating for each pixel the normal approximation of difference in log odds ratio and standard error of overlap; this Z-score was then converted to a P-value and corrected for multiple comparisons across pixels. We then performed gene ontology analysis for genes with high coincidence among comparisons using the R packages `clusterProfiler` enrichment tool for interpreting omics data,

`org.Hs.eg.db` for human genome-wide annotations, and `org.Mm.eg.db` for mouse genome-wide annotations (Wu et al., 2021; Yu et al., 2012).

Animals

All experiments with animals were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Princeton University and of the Society for Neuroscience. All mating occurred in-house to prevent shipping stress during gestation. Mice were maintained on a 12-h light/dark cycle with *ad libitum* access to food and water. Two nulliparous C57BL/6J female mice (Jackson) were mated with one male in the animal facility. Males were removed after 5 days and females were separated into individual cages 1-3 days prior to parturition. Litters were weighed and counted and cages cleaned on the day of birth (postnatal day or P0) but otherwise undisturbed. C57BL/6J pups were assessed for sex at birth and assigned “female” or “male” at weaning on postnatal day P21 by their gonadal anatomy, or external genitalia. Assigned-females and -males were weaned separately into cages of 3-5 mice, keeping littermates together and only combining pups from different litters and of the same experimental condition to maintain at least three mice per cage. Up to only two mice of the same assigned-sex were used per litter per initial control/defeat random assignment, and up to one mouse per sex per litter was used for each treatment group.

Early-life and adult stress paradigms

The two-hit ELS and adult stress paradigm in a mouse model implemented in the current study were as previously described (Peña et al., 2017; Peña, Smith, et al., 2019). Briefly, ELS involved limited nesting from P10-17 (Gilles et al., 1996; Molet et al., 2014; Rice et al., 2008). Litters were randomly assigned to standard-rearing (Std) or ELS groups. Pups were separated together as a litter to clean cages with distinct bedding for 3-4 h/day at random times each day during the light cycle to minimize predictability and habituation. Nesting material was depleted to one-third of that provided to standard-reared cages during the days of separations and then restored on the final day. For adult stress, we adapted a social defeat paradigm that can be used for both female and male mice (Dieterich et al., 2021; Yohn et al., 2019). Adult

mice were assigned randomly to either stress or control (Ctrl) conditions, and littermates were assigned to different groups for within-litter controls. For stress in adulthood, mice experienced chronic non-discriminatory social defeat stress, an improved model for depression and social stress-related disorders. In this paradigm, female and male C57BL/6J pairs were simultaneously placed into the home cage of a novel Swiss Webster aggressor mouse (Dieterich et al., 2021; Yohn et al., 2019). An experimental male mouse was introduced to an aggressor's cage first for ~3 minutes, followed by an experimental female mouse for an additional 5 minutes. Male mice were then moved across a perforated plexiglass barrier within the aggressor cage for the remainder of the day, for sensory stress without physical interaction. Female mice were removed to individual cages with aggressor bedding for the remainder of the day. Experimental mice were introduced to a new aggressor each day for 10 days, and male and female mice rotated in different directions so that trios were unique each day. While aggressors were observed to attack female mice, attacks were predominantly directed at male mice. No copulations were observed. Control mice were housed in a standard mouse cage in pairs of the same sex, separated by a perforated plexiglass barrier. At the conclusion of social defeat, all mice were rehoused in clean cages and underwent three consecutive days of behavioral testing before tissue collection.

Behavioral testing

Overview: Mice underwent behavioral testing twice in order to assess initial susceptibility or resilience to adult social stress and the effect of antidepressant treatment: first, one day after the 10 days of social defeat and, second, one day after the conclusion of antidepressant or saline treatment (Fig. 3A). For each round of testing, one behavioral test was conducted per day. Due to the large number of animals (n=237 in total)], testing was done in two cohorts. All mice were tested in all three tests described below after social defeat for the first cohort. However, there was a strong main effect of sex on social interaction behavior such that defeat decreased social investigation among males but not females. Pilot studies in our lab also showed a stronger effect of social defeat on novelty-suppressed feeding behavior among females than

males. We thereafter tested male mice in the social interaction test and female mice in the novelty-suppressed feeding test. All mice were tested on the open field test.

Arenas used for behavioral testing measured 44 × 44 × 20 cm. Video tracking software (Ethovision, Noldus) was used to record and quantify mouse behavior in each test. To mitigate habituation in repeated testing, the patterns on the walls of the chambers were altered for each testing session.

Social interaction testing

We assessed social avoidance behavior using a two-stage social interaction test conducted. Testing was conducted in the light cycle but under red lighting, described previously (Bagot et al., 2017; Peña et al., 2017). Social avoidance has been shown to be related to depression-like behavior and altered response to antidepressant treatment (Berton et al., 2006; Krishnan et al., 2007). In the first stage with no target present, the experimental mouse explored an arena containing a novel plexiglass and wire mesh enclosure (novel object; 10×6 cm) centered against one wall of the arena for 2.5 minutes. In the second stage with the target present, the experimental mouse was immediately returned to the arena with a novel Swiss Webster mouse (aggressor strain) within the enclosure for another 2.5 minutes. A social interaction ratio (SI ratio) was calculated as time that the experimental mouse spent in an "interaction zone" (14×26 cm) surrounding the enclosure in stage two with the aggressor present divided by time in the zone during stage one with the empty novel enclosure. Mice with an SI ratio <1 were categorized as susceptible to social defeat stress, while mice with an SI ratio >1 were categorized as resilient to stress.

Open field test (OFT)

To assess general locomotor and exploratory behavior, experimental mice were allowed to freely explore a brightly lit, empty arena for 10 min as previously described (Peña et al., 2017). Time spent in the center (20 × 20 cm) of the open field arena, distance traveled in the arena, and locomotor activity were measured using Ethovision.

Novelty-suppressed feeding (NSF)

Depression- and anxiety-like behavior among female mice was assessed by NSF testing, as in (Bodnoff et al., 1989). Mice were deprived of food in a clean home cage for 18 hours before testing. Testing was conducted in the dark phase of the day when mice typically eat most. On the day of testing, mice were placed in a corner of a brightly-lit arena covered with corncob bedding on its floor. A single yogurt chip was attached to a white circular platform (10 cm diameter) located at the center of the arena. The test session lasted until mice took their first bite of the chip, which was defined as the moment the mouse sat on its haunches, grasped the chip with its forepaws, and bit into the chip; or for a maximum of 10 min. Mice that failed to consume the food within 5 min were assigned a latency score of 600 seconds. Immediately after the first bite or at 10 min if the mouse failed to eat, the mouse and the yogurt chip in its arena were transferred to its home cage with *ad libitum* access to water, and latency to eat in the home cage was then recorded by hand. An NSF ratio was then calculated as the latency to eat in the novel arena, divided by the latency to eat in its home cage. Mice with an NSF ratio > mean + 0.25*SD of the Std-Ctl mice for that cohort were categorized as susceptible to social defeat stress.

Antidepressant treatment

Mice categorized as susceptible to social defeat stress were randomly assigned to treatment with either saline, escitalopram, or ketamine, beginning the day after the first round of behavioral testing ended. Saline-treated mice received 0.1 mL sterile normal saline (i.p.) daily for 21 days. Escitalopram-treated received escitalopram (10 mg/kg, i.p.; Henry Schein 1263605, dissolved in sterile saline) for 21 days, wherein dosage was demonstrated in previous literature (Anwar et al., 2013). Mice assigned to the ketamine group were given 0.1 mL sterile normal saline (i.p.) daily for 20 days, followed by a single dose of ketamine (10 mg/kg, i.p.) on day 21 of treatment, wherein dosage was demonstrated in previous literature (Bagot et al., 2017). The second and final round of behavioral testing began the day following the last day of treatment.

Statistical analyses of behavior

Behavioral and gene expression data were analyzed with R, SPSS (IBM, version 28), or Prism (GraphPad, version 9). General linear model (two-way ANOVA) was used to determine main effects and interactions, as described. To account for within-animal change in behavior with treatment, a behavioral difference score was calculated (post-treatment minus pre-treatment). Male mice were categorized as responsive to treatment if their SI-Ratio difference score was positive; female mice were categorized as responsive to treatment if their NSF-latency difference score was negative. Differences in latency to eat in the NSF test were calculated by Log-rank Mantel-Cox survival analysis. Effects of escitalopram and ketamine were compared to saline separately. Post-hoc analyses used Tukey's HSD multiple comparisons correction. Including cohort as a covariate did not alter any statistical outcomes. Outliers were defined as > 2 standard deviations away from the mean and were removed. Fixed effects models were generated to examine the effects of ELS, treatment, and their interactions on latency to eat in the NSF test (females) and SI ratio (males). All significance thresholds were set at $p < 0.05$.

Chapter 4: Summary and discussion

ELS is a prevalent experience during childhood and adolescence that changes the brain at multiple levels, from complex behavioral models of mental illness or resilience, to genome-wide changes in transcriptional patterns and networks. Moreover, ELS reveals or develops important sex differences at these same levels (Parel & Peña, 2022). In the long term, ELS increases the risk for developing depression and lowers antidepressant treatment efficacy. These translational studies leveraged a combination of genome-wide data from humans and mice treated with different antidepressants, computational analyses, and in vivo pharmacological approaches to understand how molecular changes in the brain following early-life stress may mediate altered antidepressant response. We found that early-life stress alters gene expression patterns in the nucleus accumbens—a brain center of reward and mood—which correspond with patterns of antidepressant treatment failure (Parel et al., 2023). Transcriptional patterns predicting treatment failure were strongest among female subjects, consistent with a greater risk for depression among women. This work provides neurobiological evidence for sex- and early-life stress-related molecular adaptations in the brain that contribute to antidepressant treatment response.

Current Limitations and Future Directions

Our use of sequencing technology and bioinformatic analyses to understand the impact of ELS on the brain, and how sex may mediate these effects, is still in its infancy. We see four main areas for future research to expand, particularly regarding sex differences: 1) human studies of ELS that directly examine sex differences rather than control for sex; 2) understanding the contributions of hormone levels; 3) understanding how ELS sex-specifically alters transcriptional dynamics across development; and 4) expanding sequencing methods.

Need for Additional Human Studies

Human studies have not yet directly examined sex differences in global response to ELS, much less attempted to understand the role of gonadal hormones or sex as a nonbinary variable with intersex and transgender individuals. Some existing data may be analyzed again with the express purpose of identifying sex differences (although existing datasets may be underpowered), but there is an opportunity to generate new datasets to a priori determine how ELS and sex interact to alter transcriptional development. Of course, studying the interaction of ELS and sex in the human brain is difficult for a number of reasons, including access to brain tissue, complete history including information on childhood adversity, and variability in human genetics and life experience. Building off existing genome-wide PsychENCODE datasets or formation of a consortium specific for ELS may facilitate future research (Li et al., 2018).

Need for Understanding Hormonal Contributions

Including sex as a biological variable and even accounting for sex differences does not necessarily mean studying hormonal contributions, as it is not always the case that variability is accounted for by hormonal state (McCarthy et al., 2012; Shansky, 2019). However, steroid hormone levels directly impact gene expression through binding of their cognate receptors and initiating activity as transcription factors. Additionally, chromatin organization in the female hippocampus dynamically fluctuates across the estrous cycle, and thus hormones are likely to play a role in the sex differences observed in genome-wide response to ELS (Jaric, Rocks, Greally, et al., 2019). Hormonal contributions can be examined—particularly in animal models—by directly measuring hormone levels; tracking female cycle state; and measuring outcome variables at specific stages, artificially after gonadectomy with or without hormone clamping, or with the four core genotypes mouse model.

Need for Longitudinal Assessment

There are demonstrated acute and long-lasting effects of ELS on transcription, but longitudinal studies examining transcription across postnatal development are needed to understand how ELS interacts with and potentially disrupts the time course of transcriptional development itself. For example, expression sampled at only a single time point may erroneously appear to be suppressed when in fact development of that gene program is delayed, or there may appear to be no effect of ELS when in fact maturation was accelerated (Bath et al., 2016; Gee et al., 2013; Peña et al., 2017). A transcriptomic time course of normal and ELS-impacted neurodevelopment will also reveal sensitive periods of heightened plasticity and potential vulnerability and whether there are sex differences in the timing thereof.

Opportunities to Harness New Technology

The majority of studies to date on the genome-wide impact of ELS and sex have used bulk-tissue RNA sequencing. There is a current lack of understanding of how chromatin dynamics mediate enduring consequences of ELS on a genome-wide scale. Indeed, chromatin dynamics likely underlie the altered transcriptomic response to adult stress observed after ELS (Peña et al., 2017; Rowson et al., 2019). There is also enormous potential to apply recent advances in single-cell sequencing technologies in future studies to examine cell type–specific effects of ELS in the brain. Several genome-wide studies of depression in adults have identified a role for oligodendrocytes and microglia (Lutz et al., 2017; Seney et al., 2018). Single-cell sequencing is quickly becoming easier and less expensive and may provide important cellular resolution to understand sex-specific effects of ELS.

Conclusion

Together the bioinformatic predictive analyses and the behavioral pharmacology experiments investigated here provide a significant contribution and nuanced understanding to current research at the intersection of ELS, depression, antidepressants, and sex differences. As the understanding of sex and gender in science and society changes, it is important that the language used in discussing sex differences is careful, intentional, and precise. As the understanding of sex and gender in science and society changes, it is important that the language used in discussing sex differences is careful, intentional, and precise. The results of these studies provide new opportunities of research in the field of ELS by utilizing recent methods of quantitative and computational analyses, maximizing the "big data" of existing transcriptomic datasets, and developing animal behavioral models that have high translational value to mental health and illness.

Bibliography

Agorastos, A., Pervanidou, P., Chrousos, G. P., & Baker, D. G. (2019). Developmental Trajectories of Early Life Stress and Trauma: A Narrative Review on Neurobiological Aspects Beyond Stress System Dysregulation. *Frontiers in Psychiatry / Frontiers Research Foundation*, *10*, 118. https://doi.org/10.3389/fpsyt.2019.00118

Akil, H., Gordon, J., Hen, R., Javitch, J., Mayberg, H., McEwen, B., Meaney, M. J., & Nestler, E. J. (2018). Treatment resistant depression: A multi-scale, systems biology approach. *Neuroscience and Biobehavioral Reviews*, *84*, 272–288. https://doi.org/10.1016/j.neubiorev.2017.08.019

Alhattab, S., & English, J. (2022). *Staggering scale of grave violations against children in conflict revealed in new UNICEF analysis*. https://www.unicef.org/press-releases/staggering-scale-grave-violations-against-children-conflict-revealed-new-unicef

American Psychiatric Association. (2022). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5-TR*. American Psychiatric Association Publishing. https://doi.org/10.1176/appi.books.9780890425787

Amital, D., Fostick, L., Silberman, A., Beckman, M., & Spivak, B. (2008). Serious life events among resistant and non-resistant MDD patients. *Journal of Affective Disorders*, *110*(3), 260–264. https://doi.org/10.1016/j.jad.2008.01.006

Anacker, C., O'Donnell, K. J., & Meaney, M. J. (2014). Early life adversity and the epigenetic programming of hypothalamic-pituitary-adrenal function. *Dialogues in Clinical Neuroscience*, *16*(3), 321–333. https://doi.org/10.31887/DCNS.2014.16.3/canacker

Anwar, M. J., Pillai, K. K., Samad, A., & Vohora, D. (2013). Effect of escitalopram on cardiomyopathy-induced anxiety in mice. *Human & Experimental Toxicology*, *32*(6), 632–639. https://doi.org/10.1177/0960327112462728

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P.-F., Kavalali, E. T., & Monteggia,

L. M. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, *475*(7354), 91–95. https://doi.org/10.1038/nature10130

Bagot, R. C., Cates, H. M., Purushothaman, I., Vialou, V., Heller, E. A., Yieh, L., LaBonté, B., Peña, C. J., Shen, L., Wittenberg, G. M., & Nestler, E. J. (2017). Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. *Biological Psychiatry*, *81*(4), 285–295. https://doi.org/10.1016/j.biopsych.2016.06.012

Bagot, R. C., Parise, E. M., Peña, C. J., Zhang, H.-X., Maze, I., Chaudhury, D., Persaud, B., Cachope, R., Bolaños-Guzmán, C. A., Cheer, J. F., Deisseroth, K., Han, M.-H., & Nestler, E. J. (2015). Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. *Nature Communications*, *6*, 7062. https://doi.org/10.1038/ncomms8062

Balouek, J.-A., Mclain, C., Minerva, A. R., Rashford, R. L., Bennett, S. N., & Peña, C. J. (2022). Reactivation of early-life stress-sensitive neuronal ensembles contributes to lifelong stress hypersensitivity. In *bioRxiv* (p. 2022.12.21.521303). https://doi.org/10.1101/2022.12.21.521303

Bath, K. G., Manzano-Nieves, G., & Goodwill, H. (2016). Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice. *Hormones and Behavior*, *82*, 64–71. https://doi.org/10.1016/j.yhbeh.2016.04.010

Bath, K. G., Nitenson, A. S., Lichtman, E., Lopez, C., Chen, W., Gallo, M., Goodwill, H., & Manzano-Nieves, G. (2017). Early life stress leads to developmental and sex selective effects on performance in a novel object placement task. *Neurobiology of Stress*, *7*, 57–67. https://doi.org/10.1016/j.ynstr.2017.04.001

Becker, O. by J., & Tollkuhn, J. (2018). *Sex differences in the brain: Balancing sex in preclinical research*. https://www.brainfacts.org/-/media/SfN/Documents/NEW-SfN/Careers/2018/Career-Tools-and-Resources/Scientific-Short-Courses/20190418_2018_sc1_course_book_final.pdf#page=7

Berlanga, C., & Flores-Ramos, M. (2006). Different gender response to serotonergic and noradrenergic antidepressants. A comparative study of the efficacy of citalopram and reboxetine. *Journal of*

Affective Disorders, *95*(1-3), 119–123. https://doi.org/10.1016/j.jad.2006.04.029

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., & Krystal, J. H. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, *47*(4), 351–354. https://doi.org/10.1016/s0006-3223(99)00230-9

Bernet, C. Z., & Stein, M. B. (1999). Relationship of childhood maltreatment to the onset and course of major depression in adulthood. *Depression and Anxiety*, *9*(4), 169–174. https://www.ncbi.nlm.nih.gov/pubmed/10431682

Berton, O., McClung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., Graham, D., Tsankova, N. M., Bolanos, C. A., Rios, M., Monteggia, L. M., Self, D. W., & Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*, *311*(5762), 864–868. https://doi.org/10.1126/science.1120972

Bodnoff, S. R., Suranyi-Cadotte, B., Quirion, R., & Meaney, M. J. (1989). A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. *Psychopharmacology*, *97*(2), 277–279. https://doi.org/10.1007/BF00442264

Bordner, K. A., George, E. D., Carlyle, B. C., Duque, A., Kitchen, R. R., Lam, T. T., Colangelo, C. M., Stone, K. L., Abbott, T. B., Mane, S. M., Nairn, A. C., & Simen, A. A. (2011). Functional genomic and proteomic analysis reveals disruption of myelin-related genes and translation in a mouse model of early life neglect. *Frontiers in Psychiatry / Frontiers Research Foundation*, *2*, 18. https://doi.org/10.3389/fpsyt.2011.00018

Brachman, R. A., McGowan, J. C., Perusini, J. N., Lim, S. C., Pham, T. H., Faye, C., Gardier, A. M., Mendez-David, I., David, D. J., Hen, R., & Denny, C. A. (2016). Ketamine as a Prophylactic Against Stress-Induced Depressive-like Behavior. *Biological Psychiatry*, *79*(9), 776–786. https://doi.org/10.1016/j.biopsych.2015.04.022

Bræstrup, C., & Sanchez, C. (2004). Escitalopram: a unique mechanism of action. *International Journal of Psychiatry in Clinical Practice*, *8 Suppl 1*, 11–13. https://doi.org/10.1080/13651500410005496

Breen, M. S., Bierer, L. M., Daskalakis, N. P., Bader, H. N., Makotkine, I., Chattopadhyay, M., Xu, C.,

Buxbaum Grice, A., Tocheva, A. S., Flory, J. D., Buxbaum, J. D., Meaney, M. J., Brennand, K., & Yehuda, R. (2019). Differential transcriptional response following glucocorticoid activation in cultured blood immune cells: a novel approach to PTSD biomarker development. *Translational Psychiatry*, *9*(1), 201. https://doi.org/10.1038/s41398-019-0539-x

Brivio, E., Lopez, J. P., & Chen, A. (2020). Sex differences: Transcriptional signatures of stress exposure in male and female brains. *Genes, Brain, and Behavior*, *19*(3), e12643. https://doi.org/10.1111/gbb.12643

Bryant, D. J., Oo, M., & Damian, A. J. (2020). The rise of adverse childhood experiences during the COVID-19 pandemic. *Psychological Trauma: Theory, Research, Practice and Policy*, *12*(S1), S193–S194. https://doi.org/10.1037/tra0000711

Bunney, W. E., Jr, & Davis, J. M. (1965). Norepinephrine in depressive reactions. A review. *Archives of General Psychiatry*, *13*(6), 483–494. https://doi.org/10.1001/archpsyc.1965.01730060001001

Burke, J., Loeber, R., & Birmaher, B. (2002). Oppositional defiant disorder and conduct disorder: a review of the past 10 years, part II. *Journal of the American Academy of Child and Adolescent Psychiatry*, *41*(11), 1275–1293. https://doi.org/10.1097/00004583-200211000-00009

Burke, W. J., Gergel, I., & Bose, A. (2002). Fixed-dose trial of the single isomer SSRI escitalopram in depressed outpatients. *The Journal of Clinical Psychiatry*, *63*(4), 331–336. https://doi.org/10.4088/jcp.v63n0410

Cahill, K. M., Huo, Z., Tseng, G. C., Logan, R. W., & Seney, M. L. (2018). Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach. *Scientific Reports*, *8*(1), 9588. https://doi.org/10.1038/s41598-018-27903-2

Carrier, N., & Kabbaj, M. (2013). Sex differences in the antidepressant-like effects of ketamine. *Neuropharmacology*, *70*, 27–34. https://doi.org/10.1016/j.neuropharm.2012.12.009

Chapman, D. P., Whitfield, C. L., Felitti, V. J., Dube, S. R., Edwards, V. J., & Anda, R. F. (2004). Adverse childhood experiences and the risk of depressive disorders in adulthood. *Journal of Affective Disorders*, *82*(2), 217–225. https://doi.org/10.1016/j.jad.2003.12.013

Copeland, W. E., Keeler, G., Angold, A., & Costello, E. J. (2007). Traumatic events and posttraumatic stress in childhood. *Archives of General Psychiatry*, *64*(5), 577–584. https://doi.org/10.1001/archpsyc.64.5.577

Copeland, W. E., Shanahan, L., Hinesley, J., Chan, R. F., Aberg, K. A., Fairbank, J. A., van den Oord, E. J. C. G., & Costello, E. J. (2018). Association of Childhood Trauma Exposure With Adult Psychiatric Disorders and Functional Outcomes. *JAMA Network Open*, *1*(7), e184493. https://doi.org/10.1001/jamanetworkopen.2018.4493

Coyle, C. M., & Laws, K. R. (2015). The use of ketamine as an antidepressant: a systematic review and meta-analysis. *Human Psychopharmacology*, *30*(3), 152–163. https://doi.org/10.1002/hup.2475

Culpepper, L. (2002). Escitalopram: A New SSRI for the Treatment of Depression in Primary Care. *Primary Care Companion to the Journal of Clinical Psychiatry*, *4*(6), 209–214. https://doi.org/10.4088/pcc.v04n0601

Danielsson, I., Blom, H., Nilses, C., Heimer, G., & Högberg, U. (2009). Gendered patterns of high violence exposure among Swedish youth. *Acta Obstetricia et Gynecologica Scandinavica*, *88*(5), 528–535. https://doi.org/10.1080/00016340902846056

Demaestri, C., Pan, T., Critz, M., Ofray, D., Gallo, M., & Bath, K. G. (2020). Type of early life adversity confers differential, sex-dependent effects on early maturational milestones in mice. *Hormones and Behavior*, *124*(104763), 104763. https://doi.org/10.1016/j.yhbeh.2020.104763

De Vries, G. J. (2004). Minireview: Sex differences in adult and developing brains: compensation, compensation, compensation. *Endocrinology*, *145*(3), 1063–1068. https://doi.org/10.1210/en.2003-1504

Dieterich, A., Liu, T., & Samuels, B. A. (2021). Chronic non-discriminatory social defeat stress reduces effort-related motivated behaviors in male and female mice. *Translational Psychiatry*, *11*(1), 125. https://doi.org/10.1038/s41398-021-01250-9

Dube, S. R., Anda, R. F., Whitfield, C. L., Brown, D. W., Felitti, V. J., Dong, M., & Giles, W. H. (2005). Long-term consequences of childhood sexual abuse by gender of victim. *American Journal of*

Preventive Medicine, *28*(5), 430–438. https://doi.org/10.1016/j.amepre.2005.01.015

Duclot, F., & Kabbaj, M. (2015). The estrous cycle surpasses sex differences in regulating the transcriptome in the rat medial prefrontal cortex and reveals an underlying role of early growth response 1. *Genome Biology*, *16*, 256. https://doi.org/10.1186/s13059-015-0815-x

Duman, R. S. (2018). The Dazzling Promise of Ketamine. *Cerebrum: The Dana Forum on Brain Science*, *2018*. https://www.ncbi.nlm.nih.gov/pubmed/30746033

Elfwing, M., Nätt, D., Goerlich-Jansson, V. C., Persson, M., Hjelm, J., & Jensen, P. (2015). Early stress causes sex-specific, life-long changes in behaviour, levels of gonadal hormones, and gene expression in chickens. *PloS One*, *10*(5), e0125808. https://doi.org/10.1371/journal.pone.0125808

English, J. (2023). *More than 300,000 grave violations against children in conflict verified worldwide in past 18 years*. https://www.unicef.org/press-releases/more-300000-grave-violations-against-children-conflict-verified-worldwide-past-18

Eriksen, A. M. A., Hansen, K. L., Javo, C., & Schei, B. (2015). Emotional, physical and sexual violence among Sami and non-Sami populations in Norway: The SAMINOR 2 questionnaire study. *Scandinavian Journal of Public Health*, *43*(6), 588–596. https://doi.org/10.1177/1403494815585936

Fayez, R., & Gupta, V. (2023). *Imipramine*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557656/

Fitzgerald, P. J., Kounelis-Wuillaume, S. K., Gheidi, A., Morrow, J. D., Spencer-Segal, J. L., & Watson, B. O. (2021). Sex- and stress-dependent effects of a single injection of ketamine on open field and forced swim behavior. *Stress* , *24*(6), 857–865. https://doi.org/10.1080/10253890.2021.1871600

Fitzgerald, P. J., Yen, J. Y., & Watson, B. O. (2019). Stress-sensitive antidepressant-like effects of ketamine in the mouse forced swim test. *PloS One*, *14*(4), e0215554. https://doi.org/10.1371/journal.pone.0215554

Fuh, S.-C., Fiori, L. M., Turecki, G., Nagy, C., & Li, Y. (2023). Multi-omic modeling of antidepressant response implicates dynamic immune and inflammatory changes in individuals who respond to

treatment. *PloS One*, *18*(5), e0285123. https://doi.org/10.1371/journal.pone.0285123

Furuse, K., Ukai, W., Hashimoto, E., Hashiguchi, H., Kigawa, Y., Ishii, T., Tayama, M., Deriha, K., Shiraishi, M., & Kawanishi, C. (2019). Antidepressant activities of escitalopram and blonanserin on prenatal and adolescent combined stress-induced depression model: Possible role of neurotrophic mechanism change in serum and nucleus accumbens. *Journal of Affective Disorders*, *247*, 97–104. https://doi.org/10.1016/j.jad.2019.01.007

Gee, D. G., Gabard-Durnam, L. J., Flannery, J., Goff, B., Humphreys, K. L., Telzer, E. H., Hare, T. A., Bookheimer, S. Y., & Tottenham, N. (2013). Early developmental emergence of human amygdala–prefrontal connectivity after maternal deprivation. *Proceedings of the National Academy of Sciences*, *110*(39), 15638–15643. https://doi.org/10.1073/pnas.1307893110

Gegenhuber, B., & Tollkuhn, J. (2020). Signatures of sex: Sex differences in gene expression in the vertebrate brain. *Wiley Interdisciplinary Reviews. Developmental Biology*, *9*(1), e348. https://doi.org/10.1002/wdev.348

Gerhard, D. M., & Duman, R. S. (2018). Rapid-Acting Antidepressants: Mechanistic Insights and Future Directions. *Current Behavioral Neuroscience Reports*, *5*(1), 36–47. https://doi.org/10.1016/j.psc.2011.11.005

Giano, Z., Wheeler, D. L., & Hubach, R. D. (2020). The frequencies and disparities of adverse childhood experiences in the U.S. *BMC Public Health*, *20*(1), 1327. https://doi.org/10.1186/s12889-020-09411-z

Gilles, E. E., Schultz, L., & Baram, T. Z. (1996). Abnormal corticosterone regulation in an immature rat model of continuous chronic stress. *Pediatric Neurology*, *15*(2), 114–119. https://doi.org/10.1016/0887-8994(96)00153-1

Gillman, P. K. (2007). Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *British Journal of Pharmacology*, *151*(6), 737–748. https://doi.org/10.1038/sj.bjp.0707253

Giovanardi, G., Vitelli, R., Maggiora Vergano, C., Fortunato, A., Chianura, L., Lingiardi, V., & Speranza, A. M. (2018). Attachment Patterns and Complex Trauma in a Sample of Adults Diagnosed with

Gender Dysphoria. *Frontiers in Psychology*, *9*, 60. https://doi.org/10.3389/fpsyg.2018.00060

Glicksberg, B. S., Li, L., Chen, R., Dudley, J., & Chen, B. (2019). Leveraging Big Data to Transform Drug Discovery. In *Methods in Molecular Biology* (pp. 91–118). https://doi.org/10.1007/978-1-4939-9089-4_6

Goodwill, H. L., Manzano-Nieves, G., Gallo, M., Lee, H.-I., Oyerinde, E., Serre, T., & Bath, K. G. (2019). Early life stress leads to sex differences in development of depressive-like outcomes in a mouse model. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *44*(4), 711–720. https://doi.org/10.1038/s41386-018-0195-5

Hammen, C., Henry, R., & Daley, S. E. (2000). Depression and sensitization to stressors among young women as a function of childhood adversity. *Journal of Consulting and Clinical Psychology*, *68*(5), 782–787. https://doi.org/10.1037//0022-006X.68.5.782

Hanson, J. L., Hariri, A. R., & Williamson, D. E. (2015). Blunted Ventral Striatum Development in Adolescence Reflects Emotional Neglect and Predicts Depressive Symptoms. *Biological Psychiatry*, *78*(9), 598–605. https://doi.org/10.1016/j.biopsych.2015.05.010

Hanson, J. L., Williams, A. V., Bangasser, D. A., & Peña, C. J. (2021). Impact of Early Life Stress on Reward Circuit Function and Regulation. *Frontiers in Psychiatry / Frontiers Research Foundation*, *12*, 1799. https://doi.org/10.3389/fpsyt.2021.744690

Harris, A. Z., Atsak, P., Bretton, Z. H., Holt, E. S., Alam, R., Morton, M. P., Abbas, A. I., Leonardo, E. D., Bolkan, S. S., Hen, R., & Gordon, J. A. (2018). A Novel Method for Chronic Social Defeat Stress in Female Mice. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(6), 1276–1283. https://doi.org/10.1038/npp.2017.259

Heim, C. M., Mayberg, H. S., Mletzko, T., Nemeroff, C. B., & Pruessner, J. C. (2013). Decreased cortical representation of genital somatosensory field after childhood sexual abuse. *The American Journal of Psychiatry*, *170*(6), 616–623. https://doi.org/10.1176/appi.ajp.2013.12070950

Heninger, G. R., Delgado, P. L., & Charney, D. S. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in

humans. *Pharmacopsychiatry*, *29*(1), 2–11. https://doi.org/10.1055/s-2007-979535

Herzberg, M. P., & Gunnar, M. R. (2020). Early life stress and brain function: Activity and connectivity associated with processing emotion and reward. *NeuroImage*, *209*, 116493. https://doi.org/10.1016/j.neuroimage.2019.116493

Hillis, S., Mercy, J., Amobi, A., & Kress, H. (2016). Global Prevalence of Past-year Violence Against Children: A Systematic Review and Minimum Estimates. *Pediatrics*, *137*(3), e20154079. https://doi.org/10.1542/peds.2015-4079

Hodes, G. E., & Epperson, C. N. (2019). Sex Differences in Vulnerability and Resilience to Stress Across the Life Span. *Biological Psychiatry*, *86*(6), 421–432. https://doi.org/10.1016/j.biopsych.2019.04.028

Hodes, G. E., Pfau, M. L., Purushothaman, I., Ahn, H. F., Golden, S. A., Christoffel, D. J., Magida, J., Brancato, A., Takahashi, A., Flanigan, M. E., Ménard, C., Aleyasin, H., Koo, J. W., Lorsch, Z. S., Feng, J., Heshmati, M., Wang, M., Turecki, G., Neve, R., … Russo, S. J. (2015). Sex Differences in Nucleus Accumbens Transcriptome Profiles Associated with Susceptibility versus Resilience to Subchronic Variable Stress. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *35*(50), 16362–16376. https://doi.org/10.1523/JNEUROSCI.1392-15.2015

Hodos, R. A., Kidd, B. A., Shameer, K., Readhead, B. P., & Dudley, J. T. (2016). *In silico* methods for drug repurposing and pharmacology. *Wiley Interdisciplinary Reviews. Systems Biology and Medicine*, *8*(3), 186–210. https://doi.org/10.1002/wsbm.1337

Hoffman, B. (2014). An Overview of Depression among Transgender Women. *Depression Research and Treatment*, *2014*, 394283. https://doi.org/10.1155/2014/394283

Houtepen, L. C., Vinkers, C. H., Carrillo-Roa, T., Hiemstra, M., van Lier, P. A., Meeus, W., Branje, S., Heim, C. M., Nemeroff, C. B., Mill, J., Schalkwyk, L. C., Creyghton, M. P., Kahn, R. S., Joëls, M., Binder, E. B., & Boks, M. P. M. (2016). Genome-wide DNA methylation levels and altered cortisol stress reactivity following childhood trauma in humans. *Nature Communications*, *7*(1), 10967. https://doi.org/10.1038/ncomms10967

Hovelsø, N., Sager, T. N., & Mørk, A. (2011). Combination of escitalopram and a 5-HT1A receptor

antagonist selectively decreases the extracellular levels of dopamine in the nucleus accumbens relative to striatum through 5-HT2C receptor stimulation; suggestive of antipsychotic potential. *Pharmacology, Biochemistry, and Behavior*, *97*(3), 479–485. https://doi.org/10.1016/j.pbb.2010.10.003

Humphreys, K. L., Gleason, M. M., Drury, S. S., Miron, D., Nelson, C. A., 3rd, Fox, N. A., & Zeanah, C. H. (2015). Effects of institutional rearing and foster care on psychopathology at age 12 years in Romania: follow-up of an open, randomised controlled trial. *The Lancet. Psychiatry*, *2*(7), 625–634. https://doi.org/10.1016/S2215-0366(15)00095-4

Jaric, I., Rocks, D., Cham, H., Herchek, A., & Kundakovic, M. (2019). Sex and Estrous Cycle Effects on Anxiety- and Depression-Related Phenotypes in a Two-Hit Developmental Stress Model. *Frontiers in Molecular Neuroscience*, *12*, 74. https://doi.org/10.3389/fnmol.2019.00074

Jaric, I., Rocks, D., Greally, J. M., Suzuki, M., & Kundakovic, M. (2019). Chromatin organization in the female mouse brain fluctuates across the oestrous cycle. *Nature Communications*, *10*(1), 2851. https://doi.org/10.1038/s41467-019-10704-0

Johnston, C. J., Fitzgerald, P. J., Gewarges, J. S., Watson, B. O., & Spencer-Segal, J. L. (2021). Ketamine decreases HPA axis reactivity to a novel stressor in male but not female mice. In *bioRxiv* (p. 2021.06.29.450387). https://doi.org/10.1101/2021.06.29.450387

Jonson-Reid, M., Kohl, P. L., & Drake, B. (2012). Child and adult outcomes of chronic child maltreatment. *Pediatrics*, *129*(5), 839–845. https://doi.org/10.1542/peds.2011-2529

Kasper, S., Sacher, J., Klein, N., Mossaheb, N., Attarbaschi-Steiner, T., Lanzenberger, R., Spindelegger, C., Asenbaum, S., Holik, A., & Dudczak, R. (2009). Differences in the dynamics of serotonin reuptake transporter occupancy may explain superior clinical efficacy of escitalopram versus citalopram. *International Clinical Psychopharmacology*, *24*(3), 119–125. https://doi.org/10.1097/YIC.0b013e32832a8ec8

Kaufman, J., Wymbs, N. F., Montalvo-Ortiz, J. L., Orr, C., Albaugh, M. D., Althoff, R., O'Loughlin, K., Holbrook, H., Garavan, H., Kearney, C., Yang, B.-Z., Zhao, H., Peña, C., Nestler, E. J., Lee, R. S.,

Mostofsky, S., Gelernter, J., & Hudziak, J. (2018). Methylation in OTX2 and related genes, maltreatment, and depression in children. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(11), 2204–2211. https://doi.org/10.1038/s41386-018-0157-y

Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C., & Eaves, L. J. (1995). Stressful life events, genetic liability, and onset of an episode of major depression in women. *The American Journal of Psychiatry*, *152*(6), 833–842. https://doi.org/10.1176/ajp.152.6.833

Kessler, R. C., Davis, C. G., & Kendler, K. S. (1997). Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. *Psychological Medicine*, *27*(5), 1101–1119. https://doi.org/10.1017/s0033291797005588

Khan, A., Brodhead, A. E., Schwartz, K. A., Kolts, R. L., & Brown, W. A. (2005). Sex differences in antidepressant response in recent antidepressant clinical trials. *Journal of Clinical Psychopharmacology*, *25*(4), 318–324. https://doi.org/10.1097/01.jcp.0000168879.03169.ce

Khorramzadeh, E., & Lotfy, A. O. (1973). The use of ketamine in psychiatry. *Psychosomatics*, *14*(6), 344–346. https://doi.org/10.1016/S0033-3182(73)71306-2

Kim, J.-W., Suzuki, K., Kavalali, E. T., & Monteggia, L. M. (2023). Bridging rapid and sustained antidepressant effects of ketamine. *Trends in Molecular Medicine*, *29*(5), 364–375. https://doi.org/10.1016/j.molmed.2023.02.003

Kohn, Y., Zislin, J., Agid, O., Hanin, B., Troudart, T., Shapira, B., Bloch, M., Gur, E., Ritsner, M., & Lerer, B. (2001). Increased prevalence of negative life events in subtypes of major depressive disorder. *Comprehensive Psychiatry*, *42*(1), 57–63. https://doi.org/10.1053/comp.2001.19753

Kornstein, S. G., Schatzberg, A. F., Thase, M. E., Yonkers, K. A., McCullough, J. P., Keitner, G. I., Gelenberg, A. J., Davis, S. M., Harrison, W. M., & Keller, M. B. (2000). Gender differences in treatment response to sertraline versus imipramine in chronic depression. *The American Journal of Psychiatry*, *157*(9), 1445–1452. https://doi.org/10.1176/appi.ajp.157.9.1445

Krishnan, V., Han, M.-H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., Laplant, Q., Graham, A.,

Lutter, M., Lagace, D. C., Ghose, S., Reister, R., Tannous, P., Green, T. A., Neve, R. L., Chakravarty, S., Kumar, A., Eisch, A. J., Self, D. W., … Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018

Kronman, H., Torres-Berrío, A., Sidoli, S., Issler, O., Godino, A., Ramakrishnan, A., Mews, P., Lardner, C. K., Parise, E. M., Walker, D. M., van der Zee, Y. Y., Browne, C. J., Boyce, B. F., Neve, R., Garcia, B. A., Shen, L., Peña, C. J., & Nestler, E. J. (2021). Long-term behavioral and cell-type-specific molecular effects of early life stress are mediated by H3K79me2 dynamics in medium spiny neurons. *Nature Neuroscience*. https://doi.org/10.1038/s41593-021-00814-8

Krystal, J. H., Abdallah, C. G., Sanacora, G., Charney, D. S., & Duman, R. S. (2019). Ketamine: A Paradigm Shift for Depression Research and Treatment. *Neuron*, *101*(5), 774–778. https://doi.org/10.1016/j.neuron.2019.02.005

Labonté, B., Engmann, O., Purushothaman, I., Menard, C., Wang, J., Tan, C., Scarpa, J. R., Moy, G., Loh, Y.-H. E., Cahill, M., Lorsch, Z. S., Hamilton, P. J., Calipari, E. S., Hodes, G. E., Issler, O., Kronman, H., Pfau, M., Obradovic, A. L. J., Dong, Y., … Nestler, E. J. (2017). Sex-specific transcriptional signatures in human depression. *Nature Medicine*, *23*(9), 1102–1111. https://doi.org/10.1038/nm.4386

Labonté, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., Bureau, A., Mechawar, N., Szyf, M., Meaney, M. J., & Turecki, G. (2012). Genome-wide epigenetic regulation by early-life trauma. *Archives of General Psychiatry*, *69*(7), 722–731. https://doi.org/10.1001/archgenpsychiatry.2011.2287

Landy, K., Rosani, A., & Estevez, R. (2023). *Escitalopram*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557734/

Law, C. W., Chen, Y., Shi, W., & Smyth, G. K. (2014). voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology*, *15*(2), R29. https://doi.org/10.1186/gb-2014-15-2-r29

LeGates, T. A., Kvarta, M. D., & Thompson, S. M. (2019). Sex differences in antidepressant efficacy. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *44*(1), 140–154. https://doi.org/10.1038/s41386-018-0156-z

Lewis, C. C., Simons, A. D., Nguyen, L. J., Murakami, J. L., Reid, M. W., Silva, S. G., & March, J. S. (2010). Impact of childhood trauma on treatment outcome in the Treatment for Adolescents with Depression Study (TADS). *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(2), 132–140. https://doi.org/10.1097/00004583-201002000-00007

Liebowitz, M. R., Manley, A. L., Padmanabhan, S. K., Ganguly, R., Tummala, R., & Tourian, K. A. (2008). Efficacy, safety, and tolerability of desvenlafaxine 50 mg/day and 100 mg/day in outpatients with major depressive disorder. *Current Medical Research and Opinion*, *24*(7), 1877–1890. https://doi.org/10.1185/03007990802161923

Li, M., Santpere, G., Imamura Kawasawa, Y., Evgrafov, O. V., Gulden, F. O., Pochareddy, S., Sunkin, S. M., Li, Z., Shin, Y., Zhu, Y., Sousa, A. M. M., Werling, D. M., Kitchen, R. R., Kang, H. J., Pletikos, M., Choi, J., Muchnik, S., Xu, X., Wang, D., … Sestan, N. (2018). Integrative functional genomic analysis of human brain development and neuropsychiatric risks. *Science*, *362*(6420). https://doi.org/10.1126/science.aat7615

Lippus, H., Soo, K., Laanpere, M., Yount, K. M., Part, K., Ringmets, I., Ainsaar, M., & Karro, H. (2020). The prevalence and patterns of exposure to interpersonal violence among men and women in Estonia. *PloS One*, *15*(8), e0237562. https://doi.org/10.1371/journal.pone.0237562

Lopez, J., & Bagot, R. C. (2021). Defining Valid Chronic Stress Models for Depression With Female Rodents. *Biological Psychiatry*, *90*(4), 226–235. https://doi.org/10.1016/j.biopsych.2021.03.010

Lourenco, M. T. C., & Kennedy, S. H. (2009). Desvenlafaxine in the treatment of major depressive disorder. *Neuropsychiatric Disease and Treatment*, *5*, 127–136. https://doi.org/10.2147/ndt.s3360

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Lutz, P.-E., Tanti, A., Gasecka, A., Barnett-Burns, S., Kim, J. J., Zhou, Y., Chen, G. G., Wakid, M., Shaw, M., Almeida, D., Chay, M.-A., Yang, J., Larivière, V., M'Boutchou, M.-N., van Kempen, L. C., Yerko, V., Prud'homme, J., Davoli, M. A., Vaillancourt, K., … Turecki, G. (2017). Association of a history of child abuse with impaired myelination in the anterior cingulate cortex: Convergent epigenetic, transcriptional, and morphological evidence. *The American Journal of Psychiatry*, *174*(12), 1185–1194. https://doi.org/10.1176/appi.ajp.2017.16111286

Marrocco, J., Gray, J. D., Kogan, J. F., Einhorn, N. R., O'Cinneide, E. M., Rubin, T. G., Carroll, T. S., Schmidt, E. F., & McEwen, B. S. (2019). Early Life Stress Restricts Translational Reactivity in CA3 Neurons Associated With Altered Stress Responses in Adulthood. *Frontiers in Behavioral Neuroscience*, *13*, 157. https://doi.org/10.3389/fnbeh.2019.00157

Massart, R., Nemoda, Z., Suderman, M. J., Sutti, S., Ruggiero, A. M., Dettmer, A. M., Suomi, S. J., & Szyf, M. (2016). Early life adversity alters normal sex-dependent developmental dynamics of DNA methylation. *Development and Psychopathology*, *28*(4pt2), 1259–1272. https://doi.org/10.1017/s0954579416000833

Mastrodonato, A., Pavlova, I., Kee, N., McGowan, J. C., Mann, J. J., & Denny, C. A. (2022). Acute (R,S)-Ketamine Administration Induces Sex-Specific Behavioral Effects in Adolescent but Not Aged Mice. *Frontiers in Neuroscience*, *16*, 852010. https://doi.org/10.3389/fnins.2022.852010

McCarthy, M. M., Arnold, A. P., Ball, G. F., Blaustein, J. D., & De Vries, G. J. (2012). Sex differences in the brain: the not so inconvenient truth. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*(7), 2241–2247. https://doi.org/10.1523/JNEUROSCI.5372-11.2012

McGowan, J. C., Hill, C., Mastrodonato, A., LaGamma, C. T., Kitayev, A., Brachman, R. A., Narain, N. R., Kiebish, M. A., & Denny, C. A. (2018). Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(9), 1813–1821. https://doi.org/10.1038/s41386-018-0043-7

McGowan, J. C., LaGamma, C. T., Lim, S. C., Tsitsiklis, M., Neria, Y., Brachman, R. A., & Denny, C. A.

(2017). Prophylactic Ketamine Attenuates Learned Fear. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *42*(8), 1577–1589. https://doi.org/10.1038/npp.2017.19

McLaughlin, K. A., Conron, K. J., Koenen, K. C., & Gilman, S. E. (2010). Childhood adversity, adult stressful life events, and risk of past-year psychiatric disorder: a test of the stress sensitization hypothesis in a population-based sample of adults. *Psychological Medicine*, *40*(10), 1647–1658. https://doi.org/10.1017/S0033291709992121

McLaughlin, K. A., Koenen, K. C., Hill, E. D., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2013). Trauma exposure and posttraumatic stress disorder in a national sample of adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(8), 815–830.e14. https://doi.org/10.1016/j.jaac.2013.05.011

McLaughlin, K. A., Sheridan, M. A., & Lambert, H. K. (2014). Childhood adversity and neural development: deprivation and threat as distinct dimensions of early experience. *Neuroscience and Biobehavioral Reviews*, *47*, 578–591. https://doi.org/10.1016/j.neubiorev.2014.10.012

McLaughlin, K. A., Weissman, D., & Bitrán, D. (2019). Childhood Adversity and Neural Development: A Systematic Review. *Annual Review of Developmental Psychology*, *1*, 277–312. https://doi.org/10.1146/annurev-devpsych-121318-084950

Minami, S., Satoyoshi, H., Ide, S., Inoue, T., Yoshioka, M., & Minami, M. (2017). Suppression of reward-induced dopamine release in the nucleus accumbens in animal models of depression: Differential responses to drug treatment. *Neuroscience Letters*, *650*, 72–76. https://doi.org/10.1016/j.neulet.2017.04.028

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *17*(8), 2921–2927. https://doi.org/10.1523/JNEUROSCI.17-08-02921.1997

Molet, J., Heins, K., Zhuo, X., Mei, Y. T., Regev, L., Baram, T. Z., & Stern, H. (2016). Fragmentation and high entropy of neonatal experience predict adolescent emotional outcome. *Translational Psychiatry*, *6*(1), e702. https://doi.org/10.1038/tp.2015.200

Molet, J., Maras, P. M., Avishai-Eliner, S., & Baram, T. Z. (2014). Naturalistic rodent models of chronic early-life stress. *Developmental Psychobiology*, *56*(8), 1675–1688. https://doi.org/10.1002/dev.21230

Morrison, K. E., Cole, A. B., Kane, P. J., Meadows, V. E., Thompson, S. M., & Bale, T. L. (2020). Pubertal adversity alters chromatin dynamics and stress circuitry in the pregnant brain. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *45*(8), 1263–1271. https://doi.org/10.1038/s41386-020-0634-y

Morrison, K. E., Epperson, C. N., Sammel, M. D., Ewing, G., Podcasy, J. S., Hantsoo, L., Kim, D. R., & Bale, T. L. (2017). Preadolescent Adversity Programs a Disrupted Maternal Stress Reactivity in Humans and Mice. *Biological Psychiatry*, *81*(8), 693–701. https://doi.org/10.1016/j.biopsych.2016.08.027

Murthy, S., & Gould, E. (2020). How Early Life Adversity Influences Defensive Circuitry. *Trends in Neurosciences*, *43*(4), 200–212. https://doi.org/10.1016/j.tins.2020.02.001

Nanni, V., Uher, R., & Danese, A. (2012). Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *The American Journal of Psychiatry*, *169*(2), 141–151. https://doi.org/10.1176/appi.ajp.2011.11020335

Naseeruddin, R., Rosani, A., & Marwaha, R. (2023). *Desvenlafaxine*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK534829/

Nemeroff, C. B., & Binder, E. (2014). The preeminent role of childhood abuse and neglect in vulnerability to major psychiatric disorders: toward elucidating the underlying neurobiological mechanisms [Review of *The preeminent role of childhood abuse and neglect in vulnerability to major psychiatric disorders: toward elucidating the underlying neurobiological mechanisms*]. *Journal of the American Academy of Child and Adolescent Psychiatry*, *53*(4), 395–397. https://doi.org/10.1016/j.jaac.2014.02.004

Nemeroff, C. B., Heim, C. M., Thase, M. E., Klein, D. N., Rush, A. J., Schatzberg, A. F., Ninan, P. T., McCullough, J. P., Jr, Weiss, P. M., Dunner, D. L., Rothbaum, B. O., Kornstein, S., Keitner, G., & Keller, M. B. (2003). Differential responses to psychotherapy versus pharmacotherapy in patients with chronic forms of major depression and childhood trauma. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(24), 14293–14296. https://doi.org/10.1073/pnas.2336126100

Nichols, K. A., & Paciullo, C. A. (2019). Subdissociative Ketamine Use in the Emergency Department. *Advanced Emergency Nursing Journal*, *41*(1), 15–22. https://doi.org/10.1097/TME.0000000000000222

Oberlander, J. G., & Woolley, C. S. (2017). 17β-estradiol acutely potentiates glutamatergic synaptic transmission in the hippocampus through distinct mechanisms in males and females. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *37*(50), 12314–12327. https://doi.org/10.1523/jneurosci.3011-17.2017

O'Brien, B., Lijffijt, M., Lee, J., Kim, Y. S., Wells, A., Murphy, N., Ramakrishnan, N., Swann, A. C., & Mathew, S. J. (2021). Distinct trajectories of antidepressant response to intravenous ketamine. *Journal of Affective Disorders*, *286*, 320–329. https://doi.org/10.1016/j.jad.2021.03.006

O'Brien, B., Lijffijt, M., Wells, A., Swann, A. C., & Mathew, S. J. (2019). The Impact of Childhood Maltreatment on Intravenous Ketamine Outcomes for Adult Patients with Treatment-Resistant Depression. *Pharmaceuticals* , *12*(3), 133. https://doi.org/10.3390/ph12030133

Ordoñes Sanchez, E., Bavley, C. C., Deutschmann, A. U., Carpenter, R., Peterson, D. R., Karbalaei, R., Flowers, J., 2nd, Rogers, C. M., Langrehr, M. G., Ardekani, C. S., Famularo, S. T., Bongiovanni, A. R., Knouse, M. C., Floresco, S. B., Briand, L. A., Wimmer, M. E., & Bangasser, D. A. (2021). Early life adversity promotes resilience to opioid addiction-related phenotypes in male rats and sex-specific transcriptional changes. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(8). https://doi.org/10.1073/pnas.2020173118

Paden, W., Barko, K., Puralewski, R., Cahill, K. M., Huo, Z., Shelton, M. A., Tseng, G. C., Logan, R. W.,

& Seney, M. L. (2020). Sex differences in adult mood and in stress-induced transcriptional coherence across mesocorticolimbic circuitry. *Translational Psychiatry*, *10*(1), 59. https://doi.org/10.1038/s41398-020-0742-9

Pape, J. C., Carrillo-Roa, T., Rothbaum, B. O., Nemeroff, C. B., Czamara, D., Zannas, A. S., Iosifescu, D., Mathew, S. J., Neylan, T. C., Mayberg, H. S., Dunlop, B. W., & Binder, E. B. (2018). DNA methylation levels are associated with CRF1 receptor antagonist treatment outcome in women with post-traumatic stress disorder. *Clinical Epigenetics*, *10*(1), 136. https://doi.org/10.1186/s13148-018-0569-x

Parel, S. T., Bennett, S. N., Cheng, C. J., Timmermans, O. C., Fiori, L. M., Turecki, G., & Peña, C. J. (2023). Transcriptional signatures of early-life stress and antidepressant treatment efficacy. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(49), e2305776120. https://doi.org/10.1073/pnas.2305776120

Parel, S. T., & Peña, C. J. (2022). Genome-wide Signatures of Early-Life Stress: Influence of Sex. *Biological Psychiatry*, *91*(1), 36–42. https://doi.org/10.1016/j.biopsych.2020.12.010

Pellicane, M. J., & Ciesla, J. A. (2022). Associations between minority stress, depression, and suicidal ideation and attempts in transgender and gender diverse (TGD) individuals: Systematic review and meta-analysis. *Clinical Psychology Review*, *91*, 102113. https://doi.org/10.1016/j.cpr.2021.102113

Peña, C. J., Kronman, H. G., Walker, D. M., Cates, H. M., Bagot, R. C., Purushothaman, I., Issler, O., Loh, Y.-H. E., Leong, T., Kiraly, D. D., Goodman, E., Neve, R. L., Shen, L., & Nestler, E. J. (2017). Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science*, *356*(6343), 1185–1188. https://doi.org/10.1126/science.aan4491

Peña, C. J., Nestler, E. J., & Bagot, R. C. (2019). Environmental Programming of Susceptibility and Resilience to Stress in Adulthood in Male Mice. *Frontiers in Behavioral Neuroscience*, *13*, 40. https://doi.org/10.3389/fnbeh.2019.00040

Peña, C. J., Smith, M., Ramakrishnan, A., Cates, H. M., Bagot, R. C., Kronman, H. G., Patel, B., Chang, A. B., Purushothaman, I., Dudley, J., Morishita, H., Shen, L., & Nestler, E. J. (2019). Early life stress

alters transcriptomic patterning across reward circuitry in male and female mice. *Nature Communications*, *10*(1), 5098. https://doi.org/10.1038/s41467-019-13085-6

Plaisier, S. B., Taschereau, R., Wong, J. A., & Graeber, T. G. (2010). Rank-rank hypergeometric overlap: identification of statistically significant overlap between gene-expression signatures. *Nucleic Acids Research*, *38*(17), e169. https://doi.org/10.1093/nar/gkq636

Polter, A. M., & Kauer, J. A. (2014). Stress and VTA synapses: implications for addiction and depression. *The European Journal of Neuroscience*, *39*(7), 1179–1188. https://doi.org/10.1111/ejn.12490

Raskin, A. (1974). Age-sex differences in response to antidepressant drugs. *The Journal of Nervous and Mental Disease*, *159*(2), 120–130. https://doi.org/10.1097/00005053-197408000-00006

Rice, C. J., Sandman, C. A., Lenjavi, M. R., & Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology*, *149*(10), 4892–4900. https://doi.org/10.1210/en.2008-0633

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7), e47. https://doi.org/10.1093/nar/gkv007

Romeo, R. D., Mueller, A., Sisti, H. M., Ogawa, S., McEwen, B. S., & Brake, W. G. (2003). Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Hormones and Behavior*, *43*(5), 561–567. https://doi.org/10.1016/s0018-506x(03)00063-1

Rosenbaum, S. B., Gupta, V., Patel, P., & Palacios, J. L. (2023). *Ketamine*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK470357/

Rowson, S. A., Bekhbat, M., Kelly, S. D., Binder, E. B., Hyer, M. M., Shaw, G., Bent, M. A., Hodes, G., Tharp, G., Weinshenker, D., Qin, Z., & Neigh, G. N. (2019). Chronic adolescent stress sex-specifically alters the hippocampal transcriptome in adulthood. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *44*(7), 1207–1215. https://doi.org/10.1038/s41386-019-0321-z

Russo, S. J., & Nestler, E. J. (2013). The brain reward circuitry in mood disorders. *Nature Reviews.*

Neuroscience, *14*(9), 609–625. https://doi.org/10.1038/nrn3381

Sánchez, C., Bergqvist, P. B. F., Brennum, L. T., Gupta, S., Hogg, S., Larsen, A., & Wiborg, O. (2003). Escitalopram, the S-(+)-enantiomer of citalopram, is a selective serotonin reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities. *Psychopharmacology*, *167*(4), 353–362. https://doi.org/10.1007/s00213-002-1364-z

Sansone, R. A., & Sansone, L. A. (2014). Serotonin norepinephrine reuptake inhibitors: a pharmacological comparison. *Innovations in Clinical Neuroscience*, *11*(3-4), 37–42. https://www.ncbi.nlm.nih.gov/pubmed/24800132

Scarpa, J. R., Fatma, M., Loh, Y.-H. E., Traore, S. R., Stefan, T., Chen, T. H., Nestler, E. J., & Labonté, B. (2020). Shared Transcriptional Signatures in Major Depressive Disorder and Mouse Chronic Stress Models. *Biological Psychiatry*, *88*(2), 159–168. https://doi.org/10.1016/j.biopsych.2019.12.029

Schaeffer, C. M., Petras, H., Ialongo, N., Poduska, J., & Kellam, S. (2003). Modeling growth in boys' aggressive behavior across elementary school: links to later criminal involvement, conduct disorder, and antisocial personality disorder. *Developmental Psychology*, *39*(6), 1020–1035. https://doi.org/10.1037/0012-1649.39.6.1020

Scheuplein, M., Vermeulen, S., van Harmelen, A.-L., & Alink, L. (2023). Child maltreatment and victimization. *Handbook of Clinical Neurology*, *197*, 147–160. https://doi.org/10.1016/B978-0-12-821375-9.00001-3

Schildkraut, J. J. (1995). The catecholamine hypothesis of affective disorders: a review of supporting evidence. 1965. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *7*(4), 524–533; discussion 523–524. https://doi.org/10.1176/jnp.7.4.524

Schnarrs, P. W., Stone, A. L., Salcido, R., Jr, Baldwin, A., Georgiou, C., & Nemeroff, C. B. (2019). Differences in adverse childhood experiences (ACEs) and quality of physical and mental health between transgender and cisgender sexual minorities. *Journal of Psychiatric Research*, *119*, 1–6. https://doi.org/10.1016/j.jpsychires.2019.09.001

Scott, K. M., McLaughlin, K. A., Smith, D. A. R., & Ellis, P. M. (2012). Childhood maltreatment and

DSM-IV adult mental disorders: comparison of prospective and retrospective findings. *The British Journal of Psychiatry: The Journal of Mental Science*, *200*(6), 469–475. https://doi.org/10.1192/bjp.bp.111.103267

Self-Brown, S., Lai, B. S., Thompson, J. E., McGill, T., & Kelley, M. L. (2013). Posttraumatic stress disorder symptom trajectories in Hurricane Katrina affected youth. *Journal of Affective Disorders*, *147*(1-3), 198–204. https://doi.org/10.1016/j.jad.2012.11.002

Seney, M. L., Huo, Z., Cahill, K., French, L., Puralewski, R., Zhang, J., Logan, R. W., Tseng, G., Lewis, D. A., & Sibille, E. (2018). Opposite Molecular Signatures of Depression in Men and Women. *Biological Psychiatry*, *84*(1), 18–27. https://doi.org/10.1016/j.biopsych.2018.01.017

Shalaby, A., & Kamal, S. (2009). Effect of Escitalopram on GABA level and anti-oxidant markers in prefrontal cortex and nucleus accumbens of chronic mild stress-exposed albino rats. *International Journal of Physiology, Pathophysiology and Pharmacology*, *1*(2), 154–161. https://www.ncbi.nlm.nih.gov/pubmed/21383885

Shansky, R. M. (2019). Are hormones a “female problem” for animal research? *Science (New York, N.Y.)*, *364*(6443), 825–826. https://doi.org/10.1126/science.aaw7570

Sheridan, M. A., & McLaughlin, K. A. (2014). Dimensions of early experience and neural development: deprivation and threat. *Trends in Cognitive Sciences*, *18*(11), 580–585. https://doi.org/10.1016/j.tics.2014.09.001

Sidamon-Eristoff, A. E., Cohodes, E. M., Gee, D. G., & Peña, C. J. (2022). Trauma exposure and mental health outcomes among Central American and Mexican children held in immigration detention at the United States-Mexico border. *Developmental Psychobiology*, *64*(1), e22227. https://doi.org/10.1002/dev.22227

Smith, M. E. (2005). Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: a meta-analysis of structural MRI studies. *Hippocampus*, *15*(6), 798–807. https://doi.org/10.1002/hipo.20102

Smyke, A. T., Koga, S. F., Johnson, D. E., Fox, N. A., Marshall, P. J., Nelson, C. A., Zeanah, C. H., &

BEIP Core Group. (2007). The caregiving context in institution-reared and family-reared infants and toddlers in Romania. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *48*(2), 210–218. https://doi.org/10.1111/j.1469-7610.2006.01694.x

Song, W., Shen, Y., Zhang, Y., Peng, S., Zhang, R., Ning, A., Li, H., Li, X., Lin, G. N., & Yu, S. (2019). Expression alteration of microRNAs in Nucleus Accumbens is associated with chronic stress and antidepressant treatment in rats. *BMC Medical Informatics and Decision Making*, *19*(Suppl 6), 271. https://doi.org/10.1186/s12911-019-0964-z

Steiner, M., Dunn, E., & Born, L. (2003). Hormones and mood: from menarche to menopause and beyond. *Journal of Affective Disorders*, *74*(1), 67–83. https://doi.org/10.1016/s0165-0327(02)00432-9

Takahashi, A., Chung, J.-R., Zhang, S., Zhang, H., Grossman, Y., Aleyasin, H., Flanigan, M. E., Pfau, M. L., Menard, C., Dumitriu, D., Hodes, G. E., McEwen, B. S., Nestler, E. J., Han, M.-H., & Russo, S. J. (2017). Establishment of a repeated social defeat stress model in female mice. *Scientific Reports*, *7*(1), 12838. https://doi.org/10.1038/s41598-017-12811-8

Teicher, M. H. (2018). Childhood trauma and the enduring consequences of forcibly separating children from parents at the United States border. *BMC Medicine*, *16*(1), 146. https://doi.org/10.1186/s12916-018-1147-y

Tourigny, M., Hébert, M., Joly, J., Cyr, M., & Baril, K. (2008). Prevalence and co-occurrence of violence against children in the Quebec population. *Australian and New Zealand Journal of Public Health*, *32*(4), 331–335. https://doi.org/10.1111/j.1753-6405.2008.00250.x

UNICEF. (2020, September 24). *Violence against children*. https://www.unicef.org/protection/violence-against-children

U.S. Department of Health & Human Services, Administration for Children and Families, Administration on Children, Youth and Families, Children's Bureau. (2023). *Child Maltreatment 2021*. https://www.acf.hhs.gov/cb/data-research/child-maltreatment

von Werthern, M., Robjant, K., Chui, Z., Schon, R., Ottisova, L., Mason, C., & Katona, C. (2018). The

impact of immigration detention on mental health: a systematic review. *BMC Psychiatry*, *18*(1), 382. https://doi.org/10.1186/s12888-018-1945-y

Vythilingam, M., Heim, C., Newport, J., Miller, A. H., Anderson, E., Bronen, R., Brummer, M., Staib, L., Vermetten, E., Charney, D. S., Nemeroff, C. B., & Bremner, J. D. (2002). Childhood trauma associated with smaller hippocampal volume in women with major depression. *The American Journal of Psychiatry*, *159*(12), 2072–2080. https://doi.org/10.1176/appi.ajp.159.12.2072

Walker, D. M., Bell, M. R., Flores, C., Gulley, J. M., Willing, J., & Paul, M. J. (2017). Adolescence and Reward: Making Sense of Neural and Behavioral Changes Amid the Chaos. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *37*(45), 10855–10866. https://doi.org/10.1523/JNEUROSCI.1834-17.2017

Walker, D. M., Zhou, X., Ramakrishnan, A., Cates, H. M., Cunningham, A. M., Peña, C. J., Bagot, R. C., Issler, O., Van der Zee, Y., Lipschultz, A. P., Godino, A., Browne, C. J., Hodes, G. E., Parise, E. M., Torres-Berrio, A., Kennedy, P. J., Shen, L., Zhang, B., & Nestler, E. J. (2020). *Adolescent Social Isolation Reprograms the Medial Amygdala: Transcriptome and Sex Differences in Reward*.

White, J. D., & Kaffman, A. (2019). The Moderating Effects of Sex on Consequences of Childhood Maltreatment: From Clinical Studies to Animal Models. *Frontiers in Neuroscience*, *13*, 1082. https://doi.org/10.3389/fnins.2019.01082

Williams, L. M., Debattista, C., Duchemin, A.-M., Schatzberg, A. F., & Nemeroff, C. B. (2016). Childhood trauma predicts antidepressant response in adults with major depression: data from the randomized international study to predict optimized treatment for depression. *Translational Psychiatry*, *6*(5), e799. https://doi.org/10.1038/tp.2016.61

Witcomb, G. L., Bouman, W. P., Claes, L., Brewin, N., Crawford, J. R., & Arcelus, J. (2018). Levels of depression in transgender people and its predictors: Results of a large matched control study with transgender people accessing clinical services. *Journal of Affective Disorders*, *235*, 308–315. https://doi.org/10.1016/j.jad.2018.02.051

Wright, K. N., & Kabbaj, M. (2018). Sex differences in sub-anesthetic ketamine's antidepressant effects

and abuse liability. *Current Opinion in Behavioral Sciences*, *23*, 36–41. https://doi.org/10.1016/j.cobeha.2018.02.001

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The Innovation Journal: The Public Sector Innovation Journal*, *2*(3), 100141. https://doi.org/10.1016/j.xinn.2021.100141

Yang, B.-Z., Zhang, H., Ge, W., Weder, N., Douglas-Palumberi, H., Perepletchikova, F., Gelernter, J., & Kaufman, J. (2013). Child abuse and epigenetic mechanisms of disease risk. *American Journal of Preventive Medicine*, *44*(2), 101–107. https://doi.org/10.1016/j.amepre.2012.10.012

Yang, R. Y., Quan, J., Sodaei, R., Aguet, F., Segrè, A. V., Allen, J. A., Lanz, T. A., Reinhart, V., Crawford, M., Hasson, S., GTEx Consortium, Ardlie, K. G., Guigó, R., & Xi, H. S. (2018). A systematic survey of human tissue-specific gene expression and splicing reveals new opportunities for therapeutic target identification and evaluation. In *bioRxiv* (p. 311563). https://doi.org/10.1101/311563

Yohn, C. N., Dieterich, A., Bazer, A. S., Maita, I., Giedraitis, M., & Samuels, B. A. (2019). Chronic non-discriminatory social defeat is an effective chronic stress paradigm for both male and female mice. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *44*(13), 2220–2229. https://doi.org/10.1038/s41386-019-0520-7

Yu, G., Wang, L.-G., Han, Y., & He, Q.-Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics: A Journal of Integrative Biology*, *16*(5), 284–287. https://doi.org/10.1089/omi.2011.0118