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Modulation of Acute Stress Transcriptional Signatures by Sex and History of Stress at Single-Cell Resolution

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Publication Statement

The work presented in this PhD thesis is currently part of two manuscripts in preparation for submission to peer-reviewed journals with the following titles “Sex shapes cell-type-specific transcriptional signatures of stress in the mouse hypothalamic paraventricular nucleus”, and “Female-specific GABAergic response is estrogen-dependent”. The appendix also includes a first-author review article (Brivio et al., Genes, Brain Behav., 2020) and a first-author research article (Karamihalev & Brivio et al., eLife, 2020) that were published during the PhD studies.

Most of the content and figures in the Results, Materials and Methods sections were adapted from said manuscripts.

Abstract

Psychiatric disorders are the rising medical challenge of the 21st century due to their recent rise in prevalence, world-spread incidence, and burden on patients' life. Among all psychiatric disorders, stress-related psychiatric disorders rank the highest for societal burden. This category includes disorders characterized by a strong influence of stress exposure onto their onset and progression, such as depression and anxiety disorders.

Stress-related psychiatric disorders clinically manifest in women and men with different incidence, symptoms, comorbidities, impact on quality of life, and treatment efficacy and compliance. While these aspects have been thoroughly characterised in the recent years from a clinical point of view, we still lack a lot of information on how sex is able to shapes so many aspects of these disorders and how it interacts with main environmental factors that influences them: stress.

Molecularly speaking, sex has been shown to impact on the stress response of several brain regions. Good evidence suggests that some of these sex differences might stem by sex-specific response at a cell type level. This thesis aims to verify this hypothesis in the adult mouse paraventricular nucleus of the hypothalamus using single-cell RNA-sequencing. As such, we generated a large single-cell RNA-sequencing dataset using the latest technology from male and female mice that either did not experience any stress (controls) or experienced acute restraint stress with or without a previous experience of chronic mild stress. We combined bioinformatic and molecular approaches to characterise the transcriptional response to acute stress, and describe how it differs between cell types, how it is shaped by sex, and how it is impacted by previous chronic stress exposure.

We showed that the transcriptional response to acute stress is strongly cell-type- and sex-specific with limited overlap between different cells and different sexes. We identified circulating hormones as a source for some of these sex-difference, especially for the GABAergic neurons. We further characterized the impact of chronic stress exposure in changing these transcriptional signatures, identifying a different degree of change based on the cell type and the sex. We also identified the oligodendrocytes as the cell type whose response to acute stress is the most affected by a previous exposure to chronic stress. Ultimately, we further characterized the basal transcriptional and morphological sex differences of oligodendrocytes that likely resulted in different responses and different developmental states after stress.

Altogether, this thesis describes a new rich dataset and provides evidence for cell-type-specific contributions to the sex dimorphisms in stress. It also identifies several cell types of interest — such as AVP neurons, tanycytes, GABAergic neurons, and oligodendrocytes — worth of further investigation to better understand sex differences in the stress response. Finally, we also provide an interactive and easily accessible platform for anyone to explore the dataset and ask their own set of research questions. This work contributes to the general understanding of sex differences at a molecular level, providing new target cell types and genes of interest for future translational studies with the ultimate goal of enabling personalized medicine in stress-related disorders in the near future.

“Il corpo faccia quello che vuole. Io non sono il corpo. Io sono la *mente*.

E quando muore il corpo?

Sopravvive quello che hai fatto. Il messaggio che hai dato".

Rita Levi-Montalcini

“My body can do whatever it wants. I am not the body. I am the mind.

And what happens when the body dies?

What you have achieved survives. The message you spread.”

Rita Levi-Montalcini

Contents

List of Abbreviations

aCSF	artificial cerebrospinal fluid
ACTH	adrenocorticotropic hormone
AD	Alzheimer's disease
ADHD	attention-deficit/hyperactivity disorder
ANOVA	analysis of variance
ARS	acute restraint stress
ASD	autism spectrum disorder
AUC	area under the curve
AVP	arginine vasopressin
BBB	blood brain barrier
BH	Benjamini-Hochberg
BrdU	Bromodeoxyuridine
CMS	chronic mild stress
COP	committed oligodendrocytes progenitor cells
CRF	corticotropin-releasing factor
CVS	chronic variable stress
DALYs	disability-adjusted life-years
DUD	drug use disorder
ESR1	estrogen receptor α
GC	glucocorticoids
GC	glucocorticoids
GEMs	gel beads in emulsion
GO	gene ontology
GR	glucocorticoid receptor
GSEA	gene-set enrichment analysis
HPA	hypothalamic-pituitary-adrenal
ID	intellectual disability
IMZ	intermediate zone
IQR	interquartile range
ISH	in situ hybridisation
IZ	inner zone
KEGG	kyoto encyclopedia of genes and genomes
logFC	log fold change
M	mouse (antibody host)
MAST	model-based analysis of single-cell transcriptomics
MDD	major depressive disorder
MeA	medial amygdala
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging
mRNA	messenger RNA
MSigDB	Molecular Signatures Database
OFT	open field test
OL	oligodendrocytes
OPC	oligodendrocytes progenitor cells
OVX	ovariectomized
OZ	outer zone
PCA	principal component analysis
PVN	paraventricular nucleus of the hypothalamus
QC	quality control
Rb	rabbit (antibody host)
RM ANOVA	repeated measures ANOVA
RRHO	rank-rank hypergeometric overlap analysis
SABV	sex as a biological variable

scRNA-seq	single-cell RNA sequencing
SES	socioeconomic status
SI	similarity index
SPT	sucrose preference test
ST	splash test
TF	transcription factor
TST	tail suspension test
UD	use disorder
UMAP	uniform manifold approximation and projection

Statistical Significance

Notation used for indicating p-values for statistical tests:

Notation	p-value
****	< 0.0001
***	< 0.001
**	< 0.01
*	< 0.05
#	< 0.1
n.s.	> 0.1 (not significant)

1 | Introduction

For many years women have fought for being recognized equals to men and equally worth of medical care, while being underdiagnosed or experiencing unexpected side effects from drugs [1], [2]. Until the year 1990s not much was known about the female biology outside the reproductive system, and women often were excluded from medical studies preventing the acquisition of precious information about sex differences. Just a little more than a couple of decades ago, a slow revolution to improve the situation came into place and thanks to the NIH inclusion policy, women were introduced into medical trials leading to a considerable improvement in their medical care [3]. This new regulation opened up a vibrant field of research supported by new international policies all around the world, such as the NIH policy for considering sex as biological variable (SABV) in preclinical research [4]–[6]. This field is falsifying the long-thought myths that females just a mirror image of males. It is now clear that several organs, including the brain, differ between the sexes. These differences can either be persistent or stimulus-dependent and manifest as dimorphism, difference, convergence, or divergence (**Figure 1.1**, *page 1*).

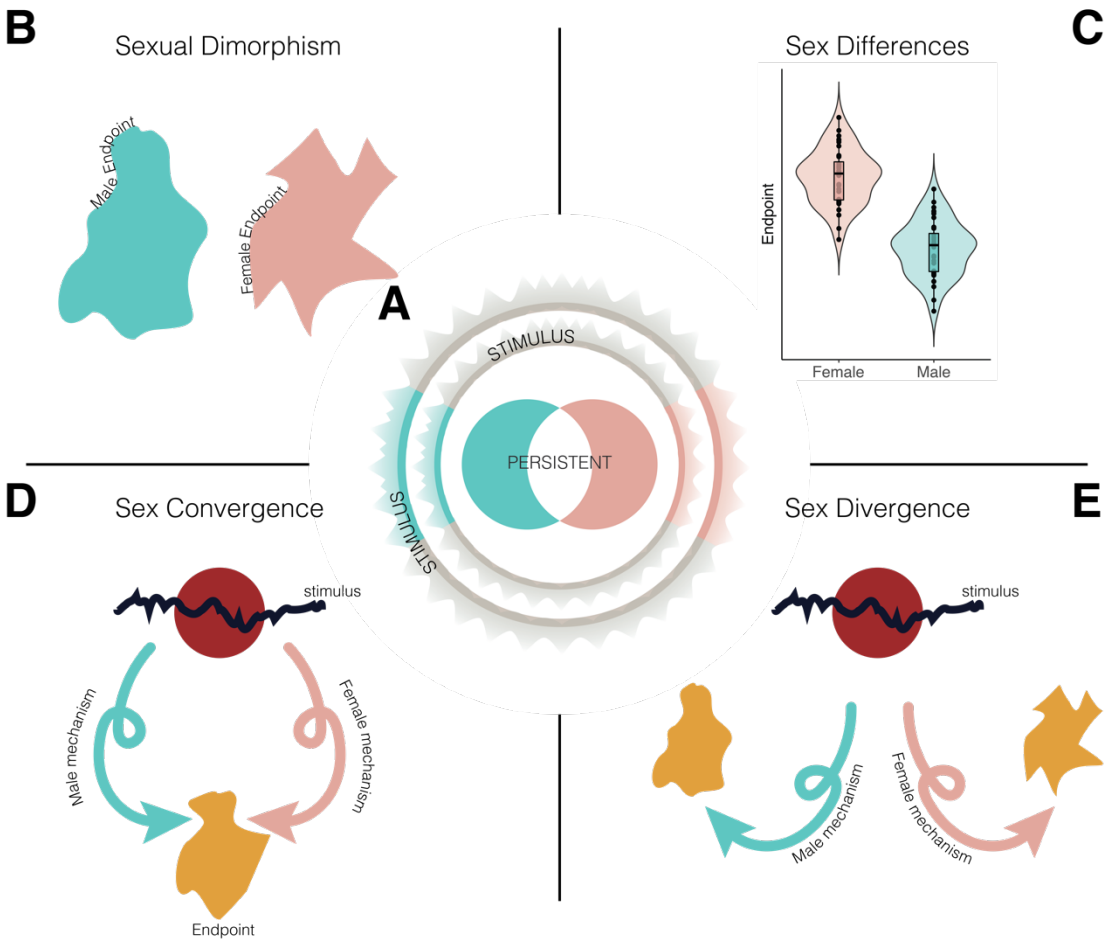


Figure 1.1: Sex Differences. (**A**) Sex differences can be either persistent or manifest in response to a stimulus. Male and female can be presented with (**B**) two manifestations of the same endpoint (sexual dimorphism), (**C**) same endpoints but shifted along the same dimension (sex difference), (**D**) different mechanisms converging on the same manifestation (sex divergence) or (**E**) similar baseline condition diverging on different endpoints (sex convergence). Figures inspired by [7], [8].

For example, female mice have been thought for long to be more variable due to their hormonal cycling. Recent studies, however, showed that housing conditions (group vs single housing) causes much more variability than sex in several physical parameters [9]–[11]. In addition, in equal conditions, males are much more variable than females on several of these parameters. Our unpublished data indicate that this true also for broad behavioural features (*unpublished data,* **Supplementary Figure 1***, page 129*).The discovery that females do not increase variability, that their inclusion in in experimental designs does not require increased resources [12] or that cycling hormones are not necessarily an experimental issue as previously thought [13] actively supports the broad use of SABV. Treating sex as a biological variable is proving to be a new source of knowledge about unknown pathological processes, especially for those disorders with limited efficacy for treatments and low available understanding of the pathophysiology such as the focus of this thesis: psychiatric disorders and stress.

1.1 Psychiatric Disorders and Sex Dimorphism

1.1.1 The Rising Burden of Psychiatric Disorders

Psychiatric disorders – defined as illnesses that affect the mental, behavioural, or emotional state of patients – include several diseases spanning a broad range of symptomatology and aetiology that often overlap. Among the most relevant, they should be mentioned eating disorders, drug and alcohol use, intellectual disabilities, and anxiety, depressive and schizophrenic disorders.

Psychiatric disorders severely impact the quality of life of almost one in ten people worldwide [14]. Prevalence in several western countries reaches even higher numbers; for example in 2017 patients affected by a mental disorder in Germany and most other European countries constituted almost one third of the whole population [15], [16]. Partially because of their high prevalence and partially because of their average severity, they also severely impact on the quality of life of these people. The impact of a disorder on the quality of life of a patient is conventionally measured worldwide by the disability-adjusted life-years (DALYs), a measure of years of full health lost – either for premature mortality or years lived with disabilities. All psychiatric disorders combined account for 4.9% worldwide DALYs and rank in the top ten leading causes of burden (7th place) [17]. Poor awareness at the population and government levels, poor efforts to curb them, and still poor understanding of etiopathologies behind these disorders are worsening this situation at a worrisome pace (DALYs of psychiatric disorders in 1990 accounted for the 3.1% worldwide, 13th place) [17], [18].

Individual disorders contribute to different extents to both global prevalence and DALYs. Depressive disorders, anxiety disorders, schizophrenia, alcohol and drug use, and self-harm, are for instance the biggest contributors and rank in the top 25 leading causes for DALYs in adults (25-49 years old) and adolescents (10-24 years old) [18]. They also share the global trend for gaining ranking positions (**Table 1.1**, *page 3*). In particular, depressive disorders affect at least 30 million people each year in Europe and are the single largest contributor to non-fatal health loss (7.5% of all years lost to disability), as well as contribute to the burden of self-harm and suicide [18]–[21].

Table 1.1: Ranking of Psychiatric Disorders by DALYs change (1990-2019). Data from [31].

Disorder	Ranking Change (1990 – 2019)
Age: 10-24	
Self-harm	▼ -1 (2 – 3)
Depressive disorders	▲ +4 (8 – 4)
Anxiety disorders	▲ +6 (12 – 6)
Drug use disorders	▲ +4 (22 – 18)
Age: 25-49	
Self-harm	▼ -6 (5 – 11)
Depressive disorders	▲ +2 (8 – 6)
Anxiety disorders	▲ +2 (17 – 15)
Alcohol use disorders	▼ -2 (18 – 20)
Drug use disorders	▲ +6 (22 – 16)
Schizophrenia	▲ +1 (23 – 22)

Altogether, mental disorders, especially major depressive disorder (MDD) and anxiety disorders, affects a big portion of the population worldwide, hindering their normal lifestyle. The poor understanding of their aetiology and pathophysiological mechanisms is still holding back on our ability to contain this phenomenon, making these disorders a new urgent and societal-relevant unmet medical need. As a results, they are considered the new 21st century medical challenge and a priority for scientific research to improve upon their diagnosis and treatments [16], [22].

1.1.2 The Role of Sex in Psychiatric Disorders

While several aspects of psychiatric disorders are still unclear, it is well know that their manifestation is the result of a combination of several factors, including genetics, environmental influences, and biological factors [23], [24]. Which factors and their interplay are so far only partially clear and mostly only superficially. However, extensive clinical research highlights sex as one of these important biological factor, especially for the top burden contributors mentioned above: depression and anxiety disorders [25].

When stratifying patients by sex, mood and anxiety disorders jump from being in the top ten to the top three causes of DALYs among females. This striking sex difference suggest that not only depression and anxiety disorders are major world-wide burden, but also that their negative impact is even higher on women and girls [16], [17], [22]. One of the reasons why disease burden differ between the sexes is because these diseases manifest sex dimorphism in several aspects. Many psychiatric disorders in fact affect disproportionally one sex over the other: almost two out of three patients suffering from MDD or anxiety is a woman [26] (**Figure 1.2**, *page 4*). Besides uneven prevalence, patients also display major differences in symptoms, comorbidities, disease progression, and treatment response, which also contribute to unequal burdens [26]–[30].

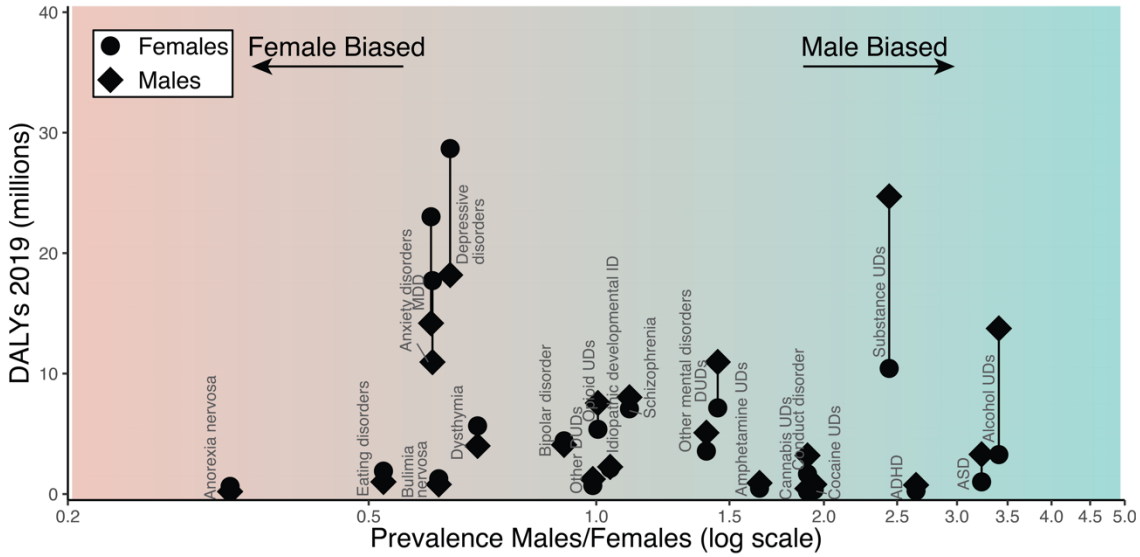


Figure 1.2: Sex Differences in Psychiatric Disorders. Disorders prevalence of psychiatric disorders if often unequal between women and men. Eating disorders and mood/anxiety disorders are female biased, with a much higher incidence in women than men. ASD and drug/substance use disorders are, on the other hand, male dominated. Most imbalances in prevalence (x-axis) are also paired with an unequal burden (y-axis) on the two sexes. MDD, major depressive disorder; ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; DUD, drug use disorder; UD, use disorder; ID, intellectual disability. Data obtained from "https://vizhub.healthdata.org/gbd-compare/", [31].

For example, MDD not only affects almost twice more European women than men [16], but the subset of symptoms and treatment efficacy are strongly moulded by the sex of the patient. Female patients manifest more withdrawal, mood, and sleep symptoms and are better responders to tricyclic antidepressants. In contrast, male patients are more prone to develop anger and substance abuse, and are more successfully treated with selective serotonin reuptake and

noradrenaline reuptake inhibitors [27], [28], [32]–[36]. Patients also benefit differently from new generation fast antidepressants such as ketamine. Ketamine acts faster in females but for longer in males, and elicits different side effects which result in a sex-specific compliance level [33], [37].

So far, no clear evidence of sex differences in genetic predisposition to depression or other stress-related disorders has been identified [38]–[41]. If this lack of results is due to the tendency of treating sex as a confounding variable rather than investigating its effect or a real absence is unfortunately still unclear. Nevertheless, current research is looking into which other factors could confer resilience or susceptibility differentially in one sex versus the other. In addition to genetics, environment is a key player in the manifestation of psychiatric disorders [24]. Investigating the role of sex in shaping the biological systems that process these experiences is beneficially contributing toward a better understanding of the sex dimorphism of these complex disorders [23].

1.2 The Stress System and the Stress Response

Years of research in clinical and preclinical settings have shown that one major environmental factor tight to the neurobiology of mood disorders such as depression and anxiety is stress exposure. This connection has been established for so long with such certainty that these disorders are often referred to as stress-related psychiatric disorders. As a consequence, basic stress neurobiology research stemmed within the field of psychiatry to investigate the stress processes and their effect on neurobiology and gain new valuable mechanistic insights on these complex diseases [23].

1.2.1 The Stress System

A real or perceived threat to our wellbeing is not an uncommon encounter in everyday life. The ability to engage metabolic resources, adapt a behaviour response, and maintain homeostasis in response to such stressor is therefore fundamental to the survival of the individual. This ability is ensured through a complex system of communicating brain regions and organ referred as stress system. The backbone of this response is the hypothalamic-pituitary-adrenal (HPA) axis, a neuro-endocrine axis with three main components: the hypothalamus, the pituitary gland, and the adrenal glands [42]–[44] (**Figure 1.3**, *page 6*). The paraventricular nucleus of the hypothalamus (PVN) receives and integrates inputs from different afferent regions to coordinate the activation of the axis. Several neuronal population here residing, including the serotonin, GABAergic, glutamatergic and norepinephrine neurons, in coordination with vasopressin (AVP) neurons regulate the overall activity of the corticotropin-releasing factor (CRF) neurons [45]–[47]. These hormone-secreting neurons project to the anterior lobe of the pituitary gland and in response to a stressor, release CRF into the hypophyseal portal vasculature. CRF, in turn, stimulates the anterior lobe of the pituitary gland to release the adrenocorticotropic hormone (ACTH) into the blood stream [46]. Through the blood stream, ACTH reaches its target organ: the adrenal glands. In the outer most part of the adrenal glands, the adrenal cortex, secretory cells are stimulated to produce and release the final product of this cascade: glucocorticoids (GC) [45]. GC, which consist mostly of cortisol in humans and corticosterone in rodents, are potent steroid able to regulate several processes including metabolic (increase in glucose metabolism, lipolysis, and proteolysis), cardiovascular (sympathetic vasoconstriction), immune (suppression of the innate immunity), and behavioural (activation of fight or flight response) through binding and activation

of two transcription factors (TFs): the mineralocorticoid (MR), and glucocorticoid (GR) receptors [48]–[50]. Due to their potency and broad effect, it is important that their activity is limited to the stress instance and rapidly shut down. The restoration of homeostasis as soon as the challenge has passed is fundamental to avoid detrimental effects on the health of the individual [49], [51]. For this reason, glucocorticoids' levels are tightly regulated through negative feedback loops activated by GRs located in the PVN and other brain regions [48], [49]. The activation of GRs in these regions in response to high levels of glucocorticoids vehicles first the reduction in GC production and eventually the shutdown of the stress response (**Figure 1.3**, *page 6*). The efficacy of this adaptive response is influenced by the amount and times of its activation and in turn influences the responses of the HPA axis to future stress [49].

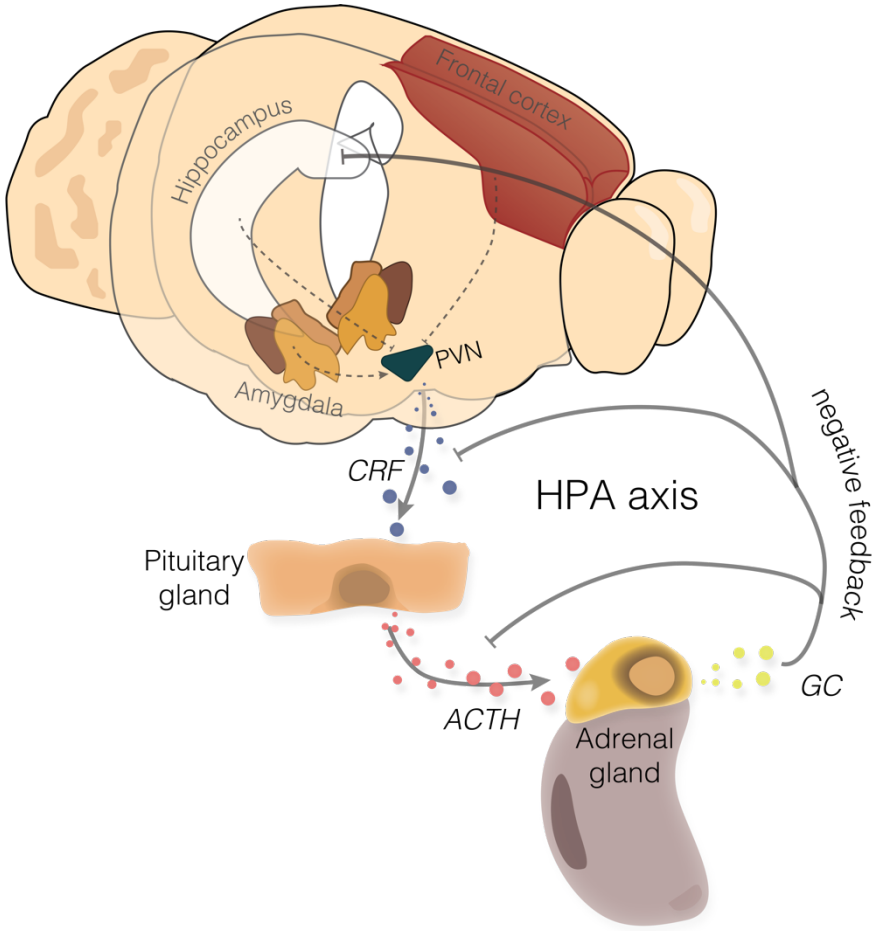


Figure 1.3: The HPA axis. The paraventricular nucleus of the hypothalamus is the initiator of the HPA axis. Its release of CRF stimulates the pituitary gland to release ACTH in the blood stream. ACTH, in turn, stimulates the cortical cells of the adrenal glands to produce glucocorticoids (GC). Glucocorticoids regulates its own levels inhibiting the release of CRF and ACTH through a negative feedback loop, eventually shutting down the stress response once the threat has passed.

In addition to the three components of the HPA axis, several other brain regions contribute to the maintenance of a correct adaptive response to stress, including the brain stem and the limbic system [52], [53]. The latter, which includes the hippocampus, the prefrontal cortex, and the amygdala, is especially important in the collection of external and internal inputs and the activation

of the negative feedback [54], [55]. For example, GRs in the hippocampus directly influence the activation levels of the PVN, inhibiting the downstream activation of the HPA axis [49].

Several types of environmental adversities can challenge the body homeostasis and hence be classified as stressors [53]. These include physical stressors, the ones that directly impact on the body homeostasis such as changes in the cardiovascular or respiratory state, pain, infections of inflammations, and psychological stressors, commonly anticipatory mental states of unpleasant experiences. These psychological stressors anticipate a real homeostatic disruption thanks to memory or intrinsic predispositions (such as recognition of dangers) generating mental states such as anxiety and fear [56]. Given their complex nature, stressors can arise from different types of experiences with different duration, intensities, and types. Short or one-timer experiences, such as a fearful encounter, a single instance of conflict, a natural disaster, and an anxiety surge for a test are referred as acute stressors. On the other hand, repeated acute stressors, that happens on a day-to-day basis, such as a war state, abuse or neglect, and repeated unpleasant situations, build up in cumulated load called chronic stress. The exposure to acute or chronic stress and the activation of the HPA axis can take a toll on the health of an individual and contribute to the development of several stress-related disorders, as explained in the next chapter.

1.2.2 Stress as a Trigger for Psychiatric Diseases

The activation of the HPA axis in response to a stressor is a well-controlled event needed to repristinate the correct homeostasis of the body. This process, called allostatic process, is beneficial in sparse activation, since it promotes healthy adaptation including learning and memory strengthening [49]. However, exposure to a particularly severe acute stressor or prolonged exposure to a stress state – either for a continuous exposure, a lack of habituation, a persistence of stress hormones or an insufficient shutdown response – leads to allostatic load, a maladaptive state which triggers detrimental effects on several organs and systems, such as the cardiovascular system, the bones, the immune system, and importantly brain plasticity and functionality [49], [51], [57]. The inability to maintain a normal response to stress is a major risk factor for disease development as much as the ability to maintain a healthy response to stress is a protective factor for diseases [51], [58], [59] (**Figure 1.4**, *page 7*).

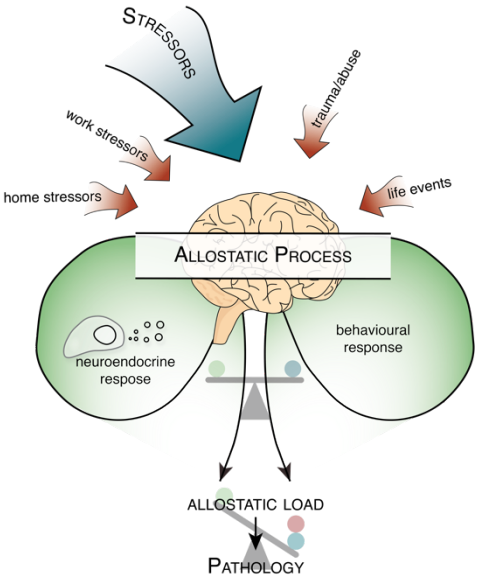


Figure 1.4: Allostatic Process. Several types of stressors are normally coped by allostatic processes which leads to neuroendocrine and behavioural responses. However, in case of particularly severe acute stressor or prolonged exposure to a stress state, the exhaustion of the allostatic system leads to allostatic load and increases risk for several pathologies including psychiatric disorders.

No life stages are safe from these processes, and from conception to old age, allostatic load becomes a relevant risk factor for stress-related disorders. For example, early life stress, exposure to prolonged and often severe stress in the first years of life, increases the chances to develop several psychiatric conditions including among others mood and anxiety disorders [55], [60]–[66]. Similarly, elevated levels of glucocorticoids, a secondary effect of stress exposure, even during prenatal stages can increase the chances of psychopathologies late in life [67]–[69]. While it is intuitive that maladaptive processes that influence brain plasticity impacts on the wellbeing of a developing individual, the same processes are similarly impactful on non-developing adult brains and bodies as well. Exposure to abusive environment and social isolation show a strong association with development of depression both in adolescence adulthood and elder [70]–[72]. A U.S.-based study, for example, found that elder individual have five times higher prevalence of depression when experiencing neglect or abuse [73]. Social status and social hierarchy, which are known also to associate with a specific allostatic load, also significantly increase the chance of developing depression and anxiety [74]–[79]. More generally, chronic stress and traumas, including prolonged anxiety traits, lead to detrimental effects on the brain and many other organs, increasing the risks of many mental disorders, especially depression and anxiety disorders [43], [44], [85], [50], [51], [66], [80]–[84], and other disorders associated to stress like cardiometabolic diseases [43], [86], [87].

In addition to being often triggered by stress exposure, stress-related psychiatric disorders are also characterised by a dysregulated stress response to novel challenges. The stress system, and the stress hormones levels are often dysregulated in patients. Both hypo- and hyper-reactivity of the HPA axis, for example, have been found in association with respectively PTSD [88], [89] and depression [67], [90], [91]. CRF levels have been found elevated in the *post-mortem* brains of depressed patients, as well as alterations in the levels of the CRF receptor [55], [92]. Glucocorticoids in patients, both in their basal levels or in response level after a challenge, were also found to be altered [55].

Given the tight connection between stress exposure, stress system functioning, and development of psychiatric disorders, a deep understanding of the mechanisms that regulated the stress system and susceptibility to negative experiences, including show sex and other factors contribute to it, can provide valuable information on disease vulnerability and mechanisms [89], [91], [93], [94].

1.3 Sex Dimorphism in the Stress System

The stress system and stress-related psychiatric disorders share a solid connection. Exposure to stress in maladaptive contexts indeed cause several structural and molecular differences in the brain [95]. Therefore, dissecting the sex dimorphism in the stress system help gain insights on the dimorphisms of the stress-related disorders.

Rodent models that closely resemble human stress response have been fundamental in dissecting these interactions and their connection with stress-related disorders [96]–[98]. They contributed to a big portion of basic research and in combination with human research they identified several instances that modulates the effect of sex on the stress response [44], [99]. Stress modalities, i.e. type, timing, and duration [100] and the combination of biological factors

such as developmental differences in the brain (often the result of the organization effect of gonadal hormones and sex chromosomes), and activational effects of circulating gonadal hormones drive this complex sex dimorphism [55]. Overall, these factors result in sex differences in the stress system at several levels: in its reactivity, in the behavioural output, in the cellular and molecular changes.

1.3.1 Reactivity and Behavioural Level

Activation of the stress response is secondary to recognition of the presence of a stressor and eventually leads to an adaptive behavioural response. Sex dimorphism can be described in both these manifestations. In human subjects, stress perception and perception of stressful emotions is strongly influence by sex [55], [101], [102]. Magnetic resonance imaging (MRI) studies have indeed shown that women have a stronger brain activation in response to stimuli with a negative valence. Among these regions, authors identified the ones responsible for emotional processing and filtering (amygdala, hippocampus, medial prefrontal cortex and cingulate cortex), and the hypothalamus itself, suggesting that female might have an intrinsic higher sensitivity to negative experiences and hence stress [103].

Interestingly a recent work from Rao and Androulakis used a semi-mechanistic mathematical model to simulate the differences between a male and a female HPA axis and its senstivity to chronic stress, using experimental data from rodents on CORT circadian rhytm, pharmacodynamic models for GR dynamics, CRF, ACTH and CORT release. *In silico* females had on average greater sensitivity and CORT response with a lower negative feedback, which ultimately made them more susceptible to chronic stress [104]. Experimental data on the other hand depict a more complex picture. Many suggest that women tend to have higher basal and stress-secreted levels of CORT [55], but several discordant results can be found in literature [105]. For example, Uhart et al. [106] showed that healthy young men and women have the same basal CORT levels. Men had a bigger increase in CORT levels after the Trier social stress test – a classic stress test in humans –, but the same women had a higher response after a pharmacological stress challenge. While conflicting results regarding CORT levels are very common in healthy individuals, studies in depressed patients consistently show increased basal and responsive CORT levels for women compared to men [107]–[109]. Other hormones along the HPA axis also show differences. ACTH levels are higher in women [106], [110]. Human studies, clearly highlight the existence of sex differences in stress response, and their discordance likely suggests that these differences are context specific. However, it is often difficult to directly compare clinical results due to the impossibility to control for all variables such as age, treatment status and the type of stress used.

Studies in rodents, on the other hand, can control more variables and consistently show that female mice and rats to have a higher basal circulating CORT levels and stronger response to acute stressor when measured in term of CORT levels [55], [111]. A weaker negative feedback in the female HPA axis, heavily regulated by circulating gonadal hormones such as estrogens, is a determining factor for these differences (**Figure 1.5**, *page 10*) [55], [111]. In support to the role of estrogens, the activation of the HPA axis in the females vary along the estrus cycle. Females in the phase of low estradiol (diestrus) appear more similar to males in their CORT and negative feedback profiles. On the contrary the differences are maximum in proestrus, when the estrogens

peak [112]. Female hormones are not the only one with potent effects on the HPA axis regulation, but also testosterone are powerful inhibitors of the HPA axis [113].

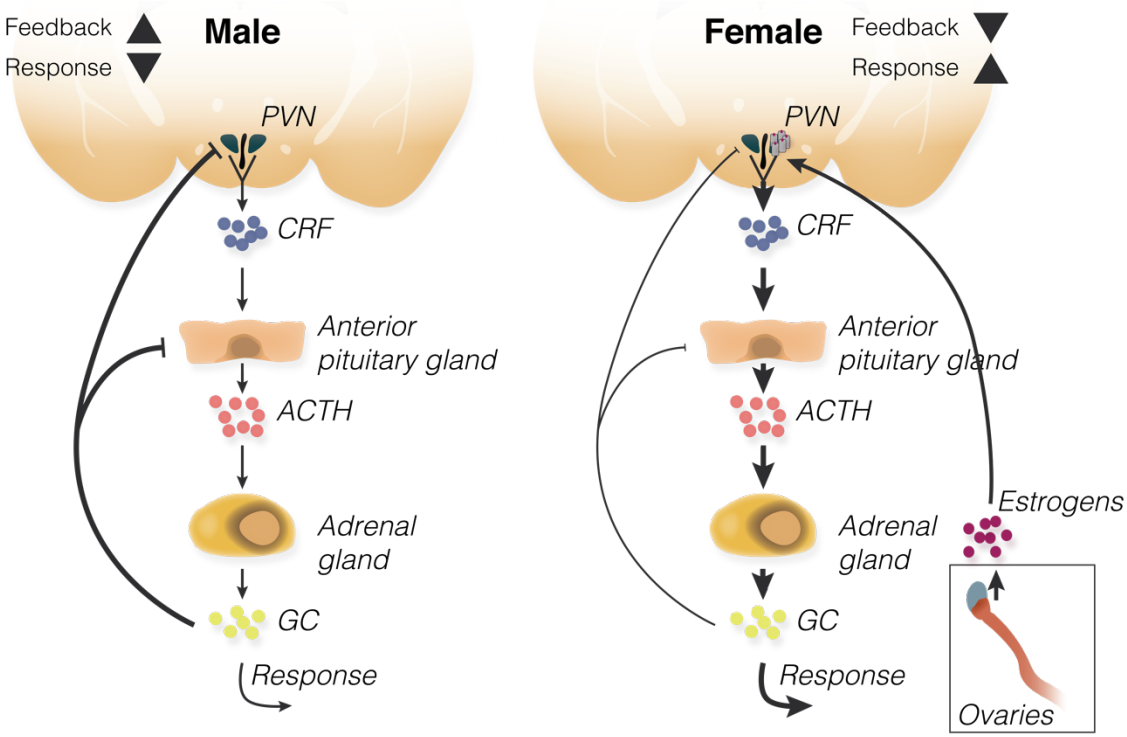


Figure 1.5: Comparison of HPA Axis Functioning in Males and Females. Males and females show overall differences in the response elicited by the HPA axis and the negative feedback that controls this process. Estrogen is a big contributor to reduced negative feedback and increased response observed in females thanks to the presence of estrogen receptors in the PVN and other upstream regions. PVN, paraventricular nucleus of the hypothalamus; CRF, corticotrophin-release factor; ACTH, adrenocorticotropic hormone; GC, glucocorticoids.

The ultimate output from the stress response is a behavioural adaptation. Differences in the activation of the stress response are accompanied by sex-specific behavioural responses. Chronic or prolonged stress, such as chronic mild stress, chronic variable stress, or social isolation, generate small but consistent sex differences [114]. For instance, females in tail suspension and forced swim tests are more prone to activate passive response, such as immobility, when compared to males [91], [93]. Part of these differences in behaviour are thought to be regulated by sexual hormones; administration of testosterone in juvenile females masculinize their behaviour in the FST and TST [115]. Other stress types also showed similar results. In response to chronic social defeat, male California mice react with more proactive strategies and females tend to have more passive strategies [99]. Importantly, clinical studies showed that that these rodent results quite match the uneven distribution of symptoms in depressed patients. Male depressed patients often develop active symptoms such as anger, while passive reactions and withdrawal are more typic of women [27], [28].

Behavioural differences can be observed in response to also different types of environmental stressors, such as social stressors. For instance, females are more affected by the isolation and males more affected by social crowding, leading to raised levels of CORT [87]. Social structure provides a stress load impacting on the wellbeing of individuals in a sex-specific way. In humans,

social structure is often measure by the combination of objective and perceived socioeconomic status (SES). This is an important social/environmental factor that determines an intrinsic stress load and ,similarly to other types of stress, impacts on susceptibility of psychiatric disorders such as depression and anxiety [74]–[79]. Human and rodent studies showed that sex modulates the influence of SES on health and its connection to psychopathologies [116]–[119]. We also contributed first-hand to the field, showing that sex interacts with social structure to shape the behavioural adaptation of male and female mice to chronic stress. Using high-throughput automated behavioural tracking [120], [121], we used social hierarchy as a proxy of social status, showing is a stable and reliable measure for both sexes, differently from what thought before. We then measure the behavioural adaptation to chronic stress and showed that sex and social hierarchy interact creating a different behavioural response in male and female mice. Overall, our results suggest that an individual's position within a social structure can influence their behavioural response to chronic stress in a sex-specific fashion. More broadly, social structure seems to have a different emotional and stress toll on the health of the individual based on their sex and therefore influence the ability of subsequent responses to stress challenges [119].

Altogether, the evidence presented here suggests that sex changes the receptivity to stress and the behavioural response, importantly interacting with other variables, such as pre-existing social structure or other stressors.

1.3.2 Cellular and Molecular Level

Behavioural differences in response to stress and different sensitivity of the stress response between the sexes is thought to be the macroscopic manifestation of several microscopic differences, divergences or convergences in mechanisms at the cellular and molecular levels, as it is true for several other brain disorders [122].

For instance, exposure to acute or chronic stress has been shown to change the number of spines, their morphology and the branching complexity in the prefrontal cortex of male rats, causing reduced complexity and arborization [57], [96], [123]–[126] These changes in response to chronic restraint have been shown to be male-specific. Female rats not only did not show a simplified phenotype, but showed even the opposite: hypertrophy, longer and more dendrites and more spines. This dimorphism depends on circulating estrogen [123], [126]–[128]. Similar changes to chronic stress in males have also been observed in the hippocampus. Chronic stress reduction in apical dendrites of CA3 pyramidal neurons in male rats in this case has been associated to impairments in learning, typical phenotype of stress and stress-related psychopathologies. In females no dendritic remodelling after chronic stress was observed [129]–[131]. While morphological changes after chronic stress are widely studied, less attention has been given to the same alterations after acute stress. The few published studies, however, suggest that even after a short, contained stressors cellular changes take place in a sex-specific fashion.

After an acute stress such as tail shock, the density of spines of hippocampal CA1 in male mice increases. Females in low estrogen phase mirror the male results, even if to a lesser extent, while females in high estrogen had an opposite effect. All in all, morphological changes in neurons might be a direct stress effect both with acute and chronic modalities [132]. The literature does not point at either sex as the most susceptible, but rather describes a wide range of sex

differences in cellular susceptibility in relation to gonadal hormones, stress type and timing. Because spines represent the actual neuronal connections, changes in the number of spine or the neuronal arborization in regions important for HPA axis regulation (such as hippocampus and prefrontal cortex) is likely participating in the macroscopic sex differences mentioned in the previous chapter. In addition, aside from these regulatory regions, exposure to stress has been shown to cause synaptic changes also in the hypothalamus, involving most of the subtype of neuropeptide-producing neurons of this region [133], [134]. However, these studies have been conducted in males only and studies systematically comparing the two sexes are still lacking.

A consistent body of research is also dissecting the intracellular molecular processes regulated by stress that could be causing these sex differences. GR and MR receptors have been among the first genes and proteins investigated, given their direct involvement in the stress system and their ability to regulate many transcriptional processes. Indeed sex interacts with the type of stressor to dynamically regulate GR and MR ratio after exposure to acute stressor [55], [112], [135]. The hypothalamic mRNA levels of Nr3c1, the gene that codifies for GR, or Nr3c2, which codifies for MR, are upregulated in males after different types of acute stressors such as forced swim test. On the other hand, females either do not show any regulation or downregulate these genes in response to the same stressors [136], [137]. The lack of molecular regulation of these important receptors in females could be contributing to the lower negative feedback detected in females and the increased sensitivity [55]. Aside from the genes Nr3c1 and Nr3c2, other transcriptional differences have been widely observed in the hypothalamus and other sex-related brain regions.

Several key regulatory neuropeptides such as *Avp*, *Crf*, *Oxt* and neurotransmitters such as monoamines, glutamatergic and GABA, and epigenetic processes have been selectively investigated in relation to stress exposure and sex with both candidate gene and genome wide approaches [96], [138]–[143]. Several reviews have been published recently that summarise all these changes in detail, including ours [138]. Of particular interest, Borrow et al. found that the two key hypothalamic neuropeptides, *Oxt* and *Avp* have a strong sex-specific regulation after chronic variable stress (CVS) [144]. Females upregulate *Oxt* and downregulate *Avp*, while males downregulate *Oxt*. These genes contribute to the regulation of the specific stress neurocircuitry, thus a sex-specific change in their levels might be an upstream regulatory process that contribute to the final activational sex dimorphism. Indeed, evaluating the activation levels of such region using cFos levels as a proxy, identifies sex-specific changes. For instance, the elevated platform stress elicits a strong upregulation of cFos in the rat PVN, representative of a strong activation, quite similarly between male and female. Nonetheless, this activation is reduced for males if the animals experience chronic restraint before, but increased for females [145]. This work from Moench et al. shows once again that HPA sensitivity is strongly sex-specific and influenced by past stressors in a sex-specific way as well. In addition, this activation is clearly detectable at the molecular level. Several other transcriptional sex differences have been found by studying individual candidate genes can be observed (**Figure 1.6**, *page 13*).

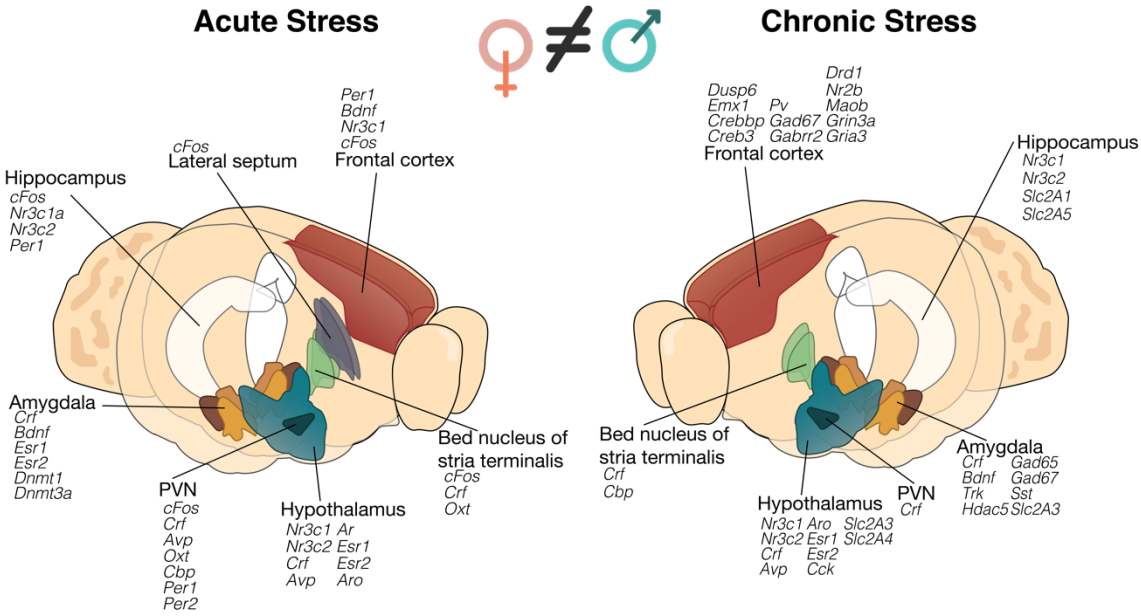


Figure 1.6: Sex-Specific Deregulated Genes after Stress. Schematic representation of sex-specific changes in gene expression by either acute (*left* panel) or chronic (*right* panel) stress in the rodent. Acute stress affects several stress-related genes (*Nr3c1*, *Crf*, *Avp)*, and activity-dependent genes (*Bdnf* and *cFos)* in opposite directions in several brain regions of male and female rodents. After chronic stress, on the other hand, the GABAergic system (*Pv*, *Gad65*, *Gad67*, and *Gabrr2*), the dopaminergic system (*Drd1*, *Nr2b*, and *Maob*) and stress-related genes (*Nr3c1*, *Nr3c2*, *Crf*, and *Avp*) are often found deregulated in opposite directions in the two sexes. Figure adapted from [138].

Despite being informative and giving precious insights, the gene targeted studies are strongly limited by their low power and low throughput and their inability to paint a complete representation of the molecular mechanisms happening simultaneously. High-throughput sequencing, on the other hand, allows to explore the sex-dependent differences in an unbiased and comprehensive manner, enabling to implicate new pathways and genes in the stress response and identify new sex-dependent molecular mechanisms and new possible therapeutic targets. High-throughput studies are unfortunately still the minority of the published work about sex differences in stress and stress-related psychiatric disorders, but they are slowly emerging and adding a lot to our understanding of sex differences. As expected, they show that sexual dimorphism in stress takes place at the transcriptional level on a big scale. Male and female brains (human and mouse) show just a small overlap in the deregulated genes by stress exposure or psychiatric disorder, approximately 30% of all differentially expressed genes (DEGs). In addition, often these DEGs are regulated in opposite directions [140], [146]–[151] These studies are also showing that different pathways and neuronal functions are likely to be deregulated in the two sexes [146], [152], because pathways are often either deregulate in only one sex or found changed in opposite directions. Labonté et al. with their seminal work showed that studying male and female post-mortem human samples and stressed rodent brains is a promising approach to better understand how and where sex differences arise in the brain and lead to pathology [146]. Authors combined human and rodent data with advanced network and pathway analyses and identified sex-specific pathways altered by depression and chronic stress exposure. Authors also showed that these changes were selectively impacting on neuronal functioning in one or the other sex. They also identified two hub genes, *Dusp6* and *Emx1*, not previously implicated in stress and depression

could and successfully attenuated the stress phenotype in mice by manipulating them in a sex-specific fashion. Importantly, this work found different pathways, regulated by different genes in males and females, which however led to similar phenotypes on neuronal functioning, confirming the existence of convergent mechanisms in males and females.

Overall, these studies highlight important facts regarding studying the molecular pathways underlying psychiatric disorder. First they enforce the idea that rodent stress models are informative for stress-related psychiatric disorders, since they share many deregulated pathways and have less limitation associated to confounding variables such as low quality tissue, treatments, and type of experiences [98]. Secondly, they confirm the need of designing studies with a direct comparison of independent male and female samples, since using mixed sex samples would hide important information. Finally, they confirm that several divergent and convergent mechanisms between males and females take place in the brain which involve complex circuitry and gene networks.

This thesis just barely started to scratch the surface of all molecular differences between male and female response to stress. Despite the growing body of literature that supports the idea that differences at a smaller resolution exists, we still lack knowledge of how these small differences add up to complex behavioural and susceptibility differences. New high-throughput resolution technologies and advanced techniques that are not limited to few genes or hypothesis at a time, are a promising new reservoir for new discoveries and could lead us one step closer to understand the mechanisms behind these processes.

1.4 Single Cell Transcriptomics for the Investigation of the Brain

1.4.1 A New Direction for Studying Sex Differences

Next generation transcriptomic studies have been very successful in identifying a big range of sex differences in response to stress and stress-related psychopathologies and laying the grounds for dissecting the origin of sex differences in stress. Within novel sex-specific gene players and pathways, these studies have also hinted at the possibility of cellular mechanisms contributing to the sex dimorphism. For instance cell-type-specific pathways analyses of deregulated genes after chronic stress exposure or in depressed patients found several glial and neuronal pathways that were uniquely regulated in males or females [146]–[148]. Female MDD patients showed downregulation of microglia-associated genes, whereas male showed upregulation of microglia-associated genes paired with reduced markers of synaptic function and neuronal genes [147]. Endothelial pathways also seemed to be exclusively affected in male samples. Further suggestions that stress could be processed in different cell types according to the sex come from other studies about cell-specific proliferation. Hippocampus proliferative abilities are selectively affected in male rats after stress, suggesting proliferative cells, such as glia or neuronal progenitors, are differentially affected in the two sexes [153]. These studies suggests that stress response differences in the two sexes start already at the cell level. However, these studies are performed on brain regions as homogenate – referred as bulk transcriptomics – combining RNA from a diverse population of cells and. As such, the technique is unable to really differentiate what

changes are happening in which kind of cells but have to rely on enrichment analysis and several statistical assumptions for inferring contributions of individual cell types. Since the brain is a highly heterogenic tissue composed of many different cell types and subtypes, cell-specific alterations and their contribution to the total changes observed are diluted and mostly lost (**Figure 1.7**, *page 15*). Bulk transcriptomics cannot differentiate if a lowly expressed gene is widely expressed at low levels (**Figure 1.7**, gene A, *page 15*) or just highly selective for a rare cell type (**Figure 1.7**, gene B, *page 15*). In the same way, for genes widely expressed, transcriptional changes selectively in a population can be underestimated or lost completely due to dilution effect (**Figure 1.7**, gene C, *page 15*) [154], [155].

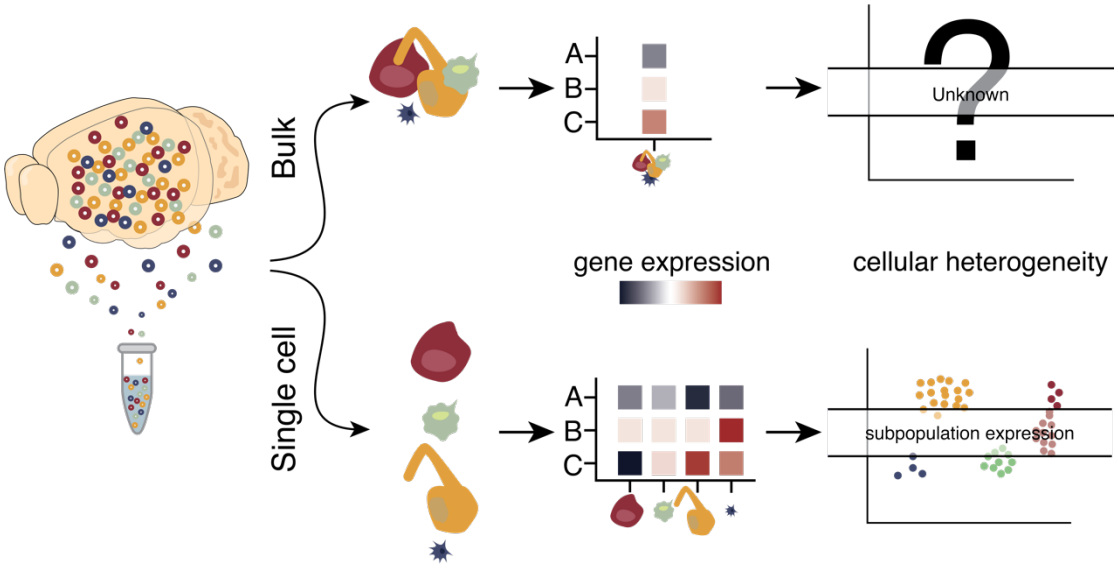


Figure 1.7: Bulk vs Single cell Transcriptomics. Bulk and single cell transcriptomics approaches use next-generation sequencing to obtain information about the whole transcriptome. In bulk transcriptomics, the whole tissue is sequenced as homogenate, obtaining gene expression values across all cell types in the tissue and losing information about cellular heterogeneity. Single cell transcriptomics, on the contrary, maintains single cell information, obtaining individual gene expression values for each cell. Single cell transcriptomics allows to differentiate gene changes that are widespread and similar among all cell types (gene A), from changes highly selective that would be diluted or lost in bulk resolution (genes B, C).

New technologies on the rise can now however overcome these issues allowing to study the cell specificity of transcriptional changes. The newly developed technique called single cell RNA-sequencing (scRNA-seq) combines sequencing and advanced bioinformatic analysis and allows to explore the transcriptome of individual cells [156]. This approach allows to decline an unbiased design both in gene expression and cell types. The high-throughput sequencing makes possible to study the whole transcriptome at once, while the single cell design allows to study each individual cell, without the need to choose in advance a cell type. In the scRNA-seq protocol, the transcriptome of each cell is tagged with unique barcodes before being amplified and process into a mRNA library. After sequencing, each RNA molecule can be assigned back to their original cell source thanks to their unique barcode. For each cell, the individual transcriptomes is recomposed and then used to determine its identity, population, and state. Different types of scRNA-seq technologies have been developed based on their methods of targeting, sorting, and tagging single cells before library preparation [154], [156], [157]. Among them, the droplet-based

system (such as the one provided by the platform 10x Genomics) have become particularly renowned thanks to the wide range of cell size they can accommodate, the simplicity of their platforms, and the high number of cells obtained per run [153], [158]. ScRNA-seq technology has been applied in several fields, such as immunology, oncology, development, and neurobiology to characterize organ or tissue populations, cell trajectory and development, cell cycling, cell interactions and specific stimulus-response [158]–[162]. In all these fields, scRNA-seq technology has been a source of new data previously difficult to collect, especially relevant in the context of complex organs such as the brain, as the next chapters will discuss.

1.4.2 Single Cell Transcriptomics Applied to the Brain

Several fields have benefitted from single cell transcriptomics, including neuroscience and neurobiology [163]. The first papers published approximately five years ago revolutionized how the field categorizes brain (neuronal and non-neuronal) cells, opening the doors for new advanced research. ScRNA-seq studies discovered that the brain is more homogenous than previously thought when observed under the mRNA lens, suggesting that characterization based on morphology or electrophysiological proprieties alone might be too simplistic. These initial works catalogued in detail the murine visual cortex, somatosensory cortex, hippocampus, and hypothalamic regions, and in addition to confirming the existence of known major neuronal types, they identified new types of neurons and several new subtypes within the major types [164]–[167]. This information broadens our understand of the molecular organization and network structure of the brain. For example, classification of neuronal subtypes based on their transcriptome can help identifying how inputs are received and processed in similar neuronal types and how this information is integrated and communicated, thanks to the detailed information encoded in the transcriptome about receptors and neurotransmitter produced by each cell.

Analogously, the technology has also been extremely useful to better characterise non neuronal populations for long overlooked in the brain [164], [167], [168]. Several works have identified new subtypes of tanycytes [169], [170], characterised the different states of inflammation for astrocytes [171], mapped the region-specific localization of subtypes of oligodendrocytes [170], [172], and defined cellular characteristics of macrophages residing at brain's border region [173].

Thanks to the growing interest of the community and the building of new bioinformatic tools, the scRNA-seq can now also be applied to study the dynamism and change in transcriptome of individual cell population in response to stimuli, as it has been done for so long with bulk transcriptomics. Few successful examples of how the technology can bring advancement in the field of neurobiology are presented in the next chapter.

1.4.2.1 Novel Applications of scRNA-seq in the Field of Neurobiology and Stress

After using scRNA-seq technology to better characterise and define brain cell identities, researchers have started to push the boundaries of what the technique can achieve, exploiting the richness of their dataset and the new refined bioinformatic techniques.

For example, scRNA-seq dataset can be used to identify new molecular regulators of a biological process and the birth of new subpopulations, as our lab did. In Lopez et al., scRNA-seq was used

to characterise the three main components of the HPA axis (PVN, pituitary gland, and adrenal glands) before and after exposure to chronic defeat stress in male mice. The use of the single cell resolution allowed to identify that chronic stress elicits the growth of a specific subtype of cortical adrenal cells that are mainly characterized by the expression of a transporter, *Abcb1b*. Complementing the scRNA-seq with classic molecular (such as qPCR) and cellular (*in vitro* cell lines), we showed that this cell population is key for the release of CORT in the blood torrent, thanks to the activity of this transporter. Without the use of single cell resolution, would have not been possible to understand that upregulation of *Abcb1* was actually associated to the generation and expansion of a specific subpopulation.

Targeting individual cells, scRNA-seq can also be paired with other RNA-based tagging approaching, to define and characterise more complex cellular processes, such as connectomes and development. Klinger and authors tagged cells based on their connectome in the developing cortex with short RNA sequences and used scRNA-seq to identify the identity of the tagged cells and their relationship with the developmental age and the surrounding cells [174]. Using pseudotime analysis, a bioinformatic tool that allows to order single cells based on their transcriptome along a pseudotime dimension, allowed them to dissect the gene programming behind maturation of each subtype of cells based on their connectome. The overall study led to the identification of new molecular players that regulate sensorimotor connectivity which would have been hardly possible to dissect without single cell technologies.

Finally, aside from identifying new cellular markers and understanding neurodevelopmental processes, scRNA-seq also hold promises to improve characterization of neurological disorders and psychiatric disorders through identification of new molecular markers and targets [175], [176]. Mathys and colleagues used prefrontal cortex *post-mortem* samples from patients suffering from Alzheimer's disease (AD) with varying degree of severity and both sexes [175]. Since human tissue cannot be processed fresh, authors relied on single nuclei RNA sequencing that allows to perform scRNA-seq using only nuclear mRNA that can be obtained from frozen tissue and therefore is the primary choice for human samples. Authors showed that all major cell types showed strongly specific DEGs and most of them would be normally masked in bulk sequencing. Bulk transcriptomics would in fact be normally dominated by genes from neurons and oligodendrocytes. The dissection of cell-specific gene changes would therefore been otherwise impossible without single cell/nuclei RNA-seq. Secondly, authors also showed that early-pathology samples, with little-to-no symptoms, exhibited already a molecular phenotype, showing the power of molecular markers to understand pathology progression. Finally, authors also used their dataset to identify cells-specific changes between the sexes, showing how scRNA-seq applied to studying sex differences can generate new valuable information. As such, they discovered that oligodendrocytes response correlates with AD severity in men but not in females, while neurons correlates in females but not males. This study demonstrates that each cell types can have different key roles in the pathology progressions, and importantly that they could do so in a sex-specific way.

What I have presented here are just examples of the creative way researchers are declining scRNA-seq technology. Overall scRNA-seq can help building a detailed connectome of the brain, by increasing the resolution of neuronal categorization and revolutionize on how we define cell types. The identification of cell-specific molecular targets for genetic targeting and manipulation

will also ultimately fill the gap with behavioural outputs. The field of stress and sex is in need of new resources and broad point of view for improve upon the understating of the molecular mechanisms behind stress and stress-related psychiatric disorders. Single cell transcriptomics has the potentiality to contribute substantially to the study of sex differences by characterizing what differentiates the stress response in male and female cell populations.

2 | Rationale and Objectives

Women and men differ in the pathological manifestations of several stress-related disorders, e.g., depression. These disorders are triggered from exposure to stressful experiences [177] and characterized by dysregulation of the stress neurocircuitry [91]. The PVN – the coordinator of the HPA axis – and the corticolimbic structures feeding into the PVN show sexual dimorphism in terms of activation, stress processing, and molecular mechanisms. Transcriptional results, so far, have suggested divergent molecular mechanisms behind the sex differences in stress processing, but a characterisation of the cellular source of these changes is lacking.

This PhD thesis aims to characterise the individual contribution of cell types to the transcriptional stress response of the paraventricular nucleus of the hypothalamus (PVN) with a particular emphasis on the role of sex in modulating this response. Since stress exposure modulates stress reactivity and shapes the risk of developing stress-related disorders, it further aims to explore the susceptibility of the different cell populations to a history of pre-existing stress.

To achieve these goals, we used well-validated mouse models and top of the field scRNA-seq technology to characterize the transcriptional stress response to acute restraint stress (ARS) of the PVN in male and female adult mice under Baseline (naïve mice) and chronic stress (mice under chronic mild stress (CMS)) backgrounds and assess robustness of their stress response.

Finally, in the spirit of open science and to enable big dataset to substantially contribute to the current and future growth of the field of sex differences in psychiatry, knowing that such a rich dataset can hold much more information that one can possibly process and exploit in a single PhD thesis experience, we provide an accessible web interface for researchers to openly access and ask their questions of choice using our data.

3 | Material and Methods

3.1 Mice Husbandry

For all experiments, we used wild-type sexually-mature (7-10 weeks old) C57BL6/N mice, which were housed in same-sex pairs in individually ventilated cages (ICV) provided with bedding and nesting material, a wood tunnel and with water and food *ad libitum* on a 12:12h dark:light schedule at the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany. Before the start of the experiment, mice were given a minimum of five days to habituate to the experimental rooms to reduce confounding stress elements. Importantly, for all procedures, both animals in one cage were always treated simultaneously, to exclude second-hand effects. All experiments were approved by and conducted in accordance with the regulations of the local Animal Care and Use Committee (Government of Upper Bavaria, Munich, Germany).

3.1.1 Estrus Cycle Monitoring

Female mice were monitored daily (minimum 10 days) around 7:00-8:00 a.m. to determine their estrus cycle stage through dry vaginal smears (**Figure 3.1A-D**, *page 22*). Vaginal opening was flushed with 30μl of 1x PBS with a filter pipette tip (**Figure 3.1A**, *page 22*). The samples retrieved were smeared on a glass coverslip, dried at 37°C for 10-15min and subsequently stained for a cytology evaluation with “modified Wright-Giemsa stain” (Sigma Aldrich, WG16-500ML). Glass coverslips were dipped in the dye solution for 30s, washed in water for 3min, rinsed one final time in water and carefully dried (**Figure 3.1B-C**, *page 22*). The amount of the three populations of exfoliated vaginal cells (nucleated epithelial cells, cornified epithelial cells and leukocytes) and their relative ratio was evaluated and the cycle stage assigned [178]. The criteria for assignment are depicted in **Figure 3.1D-E**, *page 22*:

- Proestrus: majority or near majority of nucleated epithelial cells;
- Estrus: majority of cornified epithelial cells;
- Metestrus: equal representation of nucleated, cornified epithelial cells and leukocytes;
- Diestrus: majority of leukocytes and equal amounts of nucleated and cornified epithelial cells.

For each slide, one representative picture was acquired at a Zeiss Axioplan 2 Imaging Fluorescence Microscope (1388x1040 resolution, 20x objective, **Figure 3.1F-I**, *page 22*).

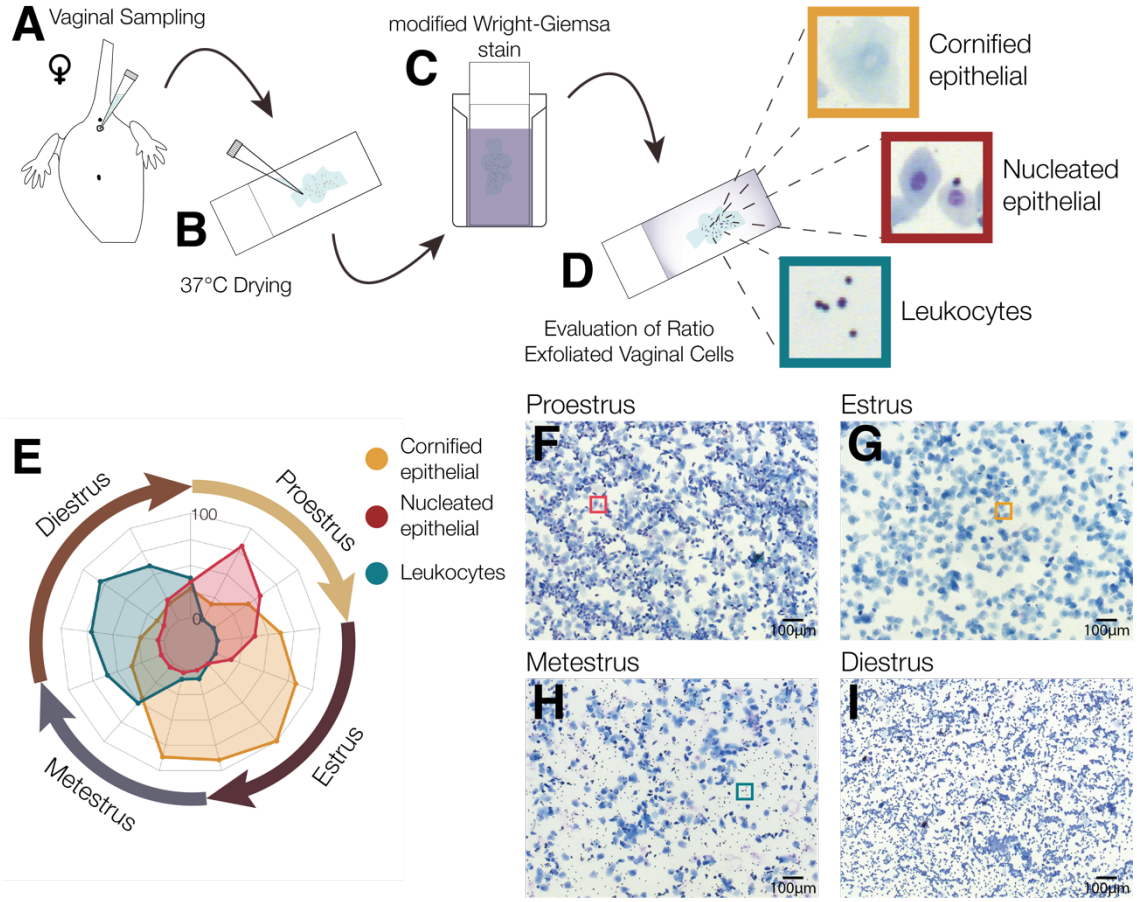


Figure 3.1: Estrus cycle determination. (**A** – **D**) Process for the assessment of estrus stage. A sample of exfoliated vaginal cells is obtained flashing the vaginal opening with PBS 1x (**A**), then later smeared on a glass coverslip, and dried at 37°C (**B**). The dried sample is first dyed with the modified Wright-Giemsa stain (**C**) and observed under the microscope to identify the ratio between the exfoliated vaginal cells (**D**). (**E**) Distribution (in %) of the three main types of vaginal exfoliated cells: cornified epithelial cells, nucleated epithelial cells and leukocytes, in dried vaginal samples in the four main stages of the estrus cycle. (**F – I**) Example of images of exfoliated cells stained with modified Wright-GIEMSA in dried vaginal samples of animals in (**F**) proestrus, (**G**) estrus, (**H**) metestrus, (**I**) diestrus.

The majority of the animals (105 out of 116, 90.5%) showed a progression through estrus stages similar to what described in literature [142](**Figure 3.2A**, *page 23*); the ones with irregular cycles (either prolonged estrus or absent estrus) were excluded from the experiment (**Figure 3.2B-C**, *page 23*). Male mice were handled at the same time to minimize confounding effects.

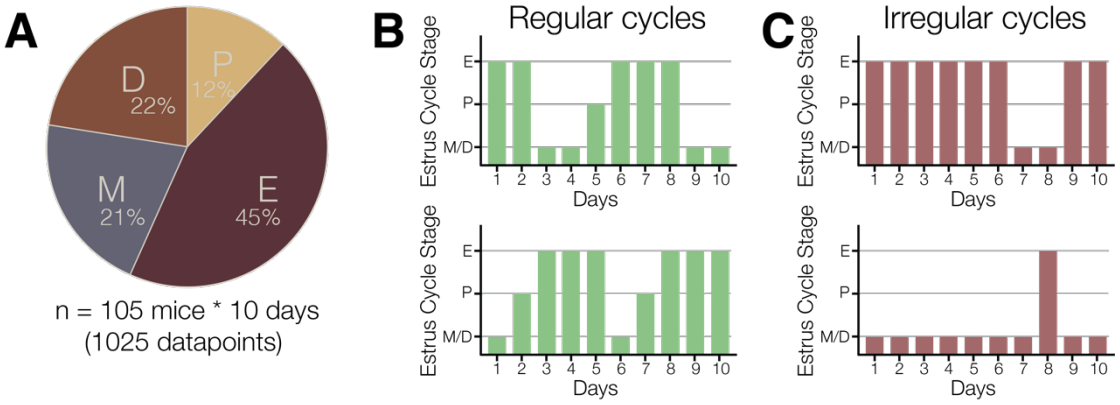


Figure 3.2: Estrus cycle of c57BL6/N mice. (**A**) Average composition in time of the estrus cycle by the four estrus phases in all cohorts combined: diestrus (D), proestrus (P), estrus (E), metestrus (M). (**B – C**) Examples of (**B**) two regularly cycling and (**C**) two irregularly cycling females.

3.1.2 Ovariectomy

To evaluate the role of estrogen in the transcriptional acute response, we performed ovariectomy in sexually-mature female mice to remove the source of circulating estrogens. Seven weeks old c57BL6/N female mice were first deeply anesthetized (intraperitoneal injection: 0.1ml/kg of 1ml 10% Ketamine, 0.25ml 2% Xylazine, 6ml 0.9% NaCl. Subcutaneous injection: 0.5 mg/kg Metacam) and ovaries were accessed through a 2cm bilateral skin incision of approximately 5mm lateral to the spine, at the anterior-posterior position corresponding to the kidneys (**Figure 3.3A-B**, *page 23*). Ovaries and oviducts were excised with sterile small scissors. Muscle incision was sutured, and the skin closed with metal clips. Ten days after surgery, we evaluated if the surgery was successful confirming that animals stopped cycling. Animals' cycle was tested with dry vaginal smear as explained above with the expectation of no exfoliated vaginal cells (**Figure 3.3C**, *page 23*). All animals in which ovariectomy was confirmed were given five extra days of rest before exposing them to the stress paradigm (total of two weeks of post-operatory rest).

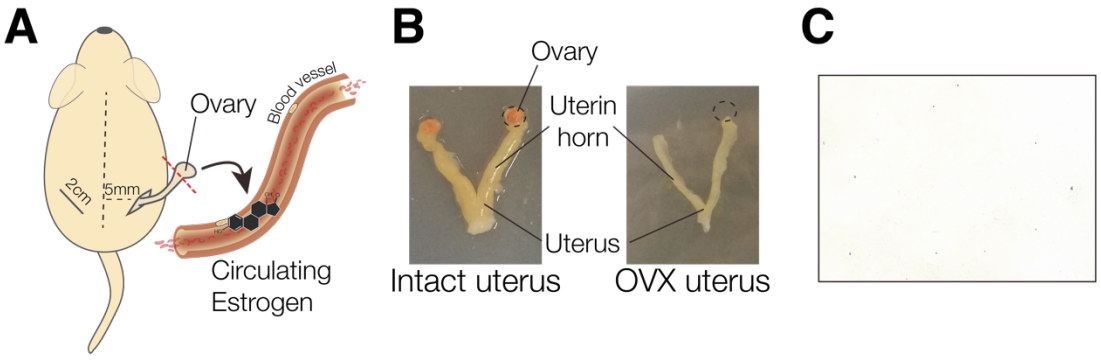


Figure 3.3: Ovariectomy. (**A**) Anesthetized mice were incised bilaterally, 5mm lateral to the spine, to access and remove ovaries as source of circulating estrogens. (**B**) Examples of an intact and ovariectomized (OVX) uteri. The latter lacks ovaries. (**C**) Representative dry vaginal smear of an ovariectomized female. No cells are present.

3.2 Stress Paradigms

For each experimental cohort, half of the mice were assigned a stress condition of either acute stress (Baseline background) or a combination of chronic and acute stress (CMS background). Schematic representation of temporal design of the different cohorts can be found in the Chapter "4 I Results", *page 37*.

3.2.1 Acute restraint stress (ARS)

As an acute stress, we selected the acute restraint stress (ARS) paradigm. Mice were restraint for 15min in a ventilated tube (created drilling holes into a 50ml falcon tube, length: 11.5cm, diameter: 3cm) in the dark at ~8:00 a.m. The ARS was given to half of the cages in the Baseline group while all stress cages in the CMS background received it on Day 22.

3.2.2 Unpredictable chronic mild stress (CMS)

For the study of the impact of background stress on the acute stress response, separate cohorts of female and male mice were exposed to the unpredictable chronic mild stress before either received a behavioural assessment or receiving ARS. Cages were randomly assigned either the control or stress condition. Each stress cage received a random combination of two stressors per day (one in the a.m. and one in the p.m. hours) for a total of 21 days.

For the first behavioural evaluation of CMS, the stressors were chosen from an original pool of 11 psychological and physical stressors (**Table 3.1**, *page 24*). We later substitute the stressors which included restraint stress (restraint in the dark, in bright light and witnessing) to be able to isolate the ARS response after CMS (see **Table 3.2**, *page 25*). In both experimental conditions, stressors were randomly combined to cover both day and night hours. However, to better isolate the signature from the acute restraint test, on Day 21, animals received the mildest stressor, the removal of nesting material for 24 hours.

Table 3.1: Stressors of the Chronic Mild Stress Paradigm – Initial Design.

Stressor	Length	Description
No nesting	24h	Removal of all nesting material and wood tunnel
No bedding	8h	Removal of all bedding and nesting material and wood tunnel
Cage tilt	6h	Cage tilt of 30°C along the vertical axis
Wet bedding	6h	200ml of 23°C water mixed in the normal bedding
Cage change	4h	Fresh cage every 30 min for a total of 4 h
Cage switching	-	Assignment to the cage of another group of the same sex
Overcrowding	1h	Mice were placed with 8-10 same-sex stranger mice in a fresh cage*
Water avoidance	15min	An empty rat cage (395x346cm) was filled with room temperature water; mice were placed on a platform (10x12 cm), 2 cm above the water level
Restraint in the dark	15min	Mice were restraint in the dark
Restraint in bright light	15min	Mice were restraint in bright light (~ 200lux)
Restraint witnessing	15min	Mice were placed in a fresh cage with their cagemate being restraint

**The rare mild episodes of aggressions witnessed in male groups were promptly interrupted to avoid injuries.*

We monitored the efficacy of the CMS paradigm on days 1, 3, 7, 10, 14, 17 and 21. During the monitoring, mice were weighed, and their coat state was scored on a scale 0 to 3 according to the following criteria:

0) Shiny/well-groomed/healthy coat (no injuries or alopecia patches),
1) Less shiny/less groomed coat or small alopecia patches, but healthy (no wounds),
2) Dull coat and/or small wounds or alopecia patches,
3) Extensive piloerection or alopecia with crusted eyes or extensive wounds.

Table 3.2: Stressors of the Chronic Mild Stress Paradigm – scRNA-Seq Design.

Stressor	Length	Description
No nesting	24h	Removal of all nesting material and wood tunnel
No bedding	8h	Removal of all bedding and nesting material and wood tunnel
Cage tilt	6h	Cage tilt of 30°C along the vertical axis
Wet bedding	6h	200ml of 23°C water mixed in the normal bedding
Cage change	4h	Fresh cage every 30 min for a total of 4 h
Cage switching	-	Assignment to the cage of another group of the same sex
Overcrowding	1h	Mice were placed with 8-10 same-sex stranger mice in a fresh cage*
Water avoidance	15min	An empty rat cage (395x346cm) was filled with room temperature water; mice were placed on a platform (10x12cm), 2 cm above the water level
Space reduction	6h	Reduction of cage space to ¼
Tail suspension	15min	Mice were hung by their tail 50 cm above the surface

**The rare mild episodes of aggressions witnessed in male groups were promptly interrupted to avoid injuries.*

A cumulative coat state per animal was calculated as the sum of the seven daily scores. Bodyweight gain was calculated as the difference of bodyweight at Day 21 – Day 1.

In addition, at sacrifice, adrenal glands were also collected, isolated from connective and surrounding fat tissue, and weighed. A mean adrenal size per animal was calculated over the two adrenal glands. Adrenal size was normalized on bodyweight at sacrifice.

For each animal, bodyweight change, cumulative coat state and mean normalized adrenal size were calculated and z-scored (for each experiment, the population of reference used was all sexes and conditions combined). Parameters were then directionally-normalized – so that positive values represented a stress state and negative values represented a non-stress state – and summed to obtain the stress score (as explained in "3.4.6 Stress Score", *page 27*).

3.3 Corticosterone assessment

To assess the corticosterone (CORT) levels in response to ARS, few µl of blood were collected in EDTA-coated tubes from the tail right before the ARS and at the end of the 15 minutes of restraint. Blood was centrifuged at 1,000g for 15 min at 4°C. Plasma was retrieved and corticosterone levels were measured using [^{125}I] radioimmunoassay kit (MP Biomedicals), according to the manufacturer's instructions.

3.4 Behavioural tests

To evaluate the impact of CMS on mice behaviour, we used a battery of tests for anxiety-related, anhedonia, depressive-like, and locomotion phenotypes (**Figure 4.2**, *page 39*). At the end of the CMS paradigm, mice were single-housed and kept that way for all behavioural tests. All tests were conducted during the dark phase of the light cycle as depicted in **Figure 4.2**, *page 39*. All analyses have been done blind to the sex and genotype of the mice.

3.4.1 Splash Test (ST)

On Day 21, approximately 10 hours after the last stressor, mice were tested in the splash test, under dim illumination (~10-15lux) looking for anhedonia and depression-like phenotypes ([179]). Each animal was sprayed twice on the back (~500µl per spray) with a solution 10% sucrose and then placed in their original cage. Mice behaviour was recorder for 5min and the latency to groom,

and the total amount of time spent grooming were manually scored using Solomon Coder 17.03.32 [180].

3.4.2 Open Field Test (OFT)

Twenty-four hours after the splash test, we tested locomotion and exploratory behaviours within an open field test for 15 minutes. Mice were placed inside a 50x50x40cm arena made of grey polyvinylchloride under dim illumination (15lux). Mice location was automatically tracked with ANYmaze Video Tracking System v. 6.13 (Stoelting, IL, USA). Because different regions of the arena have intrinsic different levels of anxiety, we virtually divided the space in four distinct areas. Inner, intermediate, outer, and corner zones which respectively are highly anxiogenic, intermediate, lowly anxiogenic, and not anxiogenic (**Figure 3.4**, *page 26*). We then calculated a total of 22 parameters for either the whole arena or each of the subdivisions (**Table 3.3**, *page 26*) across the full 15 min.

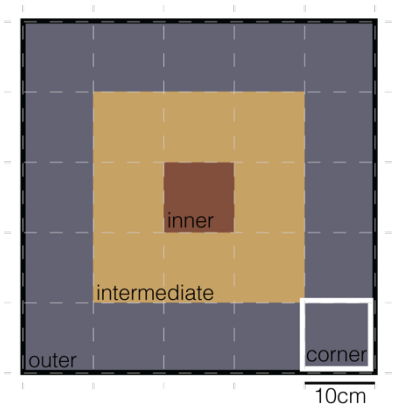


Figure 3.4: Open Field Test Arena. The OFT arena was divided in outer, intermediate, inner, and corner zones.

Table 3.3: OFT Parameters.

N.	Arena zone	Parameter
1	**Whole**	Distance
2		Time immobile
3		Immobile episodes
4	**Inner**	Entries
5		Time
6		Latency to first entry
7		Mean duration visit
8	**Intermediate**	Entries
9		Time
10		Distance
11		Latency to first entry
12		Average speed
13		Mean duration visit
14	**Outer**	Exits
15		Time
16		Distance
17		Latency to first exit
18		Average speed
19		Mean duration visit
20		Mean distance from
21	**Corners**	Entries
22		Time

3.4.3 Tail Suspension Test (TST)

Forty-eight hours after the OFT, mice were tested in the tail suspension test to assess their coping behaviour. Mice were hung by their tail approximately 50cm above the surface for 6min. Since c57BL6 mice are expert climbers and are able to turn on themselves and climb along their own tail, a small cylinder of plastic (15mm diameter, 2.5cm length) was placed at the base of their tail to avoid climbing (**Figure 3.5**, *page 27*). Immobility was automatically recorded using ANYMaze Video Tracking System v. 6.13 (Stoelting, IL, USA) with the following parameters for immobility detection: immobility sensitivity 75%; minimum immobility period 1000ms. Three parameters were evaluated: time immobile, immobile episodes, and immobility latency.

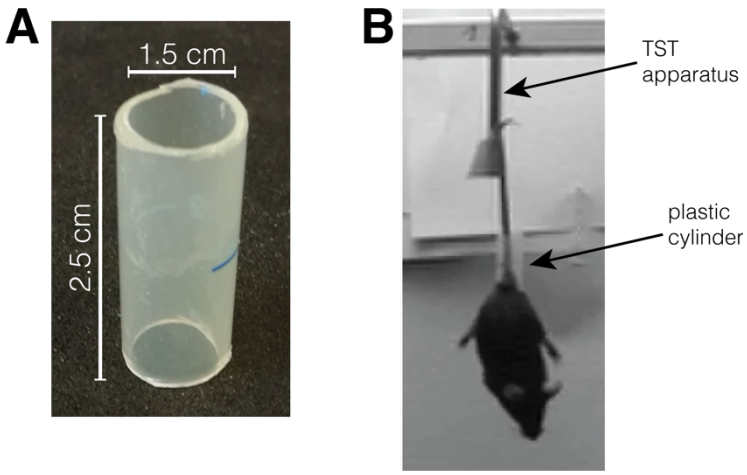


Figure 3.5: Tail Suspension Test Apparatus. To avoid climbing, a plastic cylinder was placed around the mice tail during the TST. (**A**) Photo of plastic cylinder used. (**B**) Example of a mouse during a TST wearing the plastic cylinder.

3.4.4 Sucrose Preference Test (SPT)

The sucrose preference test was used to assess anhedonia in mice after CMS. The test comprised of three parts, which were run in parallel with the other tests. At the end of ST, each home cage was provided two bottles of water for 24h as habituation. The following day, the water of one of the two bottles was substituted with a 2% sucrose solution. After 24h we inverted the position of the two bottles to exclude position-effects. The sucrose and water consumption were evaluated at the end of each day and summed across the two test days. The percentage of sucrose preference was calculated over the total intake of liquid for each mouse as follows: $\frac{sucrose}{water+sucrose} \cdot 100$.

3.4.5 Emotionality Score

At the end of the behavioural testing, mice were sacrificed and harvested for organs known to be responsive to stress exposure: the adrenal glands and the thymus. Organs were collected, cleaned from the connective tissue and surrounding fat, and their weight was normalized over bodyweight at sacrifice. In addition, bodyweight change (Day 21 – Day 1 of CMS) and cumulative coat state were also calculated. Finally, for each behavioural tests, all individual parameters specified above were calculated. All parameters were first Z-scored and directionally-adjusted as specified in "3.2.2 Unpredictable chronic mild stress (CMS)", *page 24*, and averaged to obtain one value per animal per test. This allowed to have a weighed final score per test and allowed for each test to have the same weigh in the final emotionality score. For each animal, an average score across all tests was then calculated to obtain an emotionality score which increased with higher susceptibility to stress. Splash test videos of two mice was lost due to technical issues, as a result these animals were removed from the emotionality score calculation.

3.4.6 Stress Score

For each mouse, the stress score was also calculated in a similar manner to the emotionality score. For the stress score, only the Z-scored values of bodyweight gain, the cumulated coat state (and the adrenal size when indicated) at sacrifice were averaged.

3.5 Single cell RNA-sequencing

3.5.1 Single cell suspension preparation

To maximize the collection of the second wave of transcription [181], animals were sacrificed 5 hours after receiving the acute restraint stress. Mice were sacrificed with a lethal dose of isoflurane and transcardially perfused in cold 1x PBS to remove circulating blood from the central nervous system. Control and stressed animals were sacrificed alternated, to reduce time biases. Brains were extracted and kept in cold carbonated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (aCSF: 87mM NaCl, 2.5mM KCl, 1.25mM NaH_2PO_4, 26mM $NaHCO_3$, 10mM glucose, 75mM sucrose, 2mM Mg^{2+}, 1mM Ca^{2+}) before dissection with a 0.5mm brain matrix. One slice of 1.5mm, from approximately -0.58mm Bregma to -1.22mm Bregma, containing the PVN was obtained from each brain and the PVN was manually dissected under the microscope (**Figure 3.6A-B**, *page 28*).

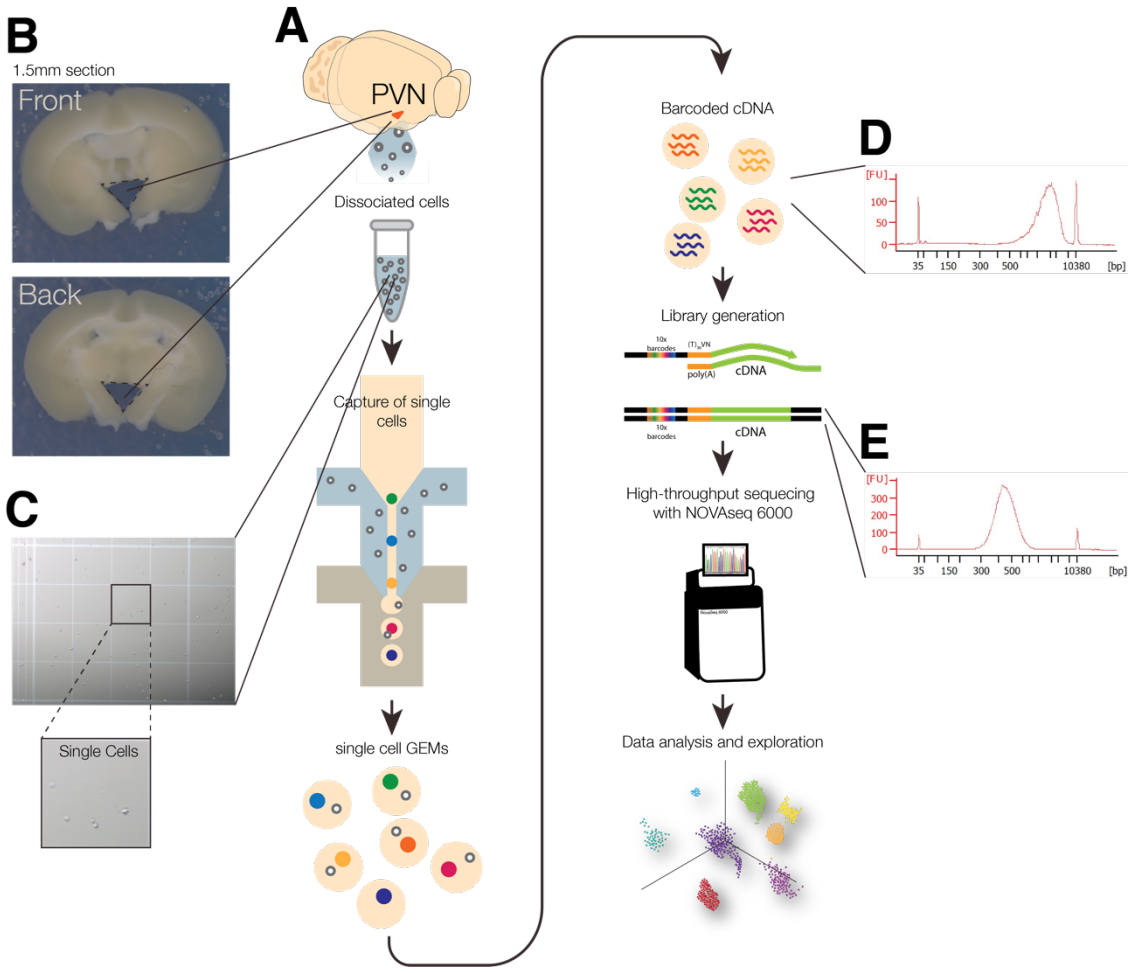


Figure 3.6: 10x Genomics protocol. (**A**) Workflow for the preparation of a scRNA-seq dataset with the 10x Genomics technology which includes the dissociation of single cells from the region of interest, the PVN; the use of the microfluidic system 10x Genomics Chromium controller to capture single cells together with a barcoded beads and generation of single cell gel beads in emulsion (GEMs); the generation of barcoded cDNA and a 3' RNA library; the high throughput sequencing and downstream data analysis. Representative pictures of: (**B**) the manually-dissected PVN from a 1.5mm-thick brain section; (**C**) the single cell suspension; (**D**) bioanalyzer traces for a representative barcoded cDNA, and (**E**) a representative library.

Due to the small size of the region, PVNs from five animals were pooled together into a single preparation per condition and sex. Pooled tissue was digested in papain supplemented with DNase I (Papain Dissociation System, Worthington BC – LK003163) for 50min at 37°C and triturated with a fire-polished glass pipette. The cell suspension was then filtered over a 30μm mesh (CellTrics 30µm, sterile, Sysmex – 04-004-2326), pelleted at 300g for 5min at 4°C, and resuspended in aCSF supplemented with more DNase I. Samples were then layered over a discontinuous density gradient of ovomucoid protease inhibitor with bovine serum albumin (Papain Dissociation System, Worthington BC – LK003163) and spun at 70g with slow acceleration and deceleration at 4°C. Cells were washed two more times in aCSF without Ca^{2+} and Mg^{2+} and finally resuspended to a final concentration of ~700.000-900.000 cells/ml (**Figure 3.6**, *page 28*). Cells were loaded on the 10x Genomics Chromium Controller, v2 chips, aiming at 10.000 cells. One chip per sex was run containing both stress and control samples to avoid batch effects.

3.5.2 Library preparation and sequencing

Library was prepared using the 10x Genomics Single Cell 3' Reagent Kits v2 (10x Genomics – PN-120237) according to the manufacturer's protocol. Molar concentration and fragment length of libraries were quantified using Bioanalyzer (Agilent High Sensitivity DNA kit – 5067-4626) and samples within each background were pooled in equal molarity for sequencing. The pooled libraries were sequenced on a NovaSeq 6000 sequencer with paired-end asynchronous sequencing, 100 cycles, 28bp/8bp/91bp on part of a lane of S2 NovaSeq with a depth of ~150 million reads per sample (**Figure 3.6**, *page 28*).

3.6 ScRNA-seq Analysis of Males and Females Samples

3.6.1 Pre-processing and Quality Control (QC)

Data was pre-processed with the 10x Genomics Cell Ranger software (v. 3.0.2 for the Baseline background and v. 3.1.0 for the CMS background) and further annotated on the mm10 reference set at the Bioinformatic Core Unit of the Weizmann Institute of Science (Rehovot, Israel). On average 27,302 (min 21,100, max 34,189) reads per cell were obtained across samples with an average saturation value of 67.12% (**Table 3.4**, *page 29*). Quality control (QC), clustering and rest of the analysis was performed within R v. 3.6.2 [182] using the package Seurat v. 3.1.3 [183], following the guidelines provided by the developers and best practice workflow in single cell data analysis [184]. Filtered count matrices were used to create one single Seurat object

Table 3.4: Pre-Processing Output from 10x Cell Ranger for Male and Female Samples.

Sample	Mean Reads/Cell	Saturation	Estimated n. Cells	Reads total
Female Baseline				
Control	25,581	60.30%	7,384	188,897,134
ARS	21,100	59.30%	7,270	158,370,920
Male Baseline				
Control	27,291	66.00%	5,225	142,599,605
ARS	34,189	70.70%	5,224	178,607,251
Female CMS				
Control	21,803	65.10%	6,021	158,653,571
ARS	24,680	74.20%	6,457	142,993,897
Male CMS				
Control	29,800	69.80%	5,324	131,275,854
ARS	30,136	71.10%	4,745	159,358,682

containing all eight samples (namely Male Baseline Control/ARS, Female Baseline Control/ARS, Male CMS Control/ARS, Female CMS Control/ARS). As first quality control, putative empty droplets, dead cells and multiplets were removed from the dataset. To remove suspect dead cells, cells with a mitochondrial gene content higher than 30% and less than 350 genes were removed. To remove suspect multiplets (doublets, triplets, or quadruplets), cells with a gene count higher than 3,500 or a UMI count higher than 15,000 were firstly removed. Subsequently, the functions *doubletCluster* and *doubletCells* from the package scran v. 1.14.6 [185] were used to estimate the probability of being a multiplet for each cell. Clusters which resulted outliers and cells with a DoubletScore > 4 were excluded. Finally, cells expressing all four blood genes *Hba-a2*, *Hbb-bs*, *Hbb-bt* and *Hba-a1* higher than 1 were considered blood cells and removed as contaminants too. This resulted in a final dataset of 35,672 single cell (**Table 3.5**, *page 30*).

Table 3.5: QC of Male and Female Samples.

Sample	Pre-QC Cell Count	Post-QC Cell Count	% Cells Retained *(n. cell lost)*
Female Baseline			
Control	6,973	5,559	80% *(1,414)*
ARS	7,020	5,777	82% *(1,243)*
Male Baseline			
Control	4,974	3,692	74% *(1,282)*
ARS	5,059	3,831	76% *(1,228)*
Female CMS			
Control	5,759	4,528	79% *(1,231)*
ARS	6,184	4,981	81% *(1,203)*
Male CMS			
Control	4,973	3,781	76% *(1,192)*
ARS	4,462	3,523	79% *(939)*
Total	**45,404**	**35,672**	**79% *(9,732)***

3.6.2 Cell Clustering

For clustering, data was lognormalize and scaled (factor 10,000) with the function *NormalizeData* in Seurat. The top 4,000 variable genes were selected using the function *FindVariableFeatures* in Seurat and used to scale the data with the function *ScaleData*. Twenty-two principal components and a resolution of 1.2 were used to cluster cells with the function *FindClusters*. Cells were than plotted in the “Uniform Manifold Approximation and Projection” (UMAP) bidimensional space and the identity of the cell clusters was identified overlapping over our clusters marker genes obtained from past experiences of the laboratory (**Supplementary Figure 8**, *page 134*) [186]. This allowed us to identify 33 clusters belonging to 17 main cell types: neurons (GABAergic (2), glutamatergic (3), vasopressin (2), and mixed (2)), astrocytes (4), microglia (3), macrophages (1), oligodendrocytes (3), committed oligodendrocytes progenitors (COPs, 1), oligodendrocytes progenitor cells (OPCs, 1), ependymal cells (2), tanycytes (1), endothelial cells (3), mixed endothelial cells (1), pericytes (2), vascular cells (1), and meningeal cells (1). The identity of the clusters was further validated exploring the representative gene for each cluster, calculated with the function *FindAllMarkers* with default settings using the Wilcoxon Rank Sum test on log-normalized scaled data.

3.6.2.1 Tanycytes Re-Clustering

For in-depth analyses of the tanycytes subpopulations, all cells belonging to the tanycytes clusters were split from the original Seurat object and re-clustered independently. These new objects contained 649 cells from all eight conditions. Clustering was executed as previously explained using the top 2000 variable genes with the following parameters: 12 PCs, 0.6 resolution. The identity of the tanycytes subtypes were assigned according to their top markers calculated by *FindAllMarkers* with default settings using the Wilcoxon Rank Sum test on log-normalized scaled data.

3.6.3 Cell Balance Analysis

To check if male and female cells equally distributed in the clusters, for each cluster and sample male and female cell counts were normalized over the size of the sample and the average sample size (equal to 4459) across the dataset as follows: $\frac{cluster\ size}{sample\ size} \cdot 4459$. For each cluster, distribution between female and male cells was calculated, expecting a 50-50 distribution. For controls conditions, in which a duplicate was available, each individual samples were normalized independently and in addition the average over the two replicates was calculated. Statistical significance of the imbalance was assessed with two-way ANOVA with Tukey's post-hoc p-value correction for controls and Fisher's exact test and Benjamini-Hochberg (BH) post-hoc corrected p-values for stress conditions. Adjusted p-values < 0.05 were considered significant.

3.6.4 Differential Gene Expression Analysis

Differential gene expression analysis was performed using Model-based Analysis of Single-cell Transcriptomics (MAST, [187], [188]) integrated in the function *FindMarkers* of Seurat to identify genes that were different between control male and female cells (combining Baseline and CMS control cells) or between stress (either Baseline ARS or CMS ARS) and control within the same sex for each of the main 17 cell types. To avoid ambient RNA noise, we tested only genes expressed in at least 50% of cells in the tested cluster in either condition. Benjamini-Hochberg (BH)-adjusted p-values less than 0.05 were used to determine significantly deregulated genes. Furthermore, average gene expression per cluster was calculated using *AverageExpression* function on log-normalized scaled data. DEGs were represented either with Upset plots (R package ComplexUpset v.1.2.1 [189], [190]) or Venn Diagrams (R package eulerr v.6.1.0 [191], [192]). All log fold changes (logFC) represent the natural logarithm of the fold change, as computed by Seurat.

To assess the similarity of the ARS response between male and female, the Szymkiewicz–Simpson coefficient was calculated according to its formula, for each cluster (C):

$$SI = \frac{|C_{male} \cap C_{female}|}{\min(|C_{male}|,|C_{female}|)}$$

To identify the enriched transcription factors interacting with the common DEGs from ARS under Baseline, the 137 genes differentially expressed in both males and females were inputted in the online platform Enrichr.com [193] and analysed in the *Transcription Factor Protein-protein interaction (PPI)* module. This module uses a literature-based approach to identify transcription

factors-protein interaction networks calculating enrichment for transcription factors in a list of protein. Results are reported in term of p-value, network, and clustergram.

3.6.5 Stress Background Susceptibility Analysis

Background influence on acute stress response was assess placing each cell type in a bidimensional space constituted of the number of DEGs and the median absolute log fold change. Distance between ARS under CMS and Baseline was calculated as Euclidean distance between the two datapoints for each sex. To have a better visualization and comparison between the sexes, distances have been Z-scored within each sex. To evaluate which cell type is the most affected across sexes, the sum of Z-scored Euclidean distance between male and female was calculated. In addition, for each cell type, we also calculated the Szymkiewicz–Simpson coefficient, as reported above ("3.6.4 Differential Gene Expression Analysis", *page 31*).

3.6.6 Rank-Rank Hypergeometric Overlap Analysis

To explore further how similar the ARS response is between males and females, we performed a rank-rank hypergeometric overlap (RRHO) analysis on the differentially expressed transcriptome of either males and females with the R package RRHO2 v.1.0 [194], [195]. Each gene was listed as the gene name and the cell type in which was identified. All genes were inputted as the product between its p-value • the sign of the fold change.

To explore how much the oligodendrocytes transcriptional stress response was affected by the background, we run a similar RRHO analysis on the differentially expressed transcriptome of either male or female oligodendrocytes. We used the R package RRHO2 v.1.0 [194], [195] for each sex on all genes present in both conditions (female = 634, male = 807). Each gene was inputted as the product between its p-value • the sign of the fold change.

3.6.7 Cell-Cell Interaction Analysis

To evaluate predicted cell–cell interaction networks, we used predicted ligand–receptor interactions from between oligodendrocytes and neuronal subclusters using the package CCInx v. 0.5.1 [196]. The package uses the Cell-Cell interaction database (http://baderlab.org/CellCellInteractions) [197] to quantify ligand-receptor interactions as edge weights. We calculated the networks within each of our eight samples (namely Control and ARS for each condition: Baseline Female, Baseline Male, CMS Female, CMS Male) for all genes with expression value higher than 1.5. To evaluate how much stress exposure perturbates interaction networks, a delta edge weight was calculated within each condition as (edge weight ARS) – (edge weight control). Delta edge weights <0.01 were removed to minimize noise and we performed a permutation analysis based on Nagy et al. [198] to test which changes in edge weight were significantly different. Specifically, we randomly permuted all control and ARS cells into two groups for 100 times and we calculated the distribution of edge weight differences between the two groups for each ligand-receptor pair in each permutation. Eleven pairs of genes were not present in the permutation results and therefore were dropped from the analysis. We then calculated a p-value for each of the stress-control edge weight differences and applied the BH post hoc p-value correction across all tests run. Edge weights with q-value<0.05 were considered

significant. Importantly two type of gene pairs could be identified. Receptor-ligands pairs whose strength was influence by the presence or absence of stress and receptor-ligand pairs present exclusively in one or the other condition (**Figure 4.45**, *page 72*). To evaluate if stress impacted in a directionality-specific way the ligand-receptor networks, the distribution of edge weights of deregulates receptor-ligand pairs for either direction (oligodendrocytes to neuron, and neuron to oligodendrocyte) were explored. Circle plots were realized using the package circlize v 0.4.10 [199].

3.6.8 Pseudotime Analysis

To study the developmental trajectory of the oligodendrocytes, we isolated the clusters belonging to the oligodendrocyte lineage (COP, OPC and Oligodendrocytes) and re-clustered them within Seurat v. 3.1.3 with the same procedure explained above, 15 PCs and a resolution of 0.6. The Seurat object was then transformed into a Monocle3's object for constructing single-cell pseudotime trajectories [200]–[202]. Trajectory analysis was performed according to authors' recommendations. Root was assigned in the far-left node in the OPC cluster (**Figure 4.49**, *page 74*). Cells projection over pseudotime was presented using ggplot density plot and cumulative plots. Statistical analysis was run on the cumulative curves for each control-stress pair using a two-sample, two-sided Kolmogorov-Smirnov test with BH p-value adjustments.

3.7 ScRNA-seq Analysis of OVX Samples

3.7.1 Pre-Processing, Quality Control and Cell Clustering

Samples from OVX control and stressed females were pre-processed with the 10x Genomics Cell Ranger software v. 3.0.2 and further annotated on the mm10 reference set at the Bioinformatic Core Unit of the Weizmann Institute of Science (Rehovot, Israel) together with the Baseline background samples. On average 29,219.5 reads per cell were obtained across the two samples with an average saturation value of 67.35% (**Table 3.6**, *page 33*). Processing of the dataset was done with Seurat as explained before (see Chapters "3.6.1 Pre-processing and Quality Control", and "3.6.2 Cell Clustering"). We obtained a final dataset of 7,693 cells that passed quality control (**Table 3.7**, *page 33*), clustered in 22 clusters.

Table 3.6: Pre-Processing Output from 10x Cell Ranger of OVX Samples.

Sample	Mean Reads/Cell	Saturation	Estimated n. Cells	Reads total
OVX Baseline				
Control	26,970	66.80%	6,042	162,955,152
ARS	31,469	67.90%	5,411	170,281,816

Table 3.7: QC of OVX Samples.

Sample	Pre-QC Cell Count	Post-QC Cell Count	% Cells Retained *(n. cell lost)*
Female OVX			
Control	5,644	4,118	73% *(1,526)*
ARS	4,989	3,575	72% *(1,414)*
Total	**10,633**	**7,693**	**72% *(2,940)***

3.7.2 Integration - Label Transfer

To allow the comparison of gene expression between correspondent cell types, the identity of OVX clusters were assigned through the label transfer procedure in Seurat. Label transfer is a form of integration that allows to overlay cell identities from one single cell dataset to a second one based on gene expression of anchor genes. Labels were transferred from the subset dataset containing only Baseline Male and Female samples to the OVX sample over 30 anchor points – calculated with the function *FindTransferAnchors* – using the function *TransferData*. The original 33 single clusters labels were transferred onto the OVX dataset. To assign the lower dimensionality labels (such as cell types and cell categories) the same relationship between original clusters and lower dimensionality clusters were maintained (e.g., Cluster 0 corresponded to Astrocytes_1 and belonged to Astrocytes in the male-female dataset. OVX cells placed in cluster 0 received these same labelled).

3.7.3 Differential Gene Expression Analysis

Differential gene expression analysis between OVX ARS and OVX Control was run within each cell type according to the same parameters as the Baseline background (see Chapter “3.6.4 Differential Gene Expression Analysis”).

3.8 Pathway Analyses

3.8.1 Gene Set Enrichment Analysis (GSEA)

Gene set enrichment analyses were used to evaluate pathway enrichment for tanycytes markers, regardless of a significance threshold. The analysis was performed in R v. 3.6.3 (2020-02-29) [203] with the package fgsea v. 1.12.0 [204]. Gene sets were retrieved from the online database Molecular Signatures Database (MSigDB) v7.4 through the R package msigdbr v. 7.0.1 [205]. For each analysis, the background was manually calculated as all genes expressed by the cells analysed and subtracted.

3.8.2 Pathway Analysis for DEGs in Oligodendrocytes and Female GABAergic Neurons

To explore which processes were mostly affected by sex in control oligodendrocytes and by ARS in GABAergic neurons in females, we looked for enriched pathways. The DEGs (250 female-upregulated DEGs; 568 male-upregulated DEGs; 329 DEGs GABAergic neurons) from each independent analysis were inputted in the online platform Metascape.org [206] and tested against the background of all tested genes. Analysis was conducted using default parameters on gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

3.9 Morphology Analysis of Oligodendrocytes

For oligodendrocytes morphology analysis, a new cohort of male and female mice received ARS after CMS or under Baseline, as previously described. Five hours after the end of stressor, mice

were lethally anesthetized in isoflurane and transcardially perfused in 4% PFA. Brains were collected, post-fixated in 4% PFA for 24h at 4°C, and then moved to sucrose 30% until sinkage. Brains were dissected in five series of forty-μm sections (for a total distance between section of 200μm) and sections containing the PVN area (-0.58mm Bregma to -1.22mm Bregma) were processed for immunofluorescence. Briefly, tissue was blocked in blocking solution (5% normal goat serum, 0.5% Triton X-100 in 1x PBS) for 1h at room temperature. Incubation with the primary antibodies (α-Tmem10, Rabbit (Rb) 1:500 (courtesy of Peles lab, Weizmann Institute of Science, Israel), α-olig2, Mouse (M) 1:250 (Millipore MABN50)) were incubated at 4°C for 20h in blocking solution. Secondary antibodies (α-Rb-Alexa Fluor 488, goat 1:500 (Invitrogen, N. A32723) and α-M-Alexa Fluor 594, goat 1:500 (Invitrogen, N. A32740)) were further incubated at room temperature for 1h. Slides were mounted with DAPI Fluoromount-G (SouthernBiotech, N. 0100-20).

Sections were first explored to identify the PVN region based on DAPI density. Two sections containing the region of interest (one frontal and one caudal, 200μm apart) were selected per animal (**Figure 4.40A**, *page 68*). Overviews of DAPI staining were acquired at a VS120 Olympus Automated Slide Scanner (15,221x7,542 resolution, 2x objective). In each selected slide, left and right PVNs were acquired as a tiled 4-field picture (1,024x1,024 resolution, 40x objective, 0.5x magnification, 2μm z-stack) at a LSM800 Zeiss confocal microscope (**Figure 4.40B**, *page 68*).

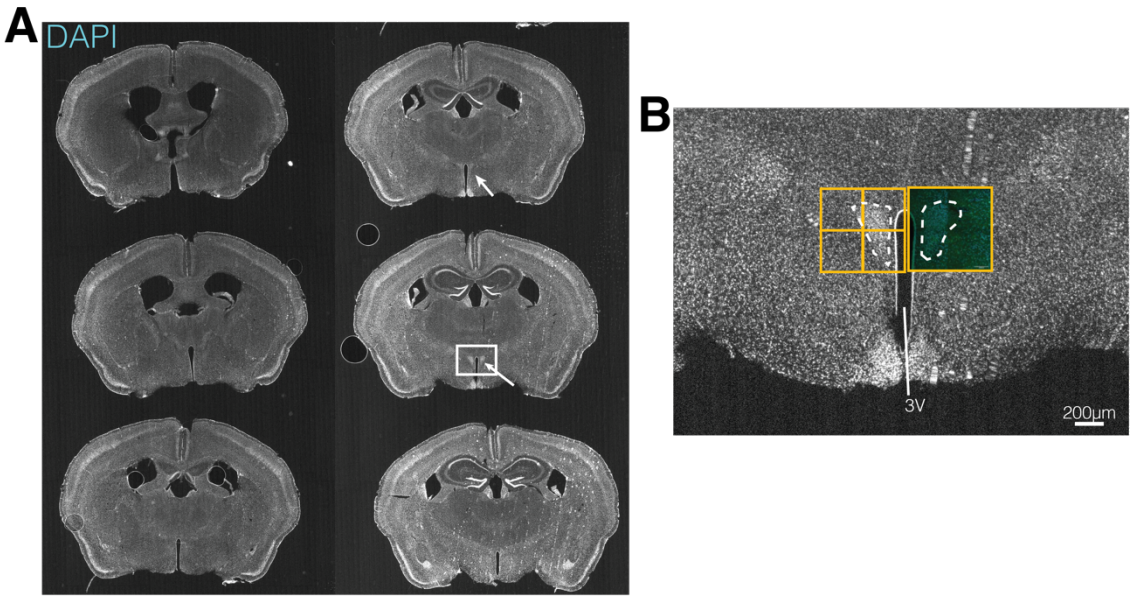


Figure 3.7: Imaging of the PVN. Several sections around the PVN per animals were imaged. (**A**) DAPI signal was used to identify the two sections containing the PVN, 200μm apart. White arrows indicate the nuclei-dense region of the PVN. (**B**) Zoom of the left and right PVNs highlighted in A. Each side was then imaged as 4-field picture (yellow squares) at a confocal microscope.

For tracing, the PVN region was first defined based on DAPI density. All visible Tmem10$^+$,Olig2$^+$ cells within the defined PVN were labelled and the Tmem10 signal was traced using the Fiji plugin Simple Neurite Tracing v. 3.1.7 [207], [208]. Number, total length, and average length of total and primary processes were computed. Sholl analysis was performed on the traces obtained using the plug-in Sholl analysis v. 4.0.1 available in Fiji [209] with default parameters and continuous sampling from the centre of soma. Concentric intersections were binned to intervals of 5 μm. To

remove intersections due to soma crossing, soma radius was calculated from circumference and intersections with radius < radius soma were removed. In addition to the intersection distribution, for each cell we also calculated the maximal distance from soma and the area under the curve. Statistical analysis was performed applying a nested design within a mixed-effects models with the R package lme4 v. 1.1-26 [210], nlme v. 3.1-144 [211], and lmerTest v.3.1-3 [212] based on the implementation in R of the Sholl analysis [213], [214]. For each condition, 6 animals were used with an average of 14.29 cells per animal (N: F CMS = 97, F ctrl = 82, M CMS = 81, M ctrl = 64). Representative cells in **Figure 4.55**, *page 79*, were generated using the skeletonize function within Simple Neurite Tracer.

3.10 Web Interactive App

To make the dataset available in a ready-to-explore manner, we created a web interactive app containing the processed and clustered dataset. For this purpose, we loaded the male and female dataset (without OVX samples) as a Seurat objected in the R package ShinyCell v. 2.1.0 [215], a package that allows to create interactive Shiny-based web applications to visualise single-cell data and directly interact with them. ShinyCell is an open-source-code based on the packages shiny[216] and shinyhelper [217]. We modified the code of the app to introduce a new homepage tab containing the description of the dataset and the references to the lab website. In addition, we matched the colour scheme to the lab website. Online deployment was done with the platform https://www.shinyapps.io/ . To allow easier exploration of the data, the online dataset was created including all detected genes, all metadata (sample, cohort, condition, n. UMIs, n. detected genes, % mitochondrial genes, clusters, cell types).

3.11 Data Analysis and Data Handling

Data manipulation and statistical analysis have been performed within R studio v. 1.2.5033 [218] with R v. 3.6.3 (2020-02-29) [203] and the support of the package tidyverse v. 1.3.0 [219]. Plots have been generated using the R packages ggplot2 v. 3.3.0 [220] and viridis v. 0.5.1 [221] if not differently specified above. ANOVA, linear models and post-hoc p-value corrections have been computed using the packages lme4 v. 1.1-26 [210], nlme v. 3.1-144 [211] lmerTest v. 3.1-3 [212], and emmeans v. 1.5.4 [222], as stated for each result. When ANOVA was used, Shapiro-Wilk normality test was used to verify normality in data distribution. In case normality was violated (as in coat state data distribution) non-parametric test such as Kruskal-Wallis rank sum test were used as indicated in each figure legend. A comprehensive list of all packages can also be found in **Supplementary Table 4**, *page 146*. Statistical models and details on the statistical tests run can be found in the section “7.2 Supplementary Tables”, *page 144*.

4 | Results

4.1 Experimental Design

To study the acute stress response in the PVN and the effect of previous stress exposure, we used sexually mature C57BL/6N male and female mice which allowed us to explore sex difference in adulthood independently form developmental sex differences. Importantly, since our goal was to dissect differences between male and females, we also chose an experimental set-up that would minimize noise in our measurements. As such, we decide to minimize social stress that has a sex-specific impact by housing the animals in same-sex pairs. Social stress can arise both by isolation and overcrowding. Isolation stress impacts on the wellbeing of single-housed animals and it does so differently on males and females [87], [223]–[225]. On the other hand, when housed in groups of three or more, complex social dynamics such as social hierarchies get established between members of the groups [226]–[229]. This social structures not only increase variability between individuals [11], but we and others also showed that it generates a stress-specific stress load [119], [228], [230]–[233]. As a result of these considerations, we housed our mice in groups of two, to minimize these confounding elements.

In addition, since gonadal adult circulating hormones (i.e., estrogen, progesterone, testosterone) strongly vary across the estrus cycle of female mice and they interact with the stress system [112], we excluded from our pools of animals all females that did not display a regular cycling (see section "3.1.1 Estrus Cycle Monitoring", *page 21* for details); anyway a rare occurrence in our adult pool (9.5% of all mice used). We exclude mice based on estrus stage for 10 consecutive days before the start of experimental procedures. Overall, the majority (105 out of 116, 90.5%) of our females cycled normally across the four stages of the estrus cycle, with cycle phase lengths similar to those observed by others [142] (**Figure 3.2A**, *page 23*).

Finally, following the same criteria, we selected acute and chronic stressors with robustness across the sexes based on literature data. We selected as acute stressor the acute restraint stress (ARS), a robust physical and psychological stress paradigm that can be effectively applied to both sexes [234]. In order to evaluate how the transcriptional stress response changes after exposure to a chronic stress paradigm, we also looked for a translatable stress paradigm with face and construct validity that would resemble human conditions and that would not be negatively impacted from the presence of both sexes. Based on a thorough literature review, we selected the unpredictable chronic mild stress (CMS, known also as just chronic mild or variable stress). While the ARS protocol was heavily standardized in the lab, the same was not true for CMS, which required further validation and design adjustments described in the next chapters ("4.2 Establishment of Stress Paradigms", *page 37*).

4.2 Establishment of Stress Paradigms

4.2.1 CMS Design and Establishment

The CMS model was developed by Willner 30 years ago and was optimized through the years to obtain a model with construct, face and predictive validity for developing depression-like symptoms in both sexes [235]–[238]. In this paradigm, mice are exposed to a series of mild

stressors over a course of several weeks (3 to 4 weeks) in a continuous and unpredictable fashion to avoid habituation. This kind of stress leads to the development of anhedonia, decrease self-care and grooming, and behavioural alterations such as helplessness and behavioural despair which makes it a robust model for depression in rodents [239]. We first selected a list of stressors that would be easily applied to both sexes and would not involve systems physiologically different aside from the stress ones (e.g., stressors that might involve changes in body temperature, circadian rhythm, and other metabolic processes).

We therefore designed an unpredictable chronic mild stress which included psycho-physical stressors impacting on homecage environment, social structure, and mobility (**Figure 4.1**, *page 38*, the detailed description of all stressor and their execution can found in the method section "3.2.2 Unpredictable chronic mild stress (CMS)", and **Table 3.1**, *page 24*) and applied the protocol for 21 days with an initial dataset of 12 males and 12 females.

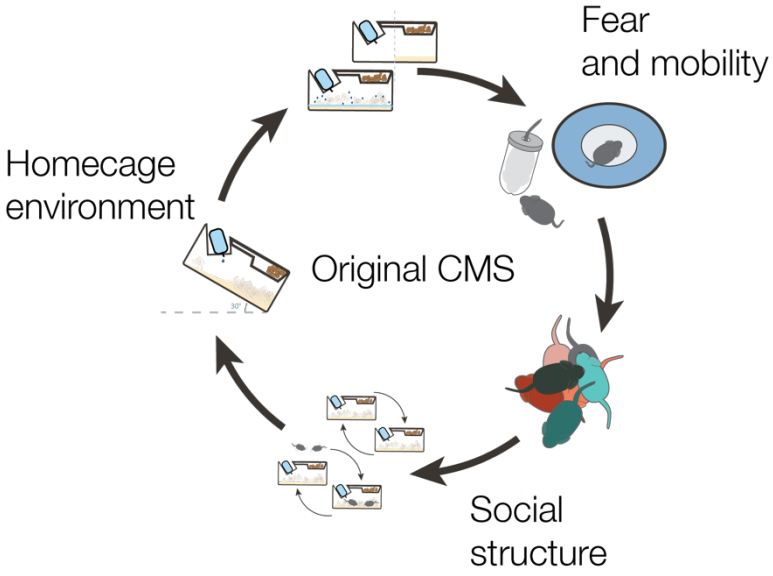


Figure 4.1: CMS paradigm. (**A**) CMS paradigm included several stressors that altered the homecage environment (no nesting, no bedding, cage tilt, wet bedding), the social structure (cage change, cage switching, overcrowding) and mobility or induced fear (water avoidance, restraint in the dark/bright light, restraint witnessing).

To validate effectiveness of our newly designed protocol, we monitored physiological and physical parameters as well as behaviours of the mice. All mice, including the control groups, were monitored twice a week for their bodyweight, coat state and their behaviour was assessed with a battery of anxiety-, anhedonia-, locomotion, and depression-related tests ("3.4 Behavioural tests", *page 25*). Finally at sacrifice, we also collected adrenal glands, thymus, and trunk blood CORT levels, all parameters known to be altered by stress states [42], [240], [241].

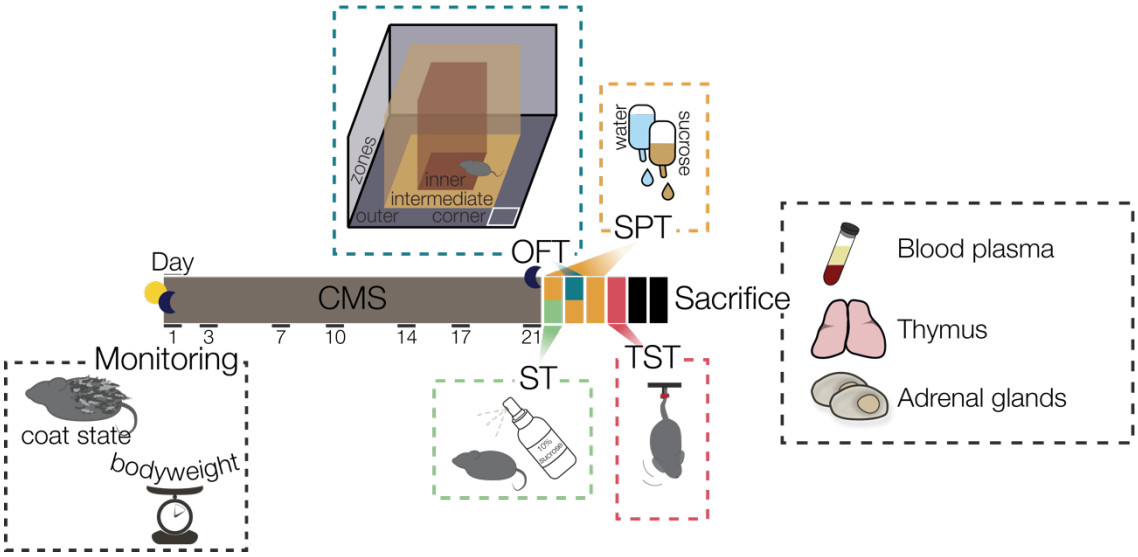


Figure 4.2: Timeline Behavioural Testing. During the 21 days of CMS, male and female mice were monitored twice per day assessing their coat state and bodyweight. Behavioural changes were assessed at the end of the stress paradigm in the open field test (OFT), the sucrose preference test (SPT), the splash test (ST), and the tail suspension test (TST). Forty-eight hours after the tests, mice were sacrificed to collect blood, thymus, and adrenal glands.

The monitoring during the CMS protocol already showed that our design was indeed impactful on the wellbeing of the mice, causing a delayed bodyweight gain so that at end of CMS mice were on average 6.20% lighter (4.88% males and 7.52% for females, **Figure 4.3A-B**, *page 39*). The coat state of these mice also clearly deteriorated during the three weeks of protocol (**Figure 4.3C-D**, *page 39*). In addition, we also found a small but significant increase in adrenal size of approximately 15% of their normalized weight (**Figure 4.4B-B'**, *page 40*). We did not see any differences in thymus size or CORT levels (**Figure 4.4A, C**, *page 40*).

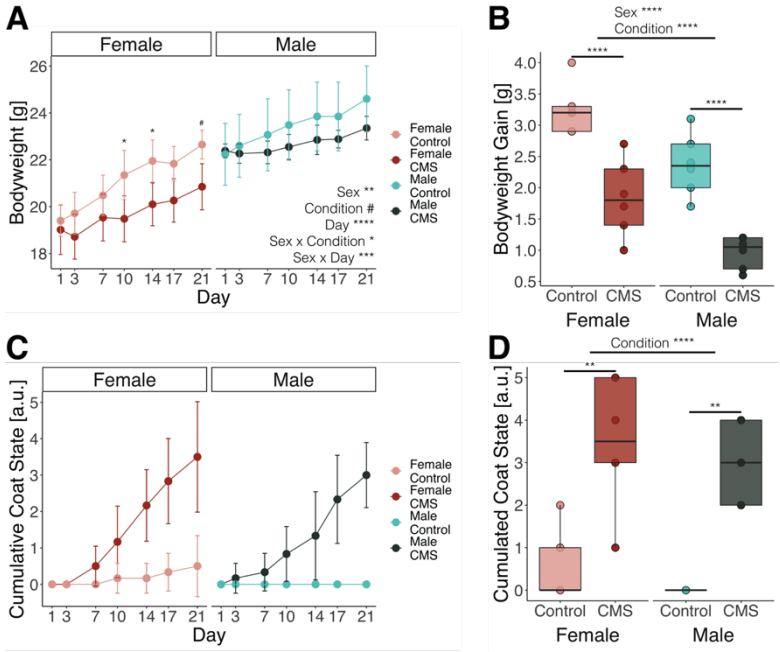


Figure 4.3: Body Weight and Coat State during CMS Monitoring. (**A-B**) Bodyweight and (**C-D**) coat state were regularly monitored during the CMS protocol. (**A**) Control male and female mice gained

considerable weight during the three weeks of protocol, while CMS mice lagged behind (RM two-way ANOVA, Tukey's post-hoc correction). (**B**) As a result, on Day 21 CMS mice were considerable lighter than controls (two-way ANOVA, Tukey's post-hoc correction). (**C**) The coat of CMS mice deteriorated across the three weeks of stress paradigm, (**D**) so that on day 21 they showed a statistically worse coat state (Kruskal-Wallis rank sum test, Dunn's post-hoc test). Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

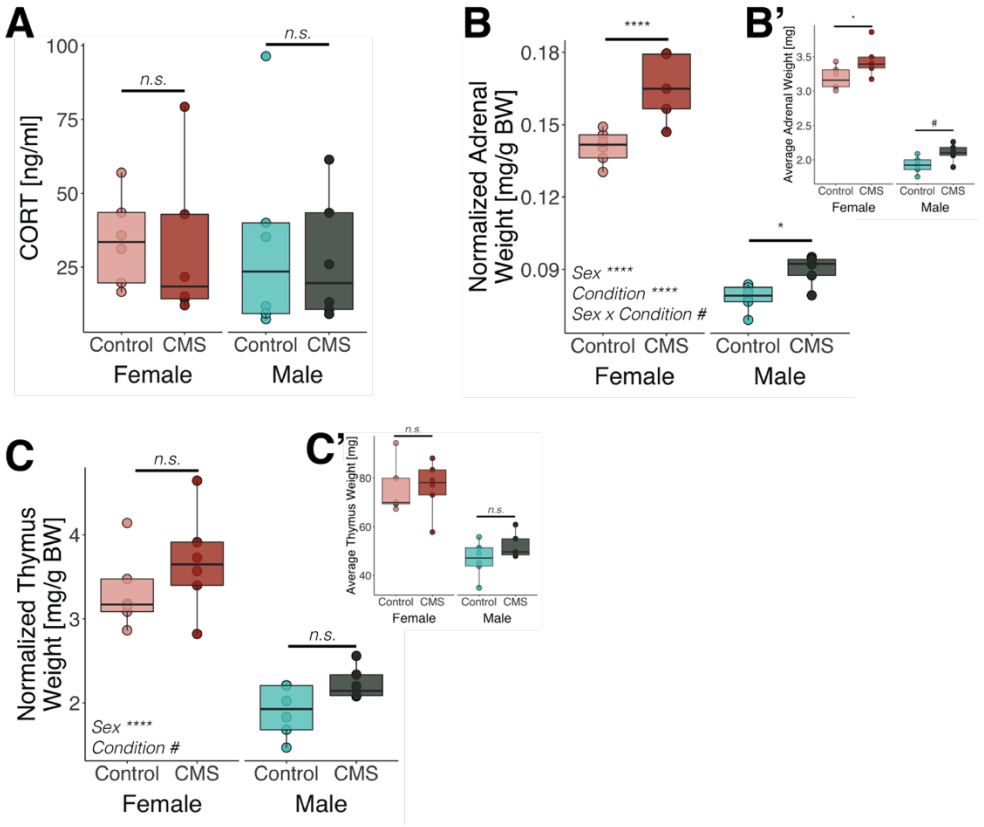


Figure 4.4: Physical Parameters at Sacrifice. CMS and control mice were sacrificed at the end the behavioural test battery. (**A**) Circulating CORT was not different between CMS and control animals (Kruskal-Wallis rank sum test, Dunn's post-hoc correction). (**B**-**B'**) Adrenals were significantly enlarged in CMS mice. (**C**-**C'**) Thymus size was not different between conditions. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

Finally, we looked for behavioural alterations of the CMS mice compared to controls. Mice did not show any differences in general locomotion and mobility (**Figure 4.5A-C, F**, *page 41*) in the open field test. But we did see that females moved in general more than males: a phenotype that has been extensively observed in literature [242]. Differences between males and females were also visible in the number of time mice entered the corner zones (**Figure 4.5D-E**, *page 41*). This effect was however probably due to the higher locomotion activity of the female mice, since both male and female explored the whole arena (**Figure 4.5F**, *page 41*). We did notice, however, that CMS mice possibly changed their corner zone exploration pattern, an indication of a change in anxiety levels (**Figure 4.5D-E**, *page 41*), since corners are a special part of the arena considered the least anxiogenic due to the presence of two walls. To further dissect anxiety-related behaviours, we also explored the behaviours associated more to anxiety dividing the arena in three zones based on distance from the outer walls of the arena (see **Figure 4.2**, *page 39* and section "3.4.2

Open Field Test (OFT)" of methods, *page 26*), and calculating time, latency to enter or exist such zones and number of entries. We did not find any differences in behaviours in the less anxiety zones, the outer zone (OZ) (**Figure 4.6**, *page 42*), aside from sex-specific differences with females having higher speed and travelling more, in accordance to the measures done at the level of the whole arena (**Figure 4.6D, F**, *page 42*).

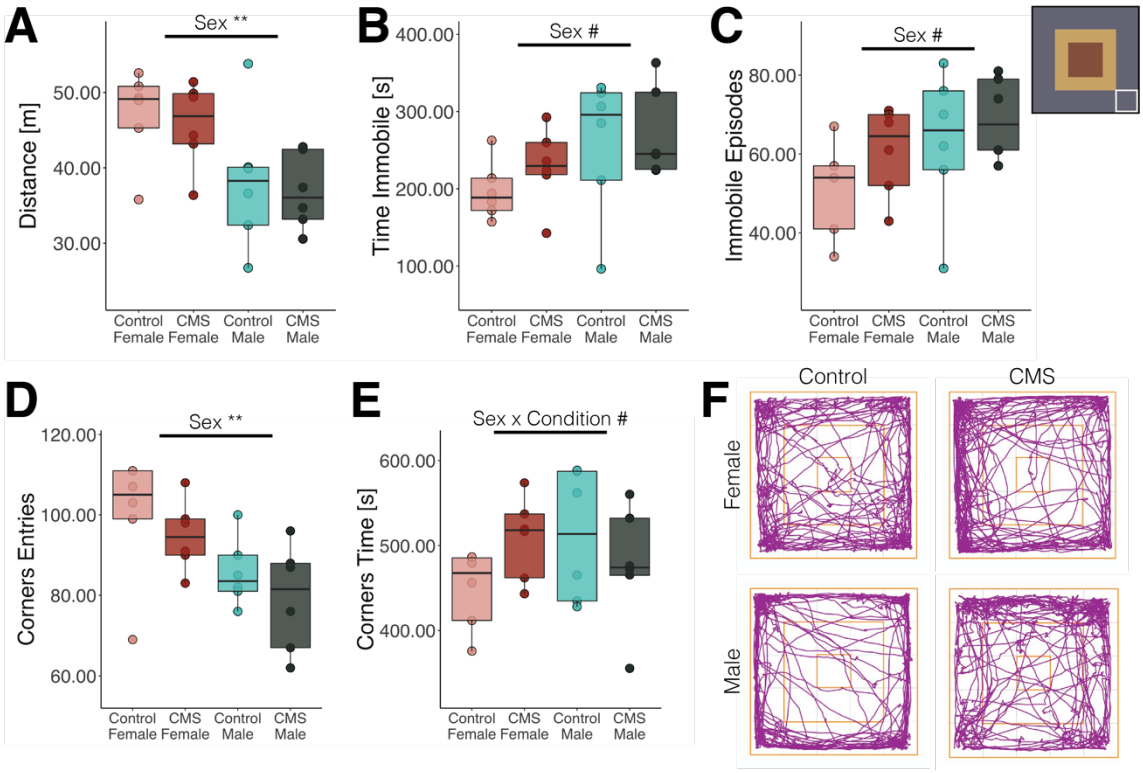


Figure 4.5: Overview Parameters from the Open Field Test. Open field test measures across 15 min. (**A**) Female mice covered on average more distance than male, but no differences between conditions were observed. (**B-C**) No statistically significant difference was observed in the immobility of mice across the whole arena. (**D**) Female mice entered the corner zones more times than males, probably as a result of their hyperactivity. However, no condition effect in the number of entries or (**E**) time spent in the corner zones was observed. (**F**) Representative tracks of animals across the 15min of OFT, showing higher activity in females. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

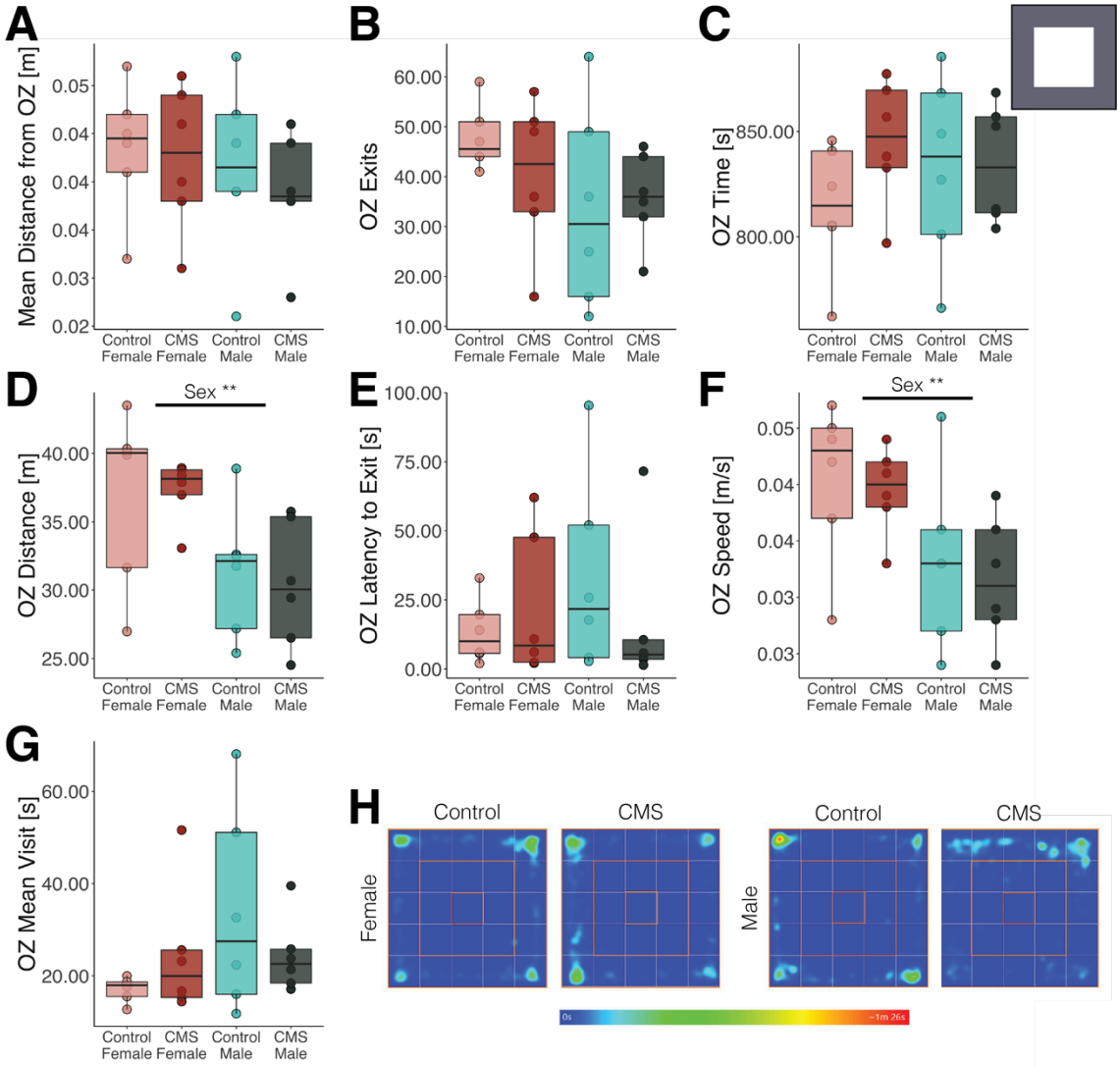


Figure 4.6: Outer Zone Measures of the Open Field Test. (**A**-**G**) Several behavioural parameters were measured within the outer zone of the OFT apparatus, the least anxiogenic zone. (**H**) Representative heatmap of mice movement within the OFT arena across the 15min showing no major differences between conditions. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values. OZ: outer zone.

The intermediate zone (IMZ), the transitioning area between the outer walls and the centre of the area showed a similar trend (**Figure 4.7**, *page 43*). Females tended to travel more than males and visit the zone more at a higher speed (**Figure 4.7C**, **E**, **F**, *page 43*). Interestingly we found a tendency for CMS mice to run faster in this area, an indication of anxiety (**Figure 4.7E**, *page 43*).

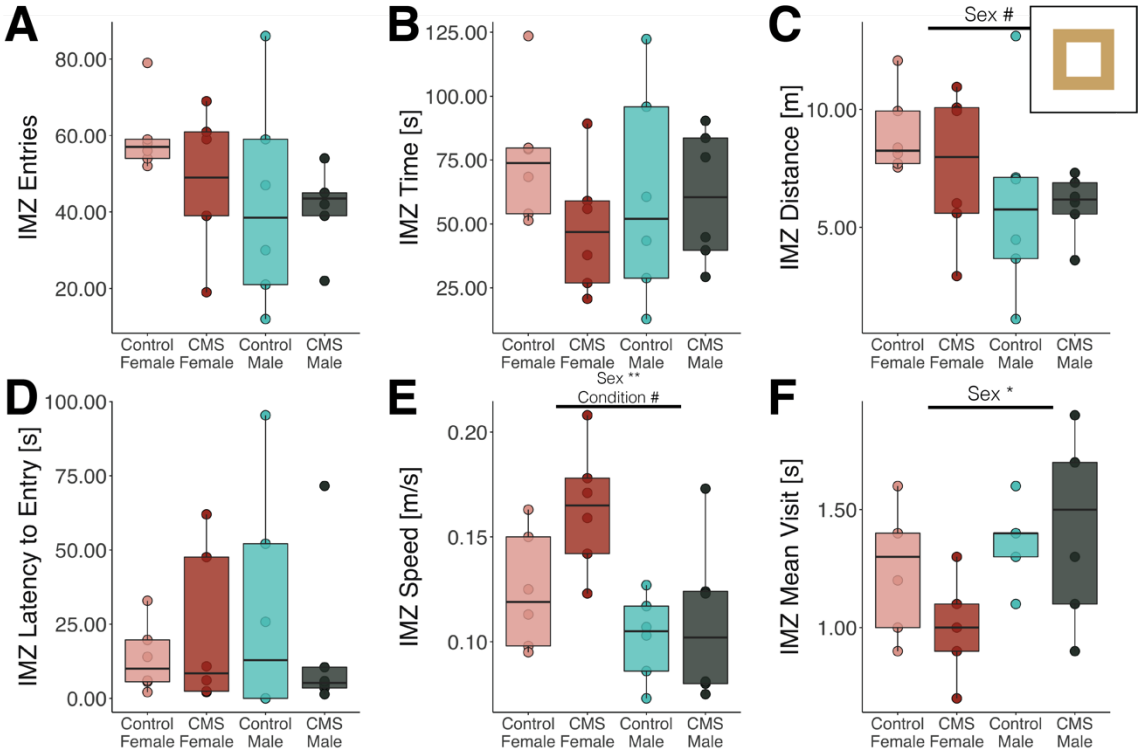


Figure 4.7: Intermediate Zone Measures of the Open Field Test. (**A**-**F**) Several behavioural parameters were measured within the intermediate zone of the OFT apparatus, the transitioning zone. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values. IMZ: intermediate zone.

Finally, we looked at the most anxiogenic area of the OFT, the inner zone (IZ), but we did not find any statistical difference between conditions (**Figure 4.8**, *page 44*) and only that male took more time than females to enter the zone for the first time (**Figure 4.8C**, *page 44*).

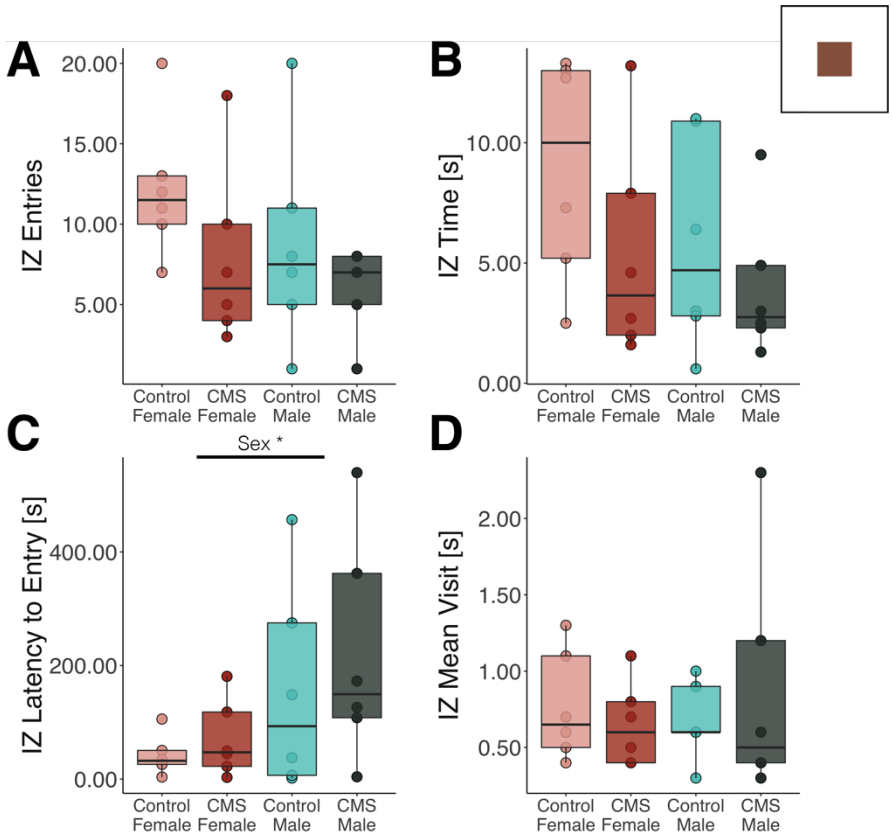


Figure 4.8: Inner Zone Measures of the Open Field Test. (**A**-**D**) Several behavioural parameters were measured within the inner zone of the OFT apparatus, the most anxiogenic zone. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values. IZ: inner zone.

Overall, the OFT did not show any major behavioural deficits due to exposure to CMS, even if we observed some small trends. However, when we combined all measures in one single OFT score (**Figure 4.9**, *page 44*), no statistically significant difference was observed.

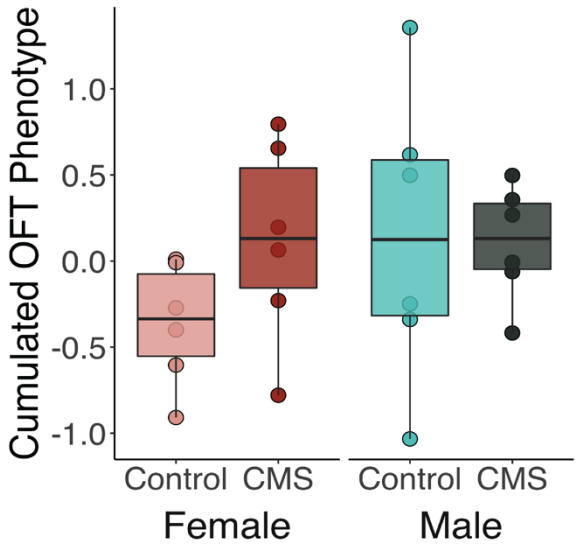


Figure 4.9: Combined OFT Phenotype. Cumulated OFT phenotype composed of all parameters measured in the 15min of OFT. No differences were observed. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA not significant.

On the other hand, we did see a clear effect of CMS on grooming behaviour in the splash test. The splash test consistent in spraying the mouse coat with sweet solution and measuring the time spent grooming and the latency to the first grooming session, evaluates self-care tendency. A reduction in these behaviours is classically associated to a depressed-like phenotype. Exposure to CMS indeed significantly increased the latency to the first grooming bout and reduced the overall time spent grooming (**Figure 4.11**, *page 45*). The phenotype was even more clear when the two measures were combined in a single ST phenotype score (**Figure 4.10**, *page 45*).

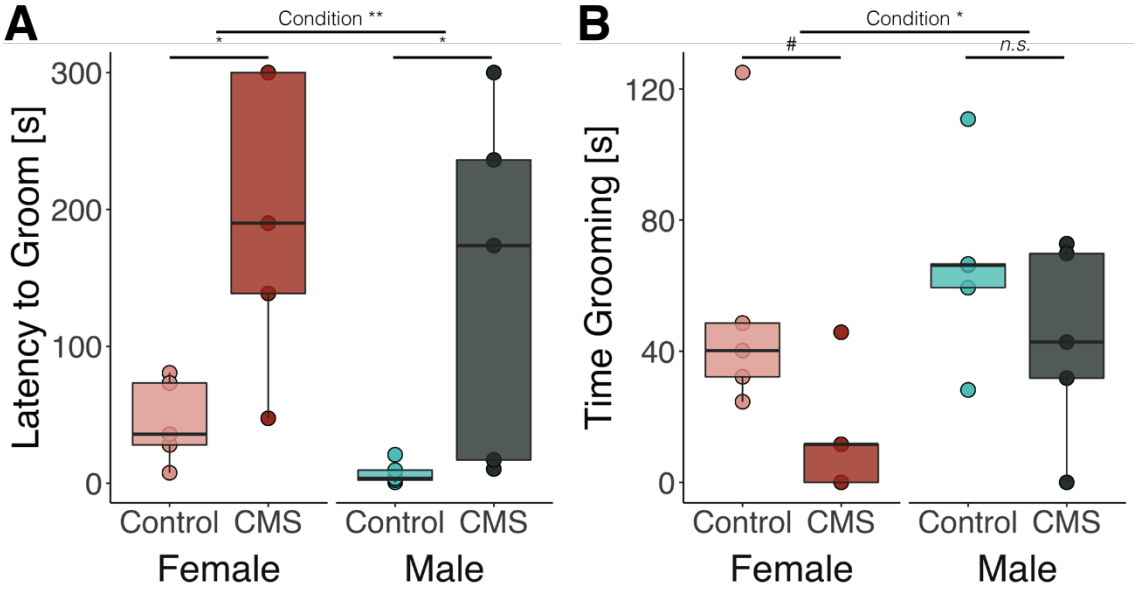


Figure 4.11: Splash Test. (**A**) Latency to the first grooming bout and (**B**) total time grooming in the splash test both showed a condition effect. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

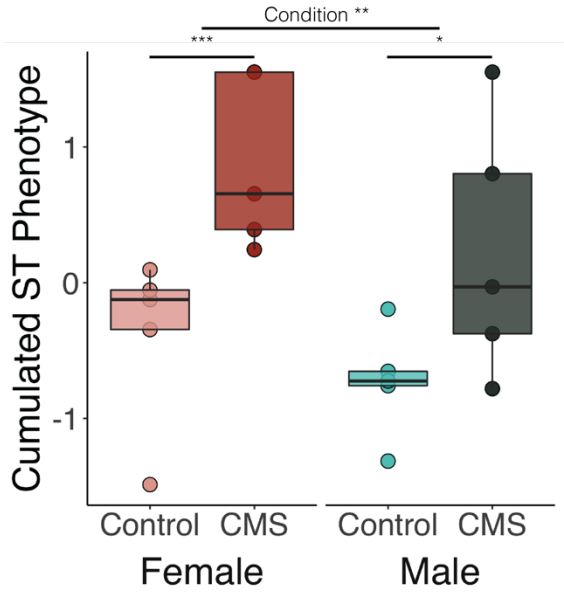


Figure 4.10: Cumulated ST Phenotype. Splash test measurements (latency to groom, and time grooming) were averaged in a cumulated ST measure that showed a significant condition effect. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

We next assessed the anhedonic phenotype of the mice through the sucrose test, a test of choice that evaluate the preference of a mouse towards a sugar solution versus normal water. Mice were first habituated to have access to two drinking bottles and tested for the following 48 hours. We Unexpectedly, we did not find any condition effect or reduction in sucrose preference for the CMS-

exposed mice (**Figure 4.12**, *page 46*).Even if we successfully confirmed that mice did have a preference for sucrose over water across both days (**Supplementary Figure 2**, *page 130*).

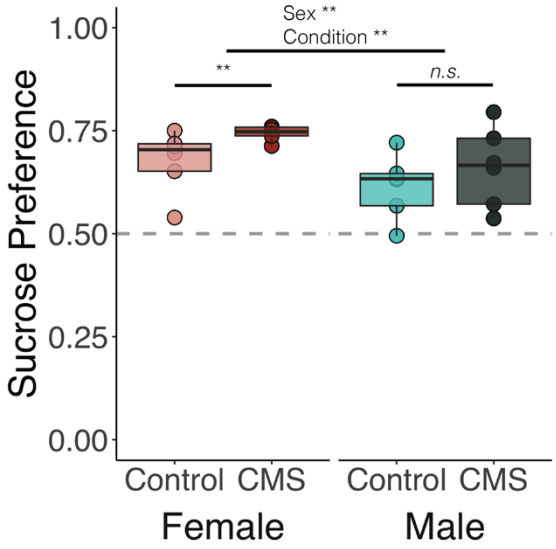


Figure 4.12: Sucrose Preference Test. Sucrose preferences in the sucrose preference test showed a condition effect opposite to the expected. Mice did not develop anhedonia, but instead increased their preferences for sucrose. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Kruskal-Wallis rank sum test, Dunn's post-hoc corrected pair-wise tests.

Finally, we explored the helplessness of the mice with the tail suspension test. Analogous to the forced swim test, in the tail suspension test the mouse is placed in an escapable situation, namely hang by its tail and the amount of time spent struggling and trying to escape from this situation is recorded. Similarly to before we did not detect any difference between conditions nor in the total time spent immobile nor in the number of immobile events, even so males showed a small tendency toward the expected phenotype (**Figure 4.13**, *page 46*, and **Figure 4.14**, *page 47*).

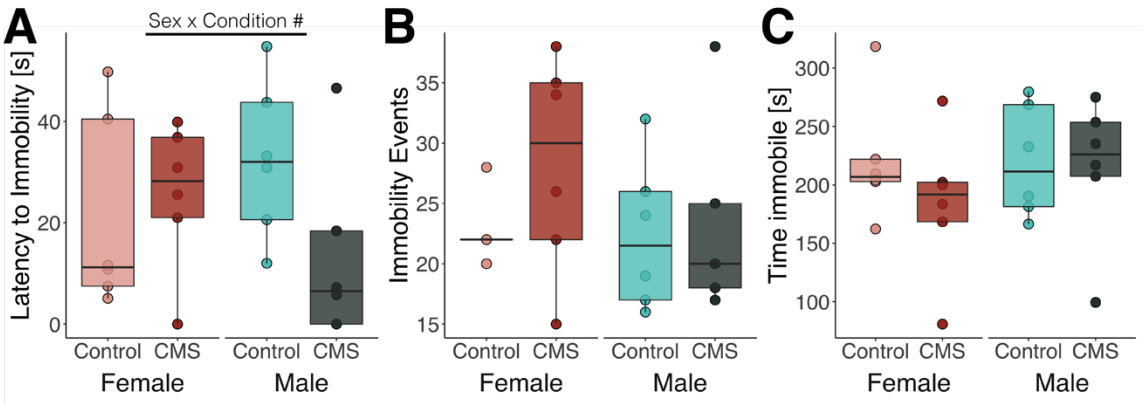


Figure 4.13: Tail Suspension Test. No clear differences were observed in any of the measurements of the TST, which included the (**A**) latency to immobility, (**B**) the number of immobility events and (**C**) the total time spent immobile. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

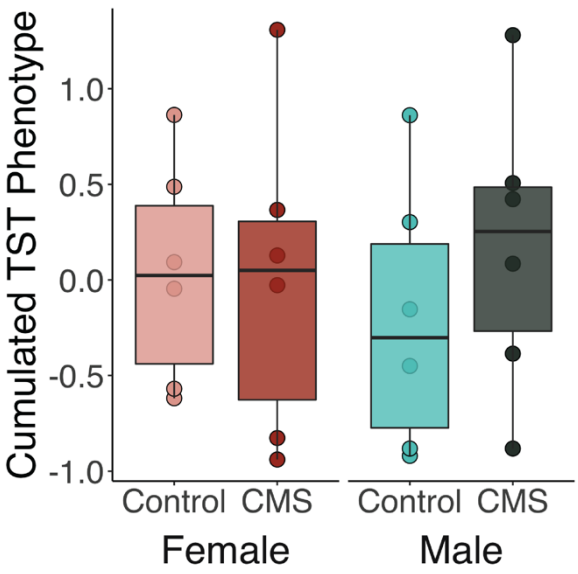


Figure 4.14: Cumulated TST Phenotype. Tail suspension test measurements (latency to immobility, immobility events, and time immobile) were averaged in a cumulated TST measure that showed no condition effect. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

It is important however to recognize that all these behaviours are not completely independent and even more importantly, small changes but identifiable in several tests and stress-related realms are as important than single results. To highlight consistency in our results, we exploited mathematical tools to calculate a combined score of stress susceptibility, the emotionality score [243]. To calculate we first z-scored all parameters measured, directionally corrected them in order to obtain higher values for stress-like phenotypes, and averaged for each individual (details on the parameters used and the normalization can be found in the Methods section "3.4.5 Emotionality Score", *page 27*). The emotionality score showed a clear increase in the CMS animals, both for males and females, confirming that the CMS protocol we devised was effective at impacting on the mouse wellbeing (**Figure 4.15A**, *page 48*). Importantly, a stress score composed of only bodyweight gain, coat state is equally powerful to detect a stress phenotype and does not require sacrifice of the mice or extensive testing (**Figure 4.15B**, *page 48* and **Figure 4.16**, *page 48*).

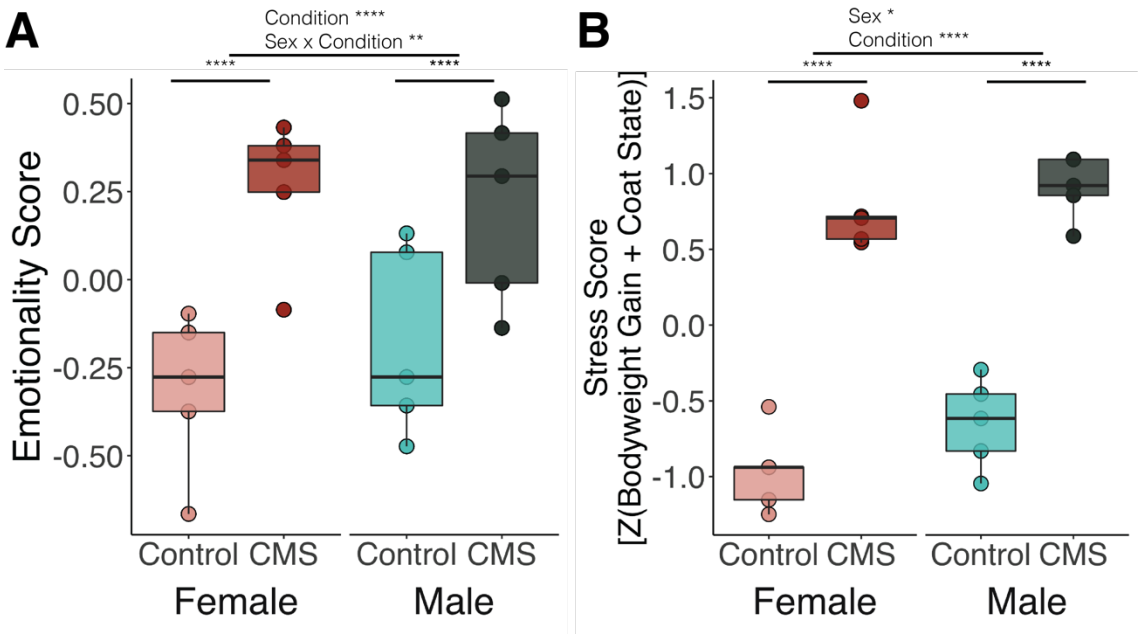


Figure 4.15: Emotionality Score and Stress Score. (**A**) Emotionality score composed as the mean of all Z-scored physical parameters and tests: bodyweight gain, cumulated coat state, normalized adrenal glands size, normalized thymus size, circulating corticosterone levels, splash test, open field test, sucrose preference test, and tail suspension test. (**B**) Stress score composed only of bodyweight gain, and coat state already detects sex and condition effects. Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.

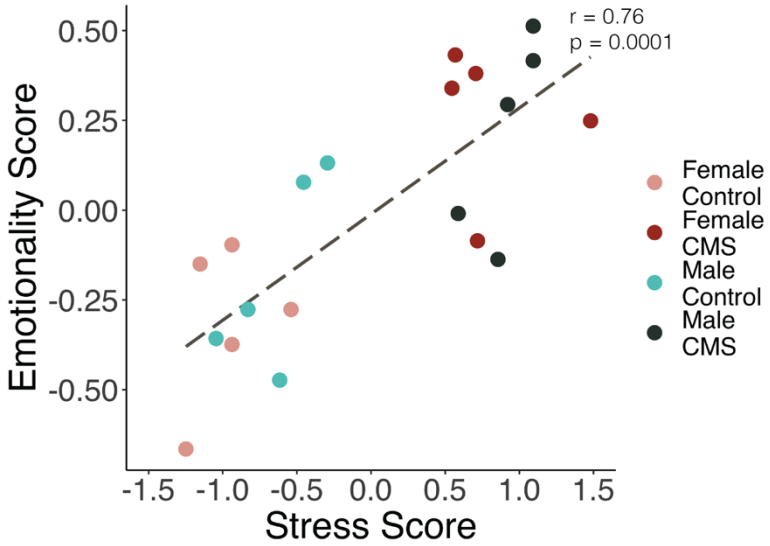


Figure 4.16: Correlation Between Emotionality and Stress Scores. The stress score is a good predictor of the emotionality score. Spearman's rank correlation test.

However based on literature knowledge, exposure to the same type of stressor elicits habituation mechanisms [244]–[246] and since we were interested in isolating and comparing the ARS response under Baseline and CMS conditions, we decided to adapt the CMS design to remove stressors related to restraint (restraint in dark/bright light, restraint witnessing), and we substitute

them with physical stressors that would influence mobility but without restraint (homecage space reduction, tail suspension, **Figure 4.17**, *page 49*, and **Table 3.2**, *page 25*).

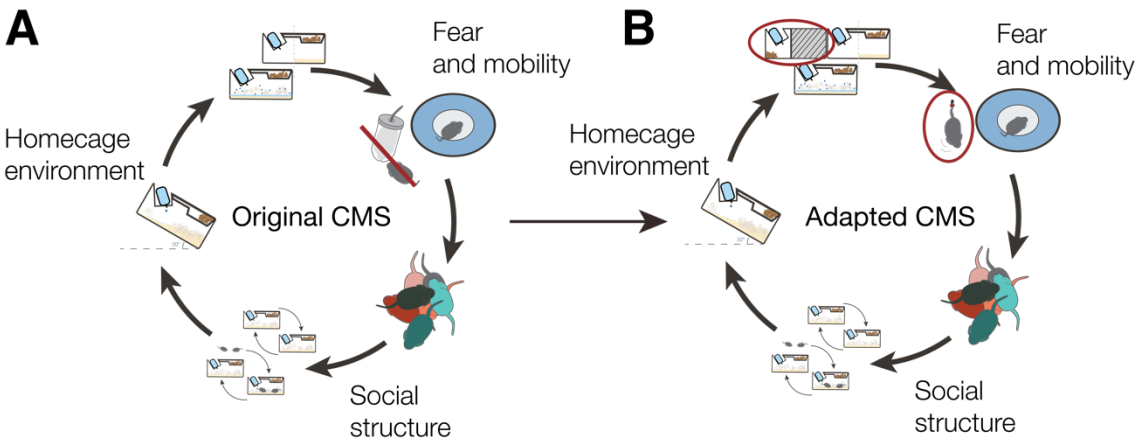


Figure 4.17: Adaptation of the CMS paradigm. (**A**) Original CMS paradigm which included several stressors that altered the homecage environment (no nesting, no bedding, cage tilt, wet bedding), the social structure (cage change, cage switching, overcrowding) and mobility or induced fear (water avoidance, restraint in the dark/bright light, restraint witnessing). (**B**) Adapted CMS protocol used in the scRNA-seq experiment. Stressors related to acute restraints were replaced with tail suspension, and homecage space reduction.

4.2.2 Validation of ARS Efficacy Across Sexes and Conditions

Exposure to an acute stressor activates the HPA axis and ultimately leads to CORT release [42]–[44]. As such, we validated the effectiveness of the exposure to ARS following the newly design CMS paradigm measuring circulating CORT levels in males and females before and after ARS exposure (**Figure 4.18**, *page 50*). We therefore exposed a new cohort of male and female mice to CMS for 21 days and exposed them to ARS alongside mice without previous experience of chronic stress. We did observe that ARS caused a rapid and steep increase in CORT circulating levels in both sexes regardless of their history of chronic stress (**Figure 4.18B**, *page 50*). As shown before ("1.3.1 Reactivity and Behavioural Level", *page 9*), females showed higher levels of CORT (**Figure 4.18B**, *page 50*) and a bigger CORT response (**Figure 4.18C**, *page 50*). Nonetheless both sexes had a robust CORT response (Female Baseline: 297 ± 78.1, Female CMS: 225 ± 113, Male Baseline: 246 ± 56.7, Male CMS: 199 ± 38.6 ng/ml). However, we did not observe an increased CORT basal level under CMS (**Figure 4.18B**, *page 50*), nor a bigger CORT response with CMS + ARS (**Figure 4.18C**, *page 50*).

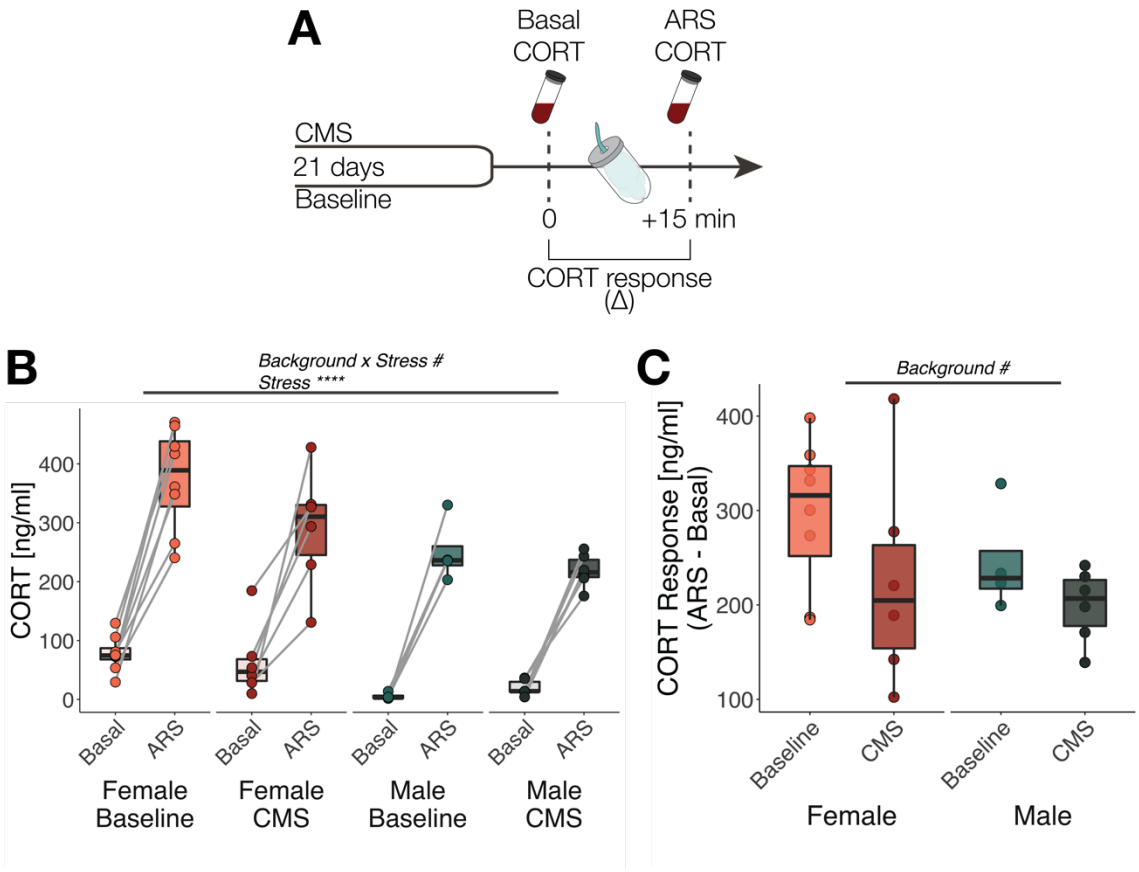


Figure 4.18: Circulating CORT after ARS. (**A**) Male and female mice were exposed to 15-min ARS after 21 days of CMS or under baseline conditions. To measure the change in CORT levels due to ARS, a blood sample was drawn at the beginning of ARS (Basal CORT) and at the end (ARS CORT). (**B**) CORT concentrations for each mouse at Basal and ARS timepoints. Exposure to ARS caused an increase in CORT levels in both sexes regardless of previous chronic stress. RM two-way ANOVA, Tukey's post-hoc corrected p-values. (**C**) CORT response calculated as ARS CORT – Basal CORT. ARS always elicited a clear increase in CORT which tended to be smaller after CMS. Two-way ANOVA, Tukey's post-hoc corrected p-values.

Our previous results showed was that the stress score was as potent as the emotionality score to identify the efficacy of the CMS paradigm (**Figure 4.15**, *page 48* and **Figure 4.16**, *page 48*), we thus validated that the new CMS design was effective in causing a stress state in mice building just a stress score composed of bodyweight change, and coat state (**Figure 4.19**, *page 51*, and **Supplementary Figure 3**, *page 131*).

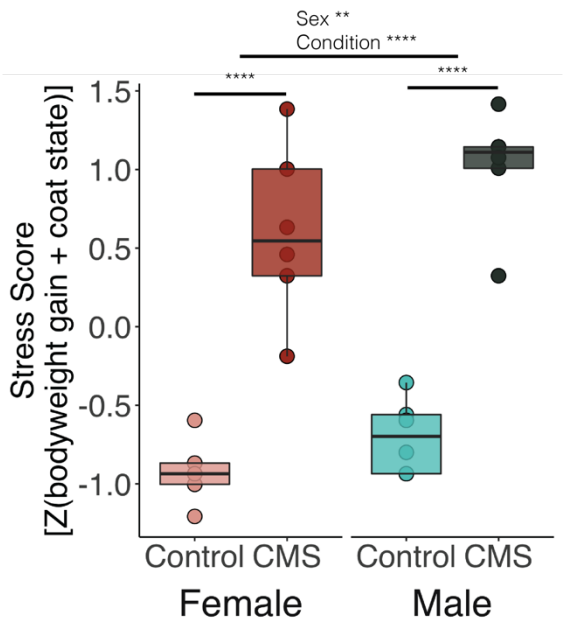


Figure 4.19: Stress Score after the Adapted CMS Paradigm. Stress Score of mice exposed to the adapted CMS paradigm composed of bodyweight gain and coat state. Two-way ANOVA, Tukey's post-hoc corrected p-values.

4.3 ScRNA-seq as a Tool for Studying the Stress Response of the PVN

Since the stress paradigms chosen proved to be robust and effective in both sexes, we designed the single cell RNA-sequencing experiment using such paradigms and appropriate controls (**Figure 4.20**, *page 51*).

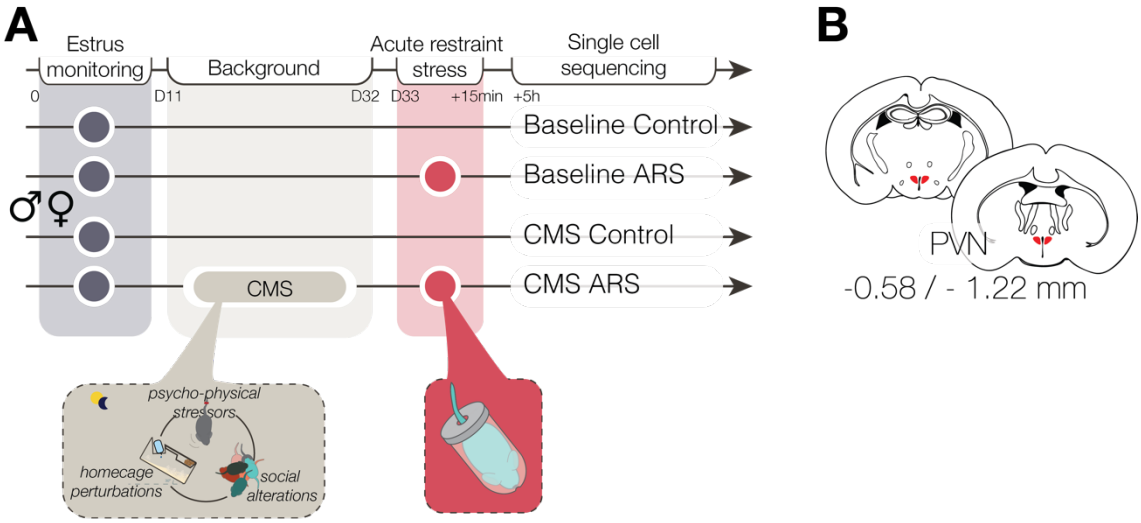


Figure 4.20: Experimental Design of the scRNA-seq Experiment. (**A**) Schematic representation of the scRNA-seq experimental design. Male and female mice received 15-mim ARS with (CMS) or without (Baseline) a previous history of CMS. Control mice which did not receive any stressors (Baseline/CMS Control) were sacrificed alongside with stressed mice five hours after ARS. (**B**) Graphical representation of the region dissected containing the PVN.

Since our goal was to define the acute stress response under different stress conditions, we used two cohorts of male and female mice and exposed them to a different combination of stress

paradigms. In order to define the stress response at Baseline, the first cohort of mice was exposed to the 15-min ARS without prior chronic stress (Baseline ARS, **Figure 4.20A**, *page 51*). A second cohort of mice, before receiving the ARS, was instead exposed to CMS paradigm (adapted CMS from **Figure 4.17**, *page 49*). As in our previous experiments, also in this cohort of mice the CMS paradigm successfully elicited a stress phenotype (**Figure 4.21**, *page 52*), since CMS mice had reduced bodyweight gain and deteriorated coat (**Supplementary Figure 4**, *page 132*). Importantly, due to the small size of the PVN (**Figure 4.20B**, *page 51*), in order to obtain enough starting material for a scRNA-seq dataset, we had to resort to animal pooling. Therefore we selected the five CMS mice and five controls from the cages with a robust response, based on their stress score (**Figure 4.21**, *page 52* and **Supplementary Figure 4**, *page 132*). After sacrifice, we also collected adrenals and calculated a combined stress score including the normalized adrenal weight **Supplementary Figure 5**, *page 132*).

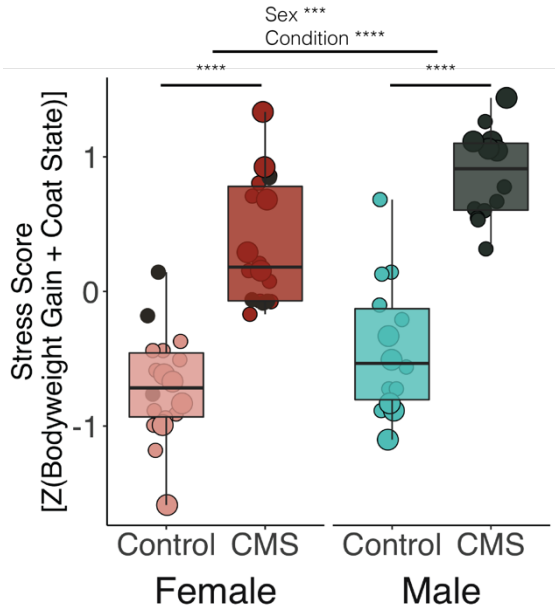


Figure 4.21: Stress Score of CMS and Control Mice in the scRNA-seq Cohort. Stress Score of mice exposed to the adapted CMS paradigm composed of bodyweight gain and coat state. Bigger dots show the mice used for the scRNA-seq dataset. Grey dots represent female mice excluded due to poor cycling. Two-way ANOVA, Tukey's post-hoc corrected p-values.

The selected mice had a clear stressed phenotype, validating that the CMS exposure did indeed affect their well-being (**Supplementary Figure 6**, *page 133*). Five hours after receiving ARS either under Baseline or CMS background, the selected mice were sacrificed alongside non-stressed controls. For each sex and condition, we prepared individual single cell suspensions from the pool of five manually-dissected PVNs and used the droplet-based system 10x Genomics to generate scRNA-seq datasets. In order to being able to directly compare cell types and cell transcriptomics across conditions, the eight individual datasets were firstly pooled in one single dataset and processed as a whole in the package Seurat (for detailed explanation about pre-processing and analysis please refer to "3.6 ScRNA-seq Analysis of Males and Females Samples", *page 29*). The complete dataset consisted of 35,672 cells that passed quality controls, eliminating roughly 30% of the initial cells, a standard percentage for good quality scRNA-seq data [184] (**Table 3.5**, *page 30*). The dataset had a median number of UMIs (unique molecular identifier) of 2118, a median number of genes of 1149, and median mitochondrial gene content of 0.045 per cell (**Figure 4.22**, *page 53*).

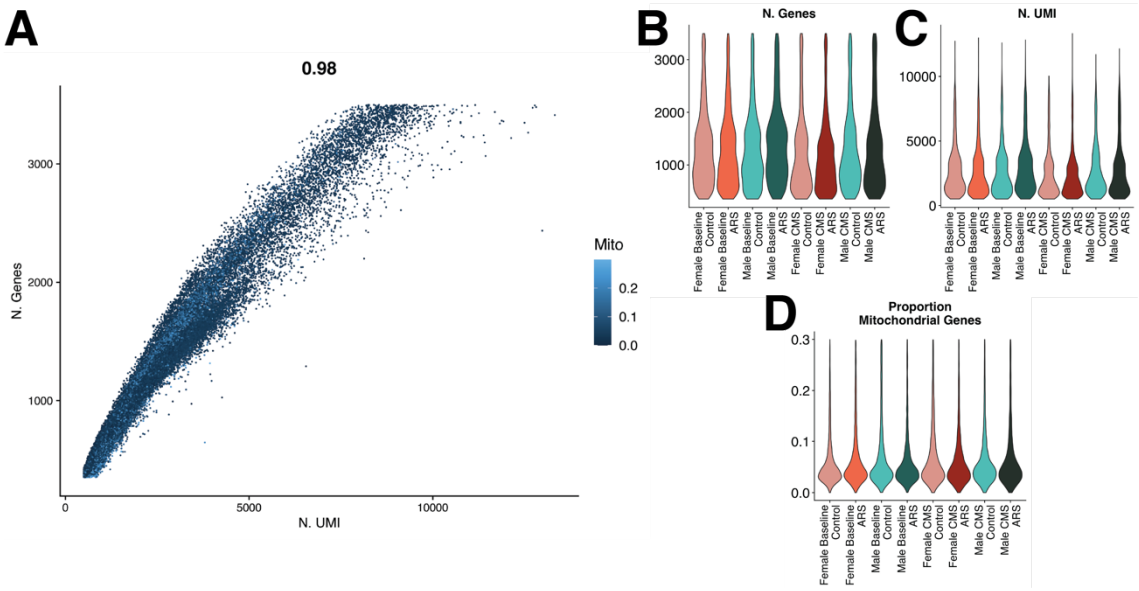


Figure 4.22: Features of the scRNA-seq Dataset. (**A**) The joint scRNA-seq was composed of 35,672 cells from eight individual samples, with (**B**) 1149 median n. of genes, (**C**) 2118 median UMI, and (**D**) 0.045 median mitochondrial gene content.

We then used the top 4000 variable genes to perform principal component analysis (PCA) and used the top 22 PCs to run non-linear dimensionality reduction followed by graph-based unsupervised clustering using the Uniform Manifold Approximation and Projection (UMAP). Doing so, we recognised the three main category of brain cell types in our dataset: neuronal, glial, and stromal cells. We identified a total of 33 cell clusters, on which we mapped known gene markers for the PVN [186] (**Figure 4.23**, *page 53* and **Supplementary Figure 7**, *page 134*).

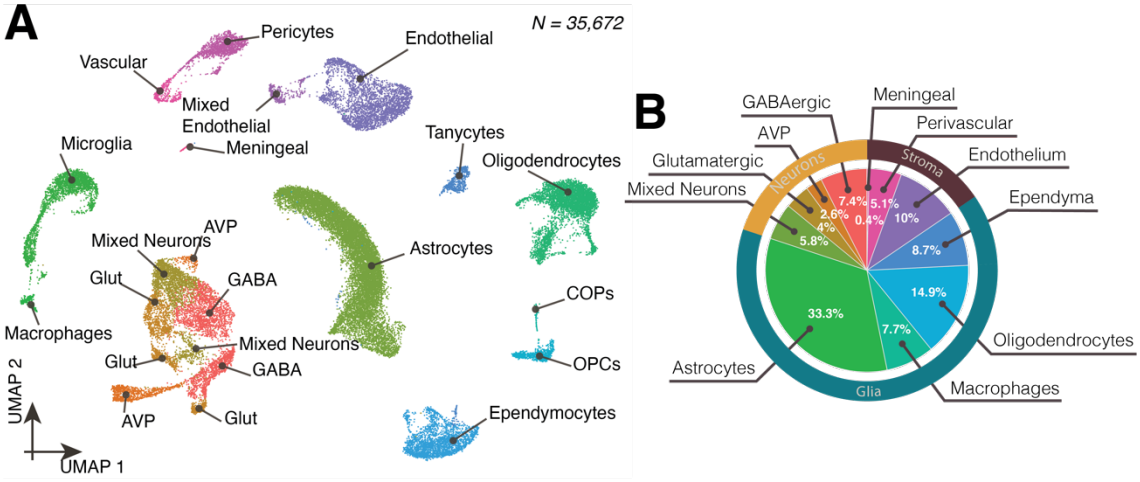


Figure 4.23: Clustered scRNA-seq Dataset. (**A**) UMAP plot of the clustered scRNA-seq dataset containing a total of 35,672 single cells. (**B**) Distribution across the major cell type lineages of the cells: Neurons (GABAergic, AVP, glutamatergic, mixed), astrocytes, macrophages (microglia, macrophages), oligodendrocytes (mature, COPs, OPCs), ependyma (ependymal cells, tanycytes), endothelium (endothelial cells, mixed), perivascular (vascular cells, pericytes), meningeal cells.

Based on their expression pattern, we could divide the clusters in 17 major cell identities: neurons (GABAergic, glutamatergic, vasopressin, mixed), astrocytes, macrophages (and microglia),

oligodendrocytes (mature, committed oligodendrocytes precursors (COPs), oligodendrocytes progenitor cells (OPCs)), ependyma (ependymal cells, tanycytes), endothelium (endothelial cells, mixed endothelial), perivascular (pericytes, vascular cells) and meningeal cells (**Figure 4.23,** *page 53*, **Supplementary Figure 8**, *page 134*, and **Table 4.1**, *page 54*). The most abundant cell population were the astrocytes (33%), followed by neurons (19.8%) and oligodendrocytes (14.9%), in proportions similar to a recent attempt at counting the absolute number of cells in the brain [247]. As expected for hypothalamic regions, we also found more GABAergic than glutamatergic neurons (51% vs 20% of all neurons). In addition, we also identified specialized subtypes of neurons enriched in the PVN such as the vasopressin-expressing neurons (AVP) (26% of all GABAergic neurons) [186], [248], [249].

Table 4.1: Cell Types and Markers.

Cell Category	Cell Type	Markers
Neurons	GABAergic	*Gad1, Gad2, Slc32a1*
	Glutamatergic	*Slc17a6*
	Vasopressin	*Avp*
	Mixed	*Nrdg4, Stmn2, Syt1, Snap25*
Glia	**Astrocytes**	*Agt, Slc1a3*
	Macrophages	*Mrc1, Pf4*
	Microglia	*Gpr34, P2ry12*
	Oligodendrocytes	
	Mature	*Mag, Mog, Cldn11*
	COP	*Bmp4, Brca1, Pak4*
	OPC	*Pdgfra, Vcan*
	Ependyma	
	Ependymal cells	*Ccdc153*
	Tanycytes	*Crym*
Stroma	**Endothelium**	
	Endothelial cells	*Cldn5, Fn1*
	Mixed endothelial	*Cldn5, Fn1*
	Perivascular	
	Pericytes	*Vtn, P2ry14*
	Vascular cells	*Acta2, Myh11*
	Meningeal Cells	*Nupr1*

4.4 Neurons and Glia Show Mild Sex Dimorphism in the PVN

4.4.1 Dimorphisms in Cell Distribution

The brain of male and females is now known to differ at several levels, including structure and cell composition [138] such as the amygdala and frontal cortex [250]. So, we first used the dataset to explore the existence of any sex dimorphisms in cell composition in the PVN. For this purposed, we used the two control groups and looked at the distribution of male and female cells within each cell type. While no clusters contained only cells from one sex, we did find mild but significant imbalances in cell distributions in two of them: tanycytes and vasopressin neurons (**Figure 4.24**, *page 55*), a difference that was consistent across the duplicates (**Supplementary Figure 9**, *page 135*).

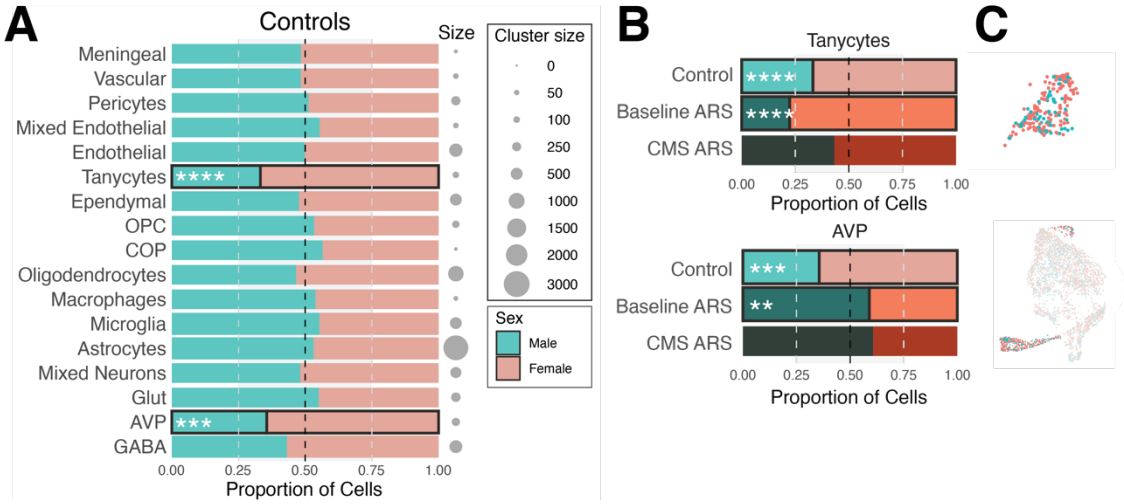


Figure 4.24: Sex Dimorphism in Cell Distribution. (**A**) Distribution between control male and female cells (bar plot) and cluster size as total number of cells per cluster (dot plot). Tanycytes and AVP neurons are significantly unbalanced (Two-way ANOVA, Sex x Cluster p = 0.036, Tukey's post-hoc corrected p-values: $p_{Tanycytes}$ = 0.0001, p_{AVP} = 0.0003). (**B**) Distribution of male and female cells in the three conditions (Controls, Baseline ARS, CMS ARS) of cell clusters unbalanced in controls: tanycytes and AVP neurons. Clusters significantly unbalanced (q-values < 0.05) are highlighted with a black border (Fisher's exact test, Benjamini-Hochberg post-hoc corrected p-values. (**C**) UMAP plots of male and female control cells in tanycytes and AVP neurons.

Of all tanycytes control cells, 66.8% of them were female, an imbalance also present in the Baseline ARS group (77.6%, q-val = 1.675 x 10^{-15}), but not in the CMS ARS group (56.8%, q-val = 0.1687) (**Figure 4.24B**, *page 55*, **Supplementary Figure 10**, *page 135*). On the other hand, vasopressin neurons were the second top unbalanced cell cluster with 64.4% of cells being female. Interestingly, AVP-positive neurons in the Baseline ARS and CMS ARS conditions inversely were enriched in male cells (Baseline ARS: 58.9%, q-val = 0.0035; CMS ARS: 61.0%, q-val = 0.0584) (**Figure 4.24B**, *page 55*, **Supplementary Figure 10**, *page 135*), a potential result of sex-specific regulation of the vasopressin gene after stress. Overall, our results suggested that the PVN of males and females is mostly homogeneous in cell composition aside from two specialized cell types, the tanycytes and the vasopressin neurons. Imbalances in these cells could be the result of the existence of sex-specific subpopulations of cells or rather just a difference in absolute numbers. We therefore further explored the tanycytes to verify this hypothesis.

4.4.2 The Case of Tanycytes

Tanycytes are specialized ependymal cells that layer the third ventricle. As a consequence they are in close contact with the CSF and the hypothalamic nuclei, such as the PVN [251]. Several subtypes, unique for their location, morphology, and function, including regenerative proprieties can be identified along the third ventricle. We wondered if the sex difference we observed were therefore restricted to a specific subtype of tanycytes or not. To do so, we isolated the tanycytes subclusters and re-clustered them in order to obtain a higher resolution to differentiate them (**Figure 4.25**, *page 56*).

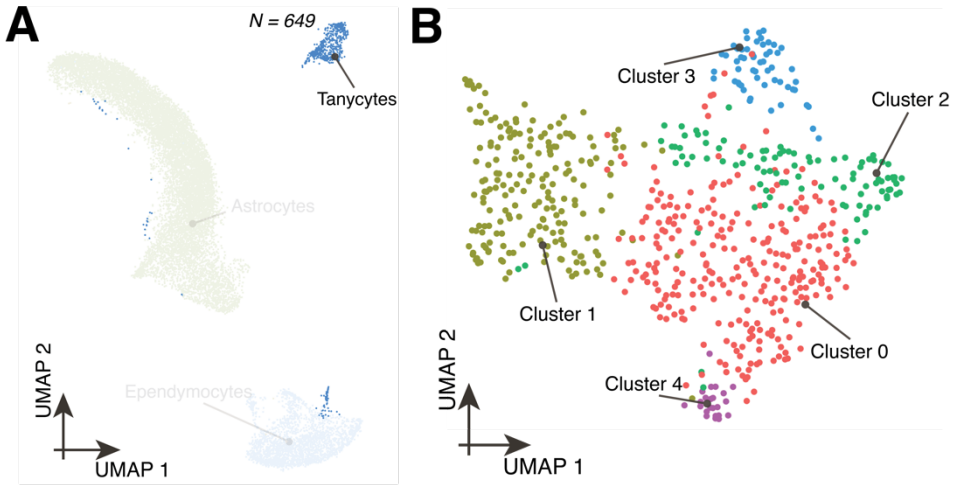


Figure 4.25: Sub-clustering of Tanycytes. (**A**) 649 tanycytes cells were extracted from the full dataset for re-clustering. (**B**) We identified a total of four subclusters of tanycytes.

We identified a total of four sub-clusters of tanycytes, with a different degree of imbalance between male and female control cells (**Figure 4.26A**, *page 56*). To explore if any of these clusters had any more relevance over the sex imbalance, we quantified the extent of this imbalance for each subcluster using only the control cells. Cluster 1 was significantly and strongly enriched for female cells (**Figure 4.26B**, *page 56*). Markers for Cluster 1 were significantly enriched for genes involved in transcription (*positive regulation of gene expression*, *DNA binding transcription factor activity*, *DNA binding transcription activator activity*), in developmental processes (*positive regulation of developmental process*, *blood vessel morphogenesis*) and downstream processes to cell activation (*response to peptide*, *response to hormone*) (**Figure 4.26C**, *page 56*). Indeed, these cells were characterized by the expression of several transcription factors known to be involved in regulating development, differentiation, and cell viability such as the Krüppel-like factors (*Klf2*, *Klf4*, *Klf6*) [252], [253]. *Fosb* [254], *Cyr61* [255], and *Btg2* [256] (**Supplementary Table 1**, *page 144*).

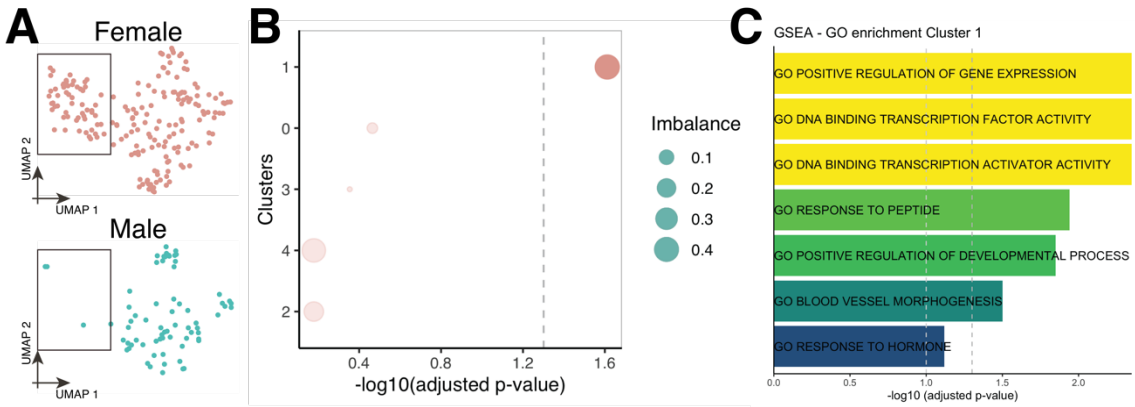


Figure 4.26: Contribution of Sub-Cluster 1 to Sex Dimorphism in Tanycytes. Cluster 1 is the biggest contributor to the imbalance between male and female cells in tanycytes. (**A**) Distribution of male and female control cells across the clusters. Black squares show cluster 1. (**B**) Cluster 1 shows the most robust enrichment for female cells. Fisher's exact test, Benjamini-Hochberg post-hoc adjusted p-values. Size

of the dots represent the extent of imbalance, colour if they are enriched of female (pink) or male (light blue) cells. (**C**) Gene markers of cluster 1 are enriched for regulators of gene expression, genes involved in development and genes involved in response to peptide and hormones. GSEA analysis over GO pathways.

Many of these markers also coincided and showed a similar expression pattern previously associated to α2 subtypes of tanycytes [257], suggesting that females might be particularly enriched in tanycytes with proliferative abilities.

4.4.3 Dimorphisms in Transcription

Aside from looking at distribution and clustering of male and female cells, sex dimorphism could be manifesting also in the basal transcriptional status of cells with otherwise the same identity. To address this possibility, we run differential gene expression analysis between control male and female cells with the same cell identity using the Model-based Analysis of Single-cell Transcriptomics (MAST) algorithm integrated in Seurat. Confirming the quality of the dataset and our approach, we were able to detect the female-upregulation of *Xist* in all cell types (**Figure 4.27**, *page 57*). *Xist* is a X-linked gene involved in the female-specific process of the X inactivation, therefore it is present only in female cells and results as strongly upregulated [258].

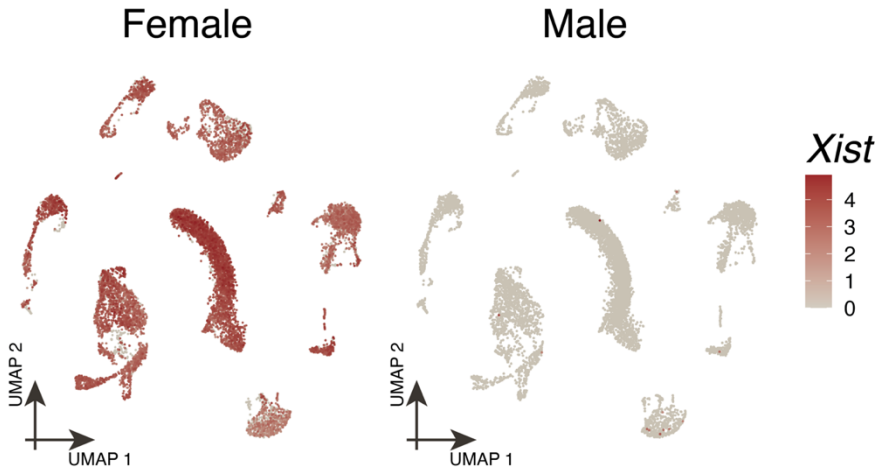


Figure 4.27: *Xist* Expression in Male and Female Control Cells. UMAP plots showing the female-exclusive expression of the X-linked gene *Xist*.

Aside from genes expressed exclusively in one sex such as *Xist*, we found that male and female cells displayed a wide range of number of DEGs, including genes upregulated in female cells and others upregulated in male cells (**Figure 4.28**, *page 58*). Only clusters with small amount of cells (COP, vascular, macrophages, mixed endothelial, and meningeal) showed a couple or no DEGs in addition to *Xist*. Among the other, oligodendrocytes showed the highest difference between male and female cells (818 DEGs), followed by ependymal and astrocytes (143, 133 respectively, **Figure 4.28A**, *page 58*). For all clusters, female cells expressed more specific genes than males did, exception made for the GABAergic neurons (**Figure 4.28B**, *page 58*).

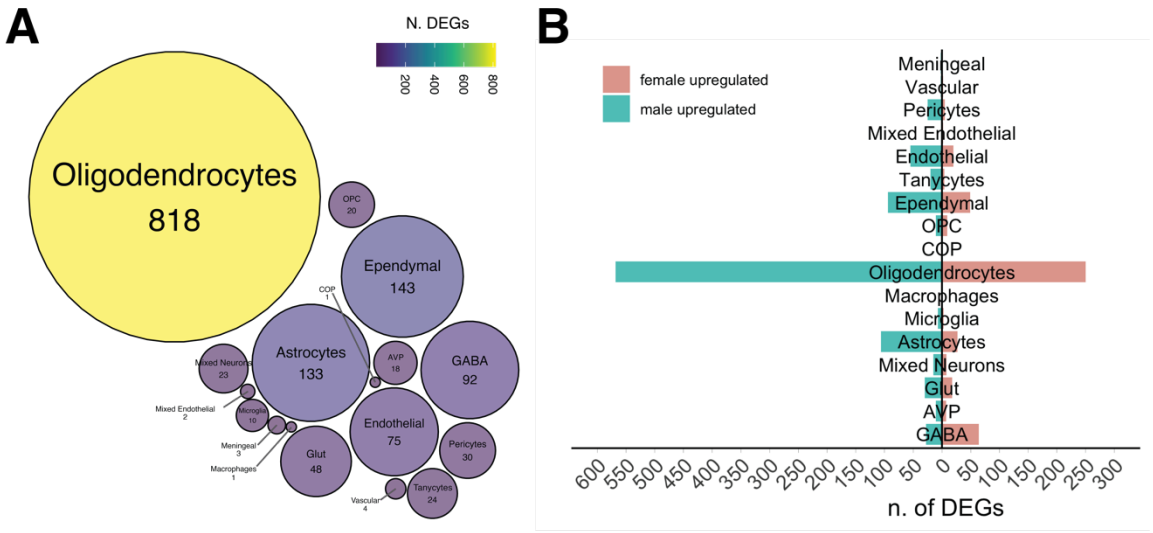


Figure 4.28: Overview of DEGs between the Male and Female Control Cells. Male and female control cells differed for several genes in almost all cell types. (**A**) Oligodendrocytes displayed the highest difference between the sexes with 818 DEGs. (**B**) We identified both female- and male-upregulated genes in all cell types.

Since oligodendrocytes showed the most striking transcriptional difference between male and female cells, we further explored which types of genes where sex-specific by pathway analysis. We analysed separately female-upregulated and male-upregulated DEGs with the goal to identify which pathways where more prominent in females and which in males, respectively (**Figure 4.29**, *page 59*). Both male and female up-regulated genes included pathways involved in development and myelin (female: *myelination*, *adherens junction assembly*, *regulation of cell morphogenesis involved in differentiation*, *synapse organization*, male: *ensheathment of neurons*), suggesting that male and female oligodendrocyte might have different morphology and myelin sheaths might be different. Additionally, male cells were enriched in genes associated to *cell responses to stress.*

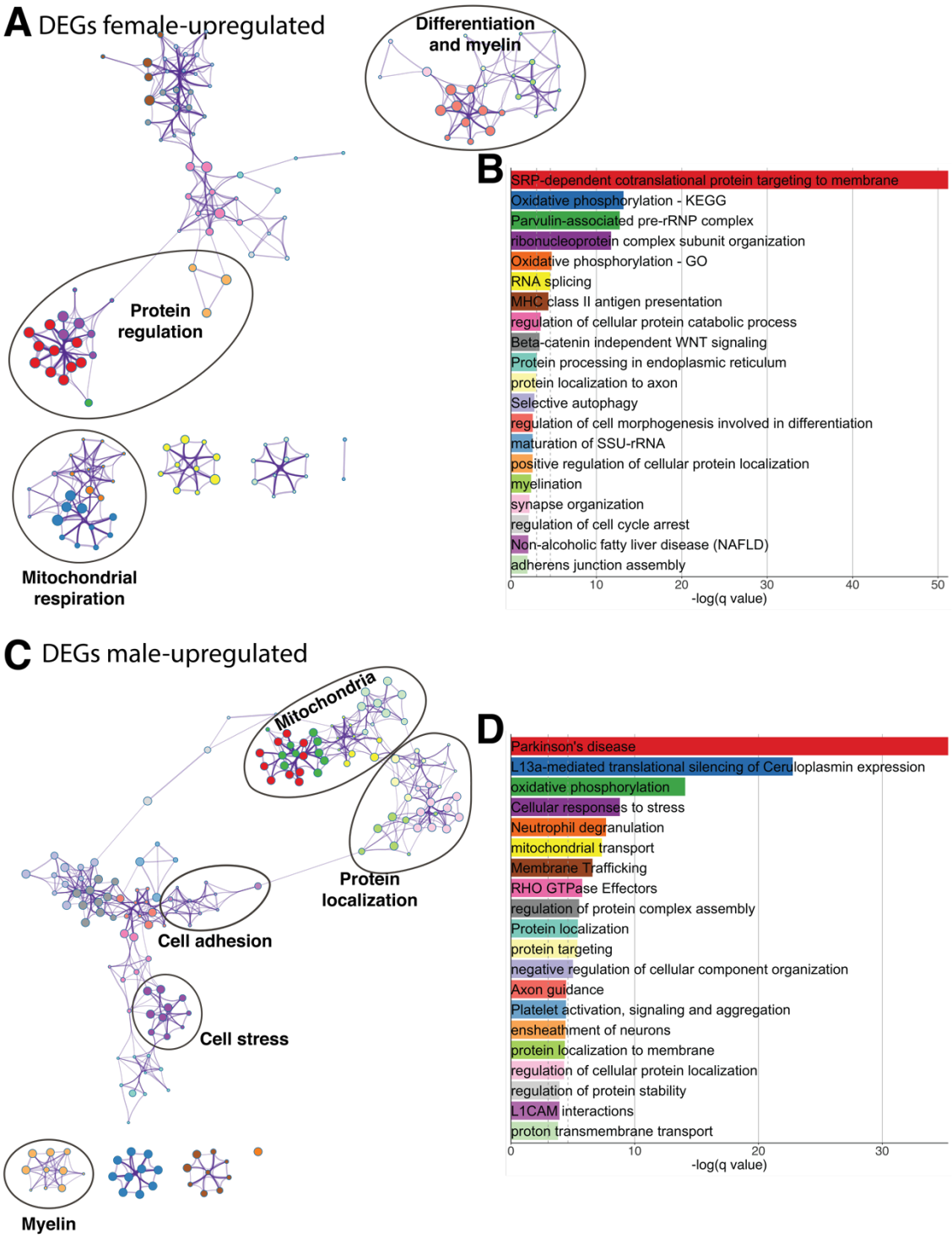


Figure 4.29: Pathway Analysis on Female- and Male-Upregulated Genes in Control Oligodendrocytes. Pathway enrichment analysis on the platform Metascape.org for the (**A-B**) female-upregulates and (**C-D**) male-upregulates genes in control oligodendrocytes. (**A, C**) Clustered network for the top enriched pathways. (**B, D**) Ranking of the pathways based on their adjusted p-values (q-value). Colours match network colouring.

4.5 The Transcriptional Response to ARS is Sex- and Cell-Type-Specific

Sex can influence the identity of brain cells, as shown above, but also their activation to stimuli, such as stressors. We used our clustered dataset to explore this hypothesis in relation to ARS, defining the baseline acute stress response of the male and female PVN. To do so, we performed differential gene expression analysis using MAST integrated in Seurat for each cell type on the samples that received ARS at Baseline and respective controls. The analysis identified several DEGs in both sexes. Interestingly, the females had 1.3 times more DEGs than males (479 vs 325) and only a small fraction of them (137 genes) was actually shared between the two (**Figure 4.30A,** *page 60*, and **Supplementary Figure 12**, *page 137*).

These overlapping DEGs were interestingly enriched for interactors of transcription factors known to coordinate stress-responsive molecular pathways such as *Esr1*, *Atf2*, *Ilf3*, *Htt*, *Ctnnb1*, *Nfkb1* and importantly *Nr3c1*, the gene that encodes for the glucocorticoid receptor – a major player in the stress response[259] (**Figure 4.30B,** *page 60*). The higher number of DEGs in females was the results of showing more responsive cell types, since overall females did not show consistent higher number of DEGs across all cell types. Instead, we found that the transcriptional response to acute stress is encoded in different cell types of the brain with a mix of up- and down-regulated genes: 15 cell clusters in females and 8 in males had at least one DEG (**Figure 4.30C,** *page 60*, and **Supplementary Table 2**, *page 145*).

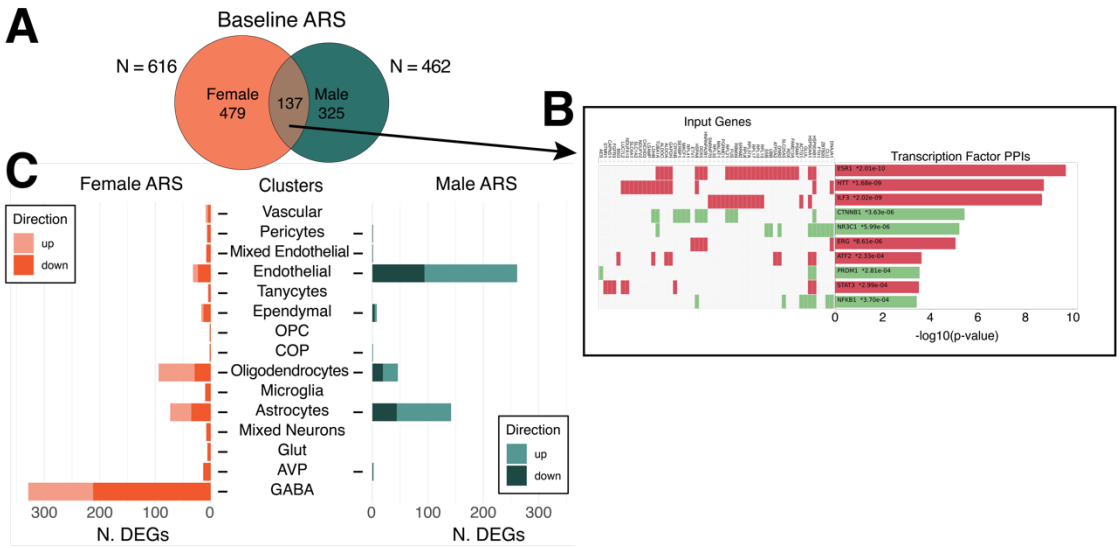


Figure 4.30: The Transcriptional Response to ARS of Male and Female Cells. Baseline ARS and respective controls were used to define the basal transcriptional response to ARS for females and males. (**A**) When collapsed across cell types, females have more DEGs than males with limited overlap. (**B**) Transcription factor protein-protein interaction analysis on the 137 DEGs shared between males and females under Baseline background. Top ten transcription factors significantly enriched. Left side shows individual contribution of DEGs to the enriched transcription factors. Right side ranks transcription factors by significance. Transcription factors are coloured based on their network of belonging. (**C**) Distribution and directionality of the DEGs is specific to the cell type and sex.

Most of these responses were largely unique to the cell type (**Figure 4.31**, *page 61*). Even the most responsive cell types shared only few DEGs with other cell types. For example, female GABAergic neurons showed a total of 329 DEGs, of which only 48 were shared with other cell types, especially astrocytes (15) and oligodendrocytes (7) (**Figure 4.31A**, *page 61*). Similarly, male endothelial cells had a total of 261 DEGs of which only 17 and 5 DEGs were in common with astrocytes and oligodendrocytes respectively (**Figure 4.31B**, *page 61*). Since some cell types (such as astrocytes, oligodendrocytes, endothelial cells, ependymal cells, vasopressin neurons, and pericytes) were stress-responsive in both sexes, we also explored how much the response for each cell type overlapped between sexes. A similarity index based on the DEGs overlap (**Figure 4.32**, left panel, *page 61*) showed that only the top 3 most responsive cell types, namely astrocytes, oligodendrocytes, and endothelial cells, had any degree of similarity (**Figure 4.32**, right panel, *page 61*). Collectively this demonstrated that exposure to an acute stressor elicits a transcriptional response in the PVN in several glial and neuronal cell types, which is mostly unique to the cell type and differs substantially between males and females.

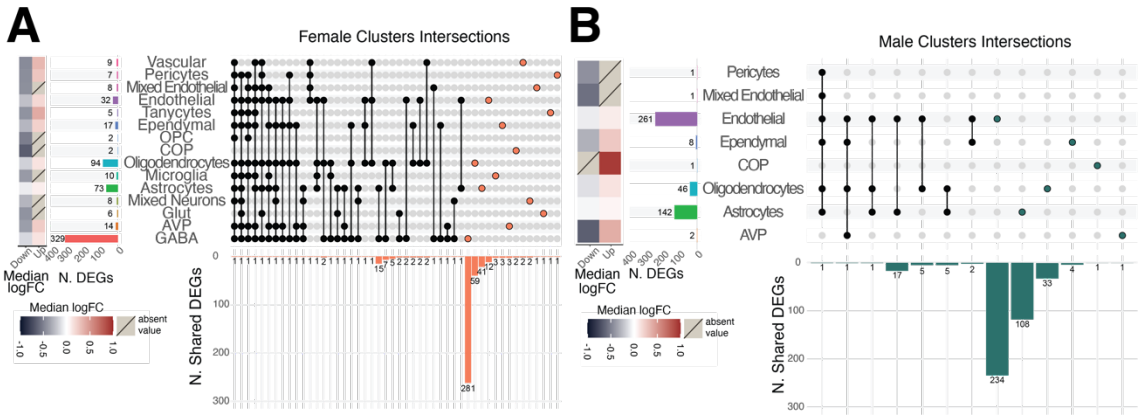


Figure 4.31: Overlap Pattern of DEGs Between Cell Types. Upset plots for the (**A**) female ARS and (**B**) male ARS response showing small overlap in DEGs between different cell types. Panels on the left show the median logFC and the number of DEGs for each cell type with DEGs. Panels on the right shows the number of DEGs shared between the different cell types.

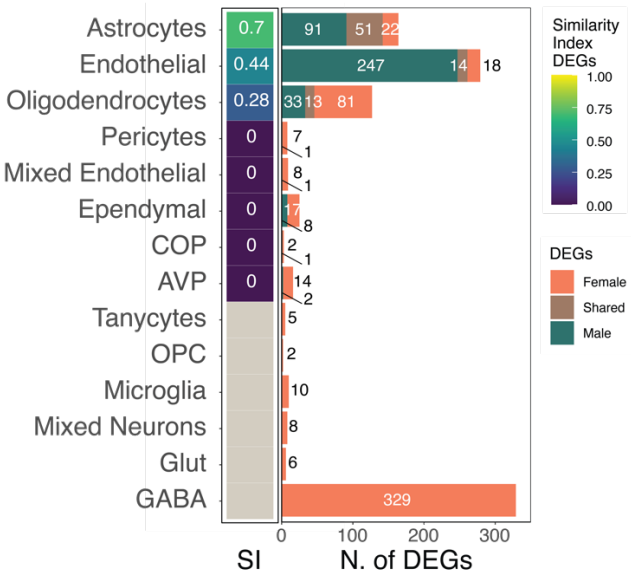


Figure 4.32: Similarity Between Male and Female ARS Response. The overlap between male and female ARS response is fairly limited, exception made for astrocytes. Right panel shows the number of DEGs unique to female, male or shared between the sexes. Left panel ranks the cell types based on their similarity index (Szymkiewicz–Simpson coefficient).

4.5.1 The Case of the GABAergic Neurons

Among all cell types that showed a transcriptional response to ARS, GABAergic neurons stood up for their high number of DEGs and their strong selectivity to females. GABAergic neurons are also the most abundant neuronal population in the PVN and known to regulate the general activity of the region [249]. A sex-specific reactivity to stress of this neuronal population could therefore contribute to the different responsivity of the HPA axis of females and males. We thus explored the DEGs we detected in the GABAergic neurons to see if they could indeed explain a different basal response to ARS.

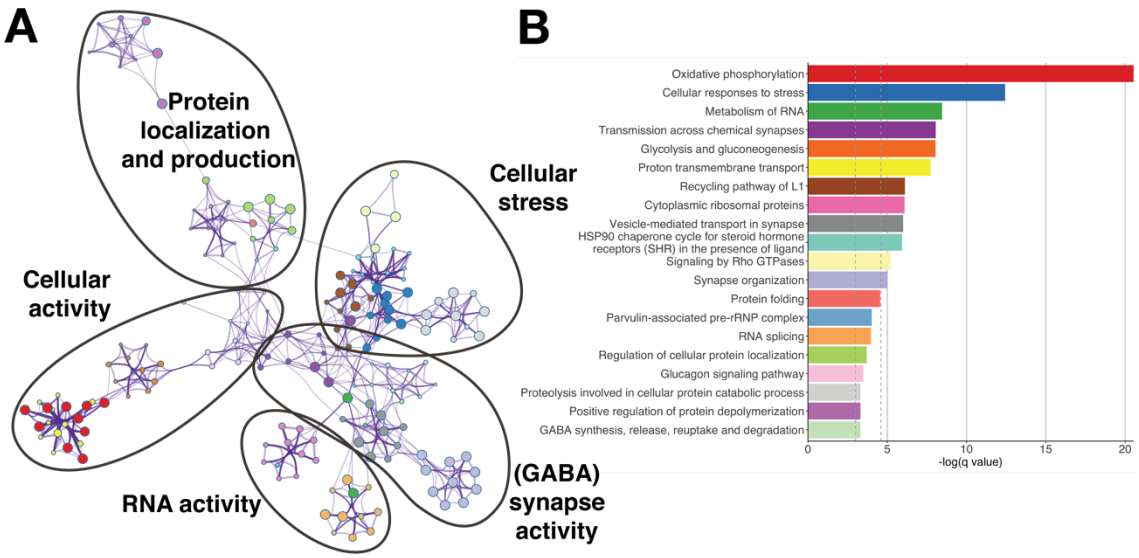


Figure 4.33: Gene Ontology Analysis on ARS-induced DEGs in Female GABAergic Neurons. Pathway enrichment analysis on the platform Metascape.org for the 329 DEGs from the female GABAergic neurons identified several pathways. (**A**) Clustered network for the top enriched pathways. (**B**) Ranking of the pathways based on their corrected p-values (q-value). Colours match network colouring.

We performed a pathway analysis with the help of the online platform Metascape.org using the 329 DEGs detected in the female GABAergic neurons. Several pathways were significantly enriched in our sample (**Figure 4.33**, *page 62*, and **Supplementary Table 2**, *page 145*), including cellular stress-related pathways such as *cellular responses to stress*, and *HSP90 chaperon cycle for steroid hormone receptors*. We also identified genes associated to *oxidative phosphorylation*, *glucagon signalling pathway*, and *glycolysis and gluconeogenesis* that suggested a change in cellular activity of these neurons. In addition, protein localization and production (*positive regulation of protein depolymerization*, *regulation of cellular protein localization*), and RNA activity (*metabolism of RNA*, *RNA splicing*, *metabolism of RNA*) were also affected. Finally and importantly, the gene ontology analysis also suggest that these neurons show a change in their GABAergic synaptic activity and organization (*synapse organization*, *transmission across chemical synapses*, *vesicle-mediated transport in synapse*, *GABA synthesis, release, reuptake and degradation*). Overall, the pathway analysis suggests that the female GABAergic neurons are engaging cellular stress systems that lead to a remodelling of their cellular and functional activity.

Given that we found a high number of genes deregulated, we wondered if these genes shared any common regulatory mechanisms. Thus, we then explored if these genes shared any TF regulators. We used the platform EnrichR to assess this question. Twenty-two transcription factors were significantly enriched as interactors of the 329 DEGs found in female GABAergic neurons (**Figure 4.34**, *page 63* and **Supplementary Table 3**, *page 146*). Interestingly, the estrogen receptor α, ESR1, was the most significant enriched TF, being overrepresented as interactor among the DEGs. Altogether our results suggest that GABAergic neurons show a prominent sex difference in their response to ARS and estrogen could be an important player for this regulation.

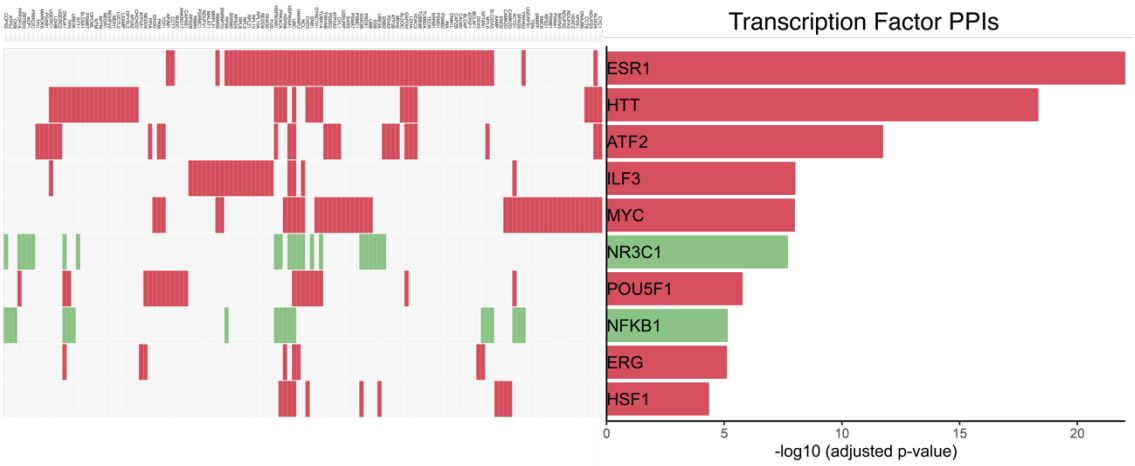


Figure 4.34: Estrogen Receptor α is Enriched among the GABA's DEGs. Transcription factor protein-protein interaction analysis on the 329 DEGs of female GABAergic neurons after ARS under Baseline background. Top ten transcription factors significantly enriched. Left side shows individual contribution of DEGs to the enriched transcription factors. Right side ranks transcription factors by significance. Transcription factors are coloured based on their network of belonging.

4.5.2 Ovariectomy Eliminates GABAergic Response to ARS

Since GABAergic neurons showed a female-specific response to ARS and that circulating steroid hormones could be involved this sex-specificity, we further explored the role of estrogens in influencing GABAergic transcriptional response to ARS. To better isolate the role of estrogens in determining the strong GABAergic response, we repeated the experiment with females without circulating estrogens. The ovaries – the source of circulating estrogens – were surgically removed from sexually mature female mice (OVX Control/ARS) before exposing them to the same stress protocol and single cell RNA sequencing procedure explained above (**Figure 4.35A**, *page 64*). Effective ovariectomy was validate through vaginal smears (see Material and Methods chapter "3.1.2 Ovariectomy", *page 23*). In addition, female mice that were not used in the single cell RNA procedure, were sacrificed to inspect their uterus. Uteri of OVX were on average less than a third in weight of normal uterus, as a result of uterus atrophy subsequent to removal of circulating estrogens [260].

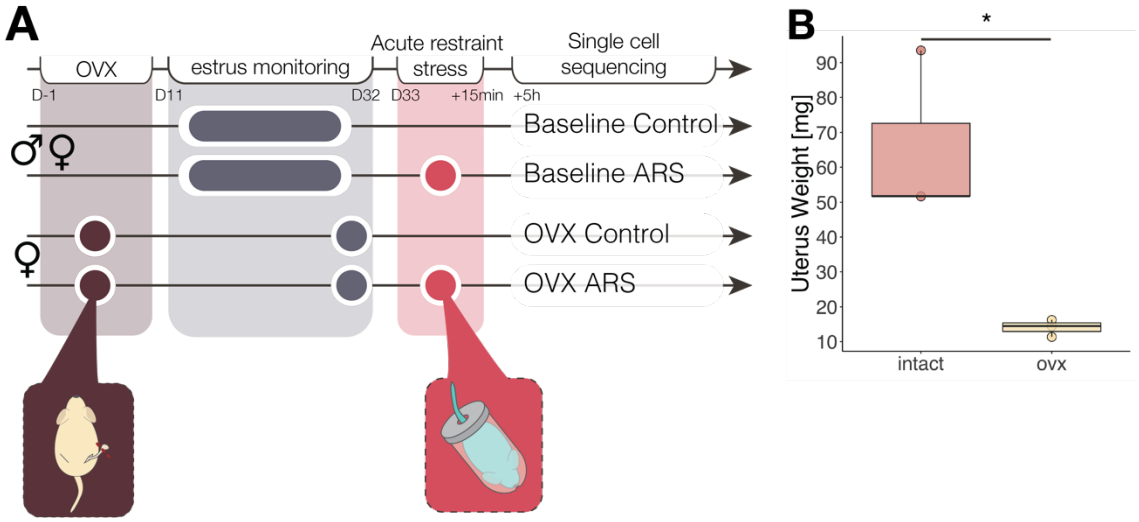


Figure 4.35: Experimental Design of the OVX scRNA-seq. (**A**) Schematic representation of the groups and timeline for the OVX scRNA-seq. Timeline was matched to the Baseline dataset already available. (**B**) Uterus size of OVX mice not used in the scRNA-seq experiment and female mice as comparison. OVX mice showed the characteristic uterus atrophy. Unpaired two-tailed t-test. p = 0.0211.

The new dataset obtained from the merged control and ARS cells consisted of 7,693 single cells that passed quality control (**Figure 4.36**, *page 64*). Quality control eliminated roughly 28 % of the initial identified cells (**Table 3.7**, *page 33*), similarly to the first dataset (**Table 3.5**, *page 30*). Overall the dataset had a median number of UMIs of 2364, a median number of genes of 1260, and median mitochondrial gene content of 0.043 per cell (**Figure 4.36**, *page 64*), very similar to the first dataset (**Figure 4.22**, *page 53*).

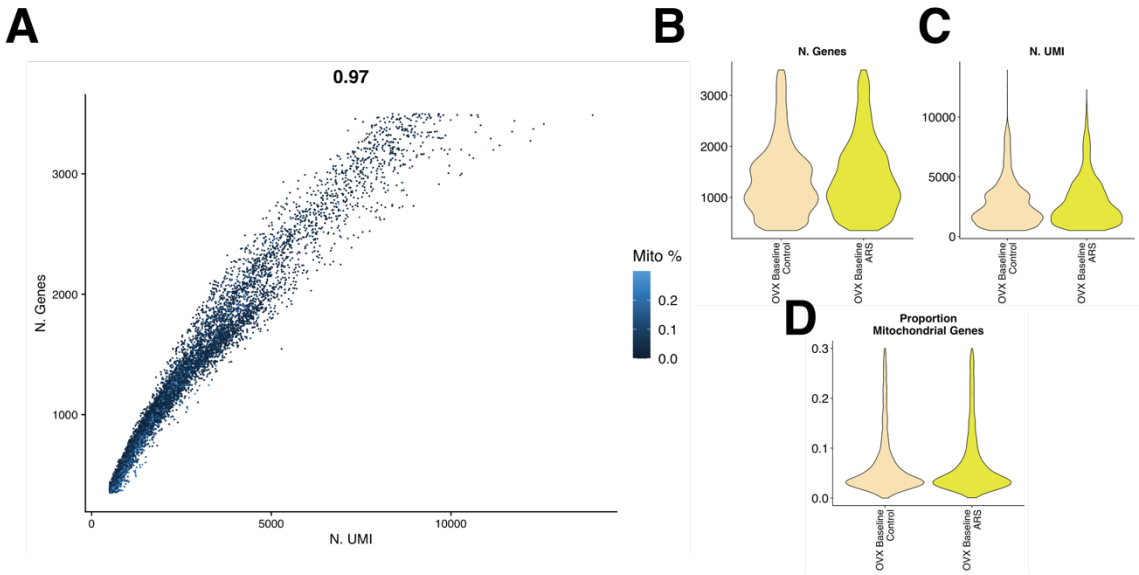


Figure 4.36: Features of the OVX scRNA-seq Dataset. (**A**) The OVX scRNA-seq was composed of 7,693 cells from eight individual samples, with (**B**) 2364 median n. of genes, (**C**) 1260 median UMI, and (**D**) 0.043 median mitochondrial gene content.

As for the previous dataset, we performed PCA using the top 4000 variable genes and used the top 21 PCs and a resolution of 1.2 to run non-linear dimensionality reduction followed by graph-based unsupervised

clustering using UMAP graphing. We identified an initial count of 22 clusters of neurons, glia and stromal cells (**Figure 4.37A**, *page 65*).

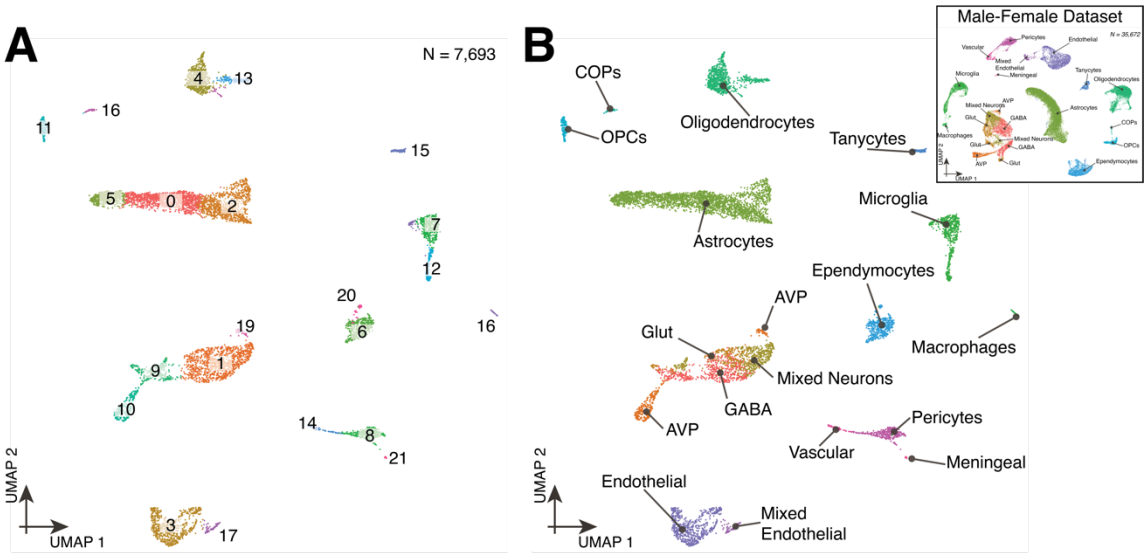


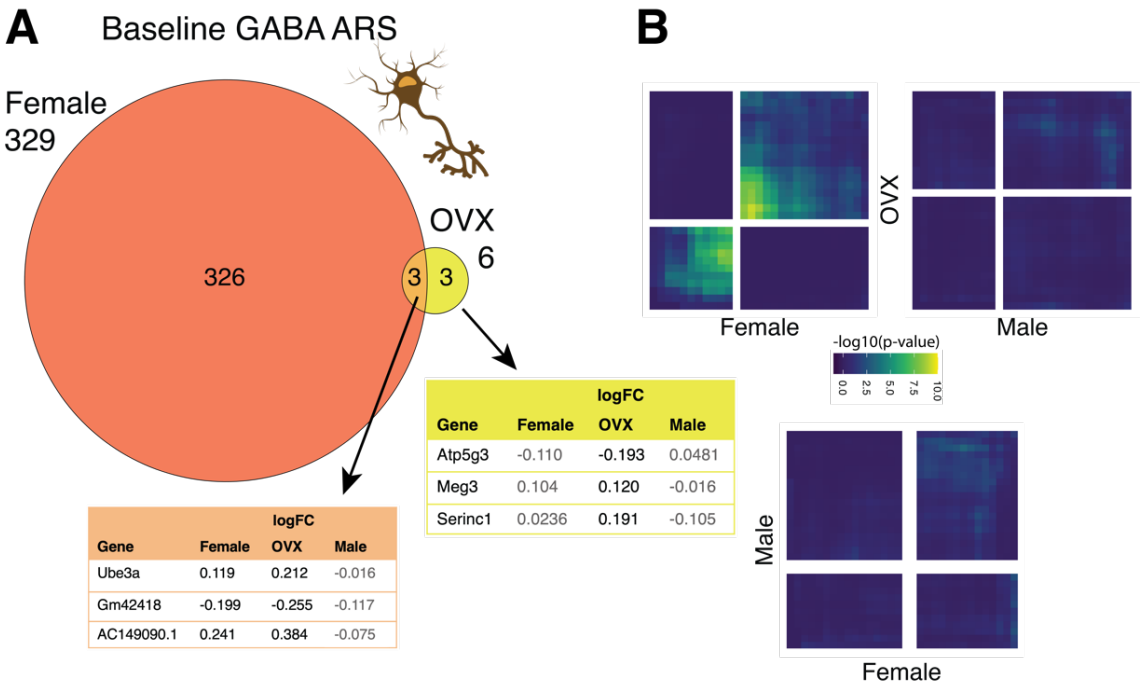
Figure 4.37: Clustering and Label Assignment of OVX scRNA-seq Dataset. (**A**) UMAP plot of the clustered OVX dataset with a total of 7,693 single cells. clustered dataset (**B**) Clustered dataset with the 17 cell identity labels transferred from the male-female dataset (inset).

In order to allow for a direct comparison between previous results and genes deregulated in the OVX samples, we had to make sure that the same type of cells was assigned the same identity. To assure this, instead of mapping known markers genes on the identified clusters, we transposed cell identities from the first dataset to the OVX cells with the label transfer functionality within Seurat. This procedure allowed us to remap all cells into the original 17 cell identities (**Figure 4.37B**, *page 65*, and **Supplementary Figure 13**, *page 138*), including the cluster of GABAergic neurons. Overall, the OVX sample had a distribution among major cell types similar to the baseline samples (**Supplementary Figure 14A, B**, *page 139*). In terms of detailed balance between individual cell types that showed imbalances between male and female cells, OVX cells distributed often more similarly to females in neuronal populations (AVP neurons, GABAergic neurons, mixed neurons) and more similarly to males in glial populations (astrocytes, oligodendrocytes, macrophages) (**Supplementary Figure 14C, D**, *page 139*).

We then performed differential gene expression analysis between controls and ARS OVX samples with the goal to identify how much of the GABAergic ARS response was changed due to the removal of circulating estrogens. As in male and female baseline samples, we were able to detect DEGs in several populations beyond GABAergic neurons, which were mostly unique to the cell type (supp **Supplementary Figure 15**, *page 140*) and had a different degree of similarity with either male or female response.

In support of the idea that GABAergic response is strongly led by circulating estrogen, OVX GABAergic neurons showed only 6 DEGs instead of the 329 of females, and only half of them were in common with females (**Figure 4.38A**, *page 66*). To confirm that this lack of overlap was not due to an artificial lower resolution in the differential gene expression and p-value calculations and to explore if the surgery had increased the similarity with males, we used a rank-rank

hypergeometric overlap (RRHO) analysis to explore the whole transcriptome. The RRHO approach allows to evaluate the correlation between two differentially expressed transcriptomes, in this case the change between OVX and females and OVX and males. While OVX females lose all female DEGs, their transcriptome was still quite similar to females and did not increase its similarity to males (**Figure 4.38B**, *page 66*).



Gene	logFC Female	OVX	Male
Ube3a	0.119	0.212	-0.016
Gm42418	-0.199	-0.255	-0.117
AC149090.1	0.241	0.384	-0.075

Gene	logFC Female	OVX	Male
Atp5g3	-0.110	-0.193	0.0481
Meg3	0.104	0.120	-0.016
Serinc1	0.0236	0.191	-0.105

Figure 4.38: Similarity of OVX Differential Expression with Male and Female in GABAergic Neurons. (**A**) OVX females had an extremely reduced number of DEGs after ARS when compared to females and only half of them were in common with non-ovariectomized females. (**B**) RRHO shows that despite the small overlap in DEGs, on a macroscopic scale, the OVX transcriptome was still quite more similar to females than males.

4.6 CMS Modulates Cell-Type-Specific Responses to ARS

Exposure to chronic stress modulates the response to an acute stressor of the individual [49]. Having shown that ARS response is cell-type-specific in the PVN and it is influenced by sex, we, explored if the modulation from chronic stress can also be detected at the single cell level and if it sex-specific. So, we explored the impact of CMS onto the previously described ARS transcriptional responses, running a differential gene expression analysis with the second half of our dataset (CMS control and CMS ARS cells). To being able to directly compare the results, we performed the analysis following the same parameters and procedure as before.

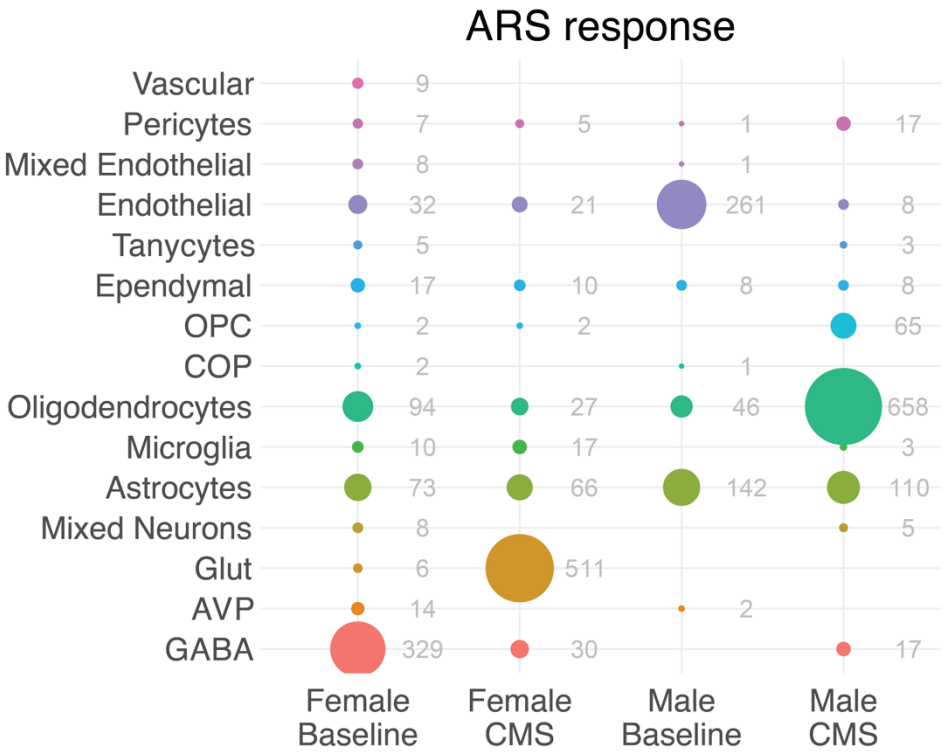


Figure 4.39: Overview of the ARS Response by Sex and Stress Background. Number of DEGs per cell type in each of the four conditions. GABAergic and glutamatergic neurons, oligodendrocytes and endothelial are the top responders.

Both sexes showed new stress cell-type-specific signatures which differed from Baseline both by extent and involvement of cell types (**Figure 4.39,** *page 67*). To identify which cell type was affected the most, we described each of our cell clusters by the extent (as in number of DEGs) and magnitude (as in the absolute median log fold change) of their stress and calculated the distance for each cluster between CMS and Baseline response (**Figure 4.40**, *page 68*). Doing so we obtained a susceptibility score for each cell type within each sex (**Figure 4.41A**, *page 68*) that allowed us to identify susceptibility range between cell types. To establish which cell type displayed the highest susceptibility to stress background across sexes, we then calculated the sum of distances between males and females (**Figure 4.41B**, *page 68*). This approach identified a range of susceptibility in which astrocytes, ependymal and pericytes ranked the lowest, while endothelial cells, GABAergic neurons, and oligodendrocytes showed a mild to prominent susceptibility to the background. Interestingly, glutamatergic neurons also showed a strong susceptibility, but selectively in females (**Supplementary Figure 16**, *page 140*). In addition, oligodendrocytes ranked the top cluster with a calculated susceptibility score of 4.92, more than twice the score of the second ranked (2.40). To support this finding, we also evaluate the susceptibility to the change in stress background with a second complementary approach. We explored how similar or different the stress response in terms of DEGs was between conditions (**Supplementary Figure 17A, B**, *page 141*) and measure this similarity by a similarity index (SI) built in the same as the one used in the previous section (**Supplementary Figure 17C**, *page 141*). In addition, to highlight if the response was changing decreasing in size or increasing in size after CMS, we gave negative SI values to the former and positive to the latter. This complementary approach confirmed that CMS effect on ARS response is cell-type- and sex-

specific and additionally highlighted that the changes we previously identified for the oligodendrocytes were happening in opposite directions for the two sexes.

Overall, these results suggest that previous exposure to chronic stress influences the ability of a cell to respond to an acute stressor. This effect is modulated not only by the identity of the cells but importantly also by sex and is especially pronounced in oligodendrocytes.

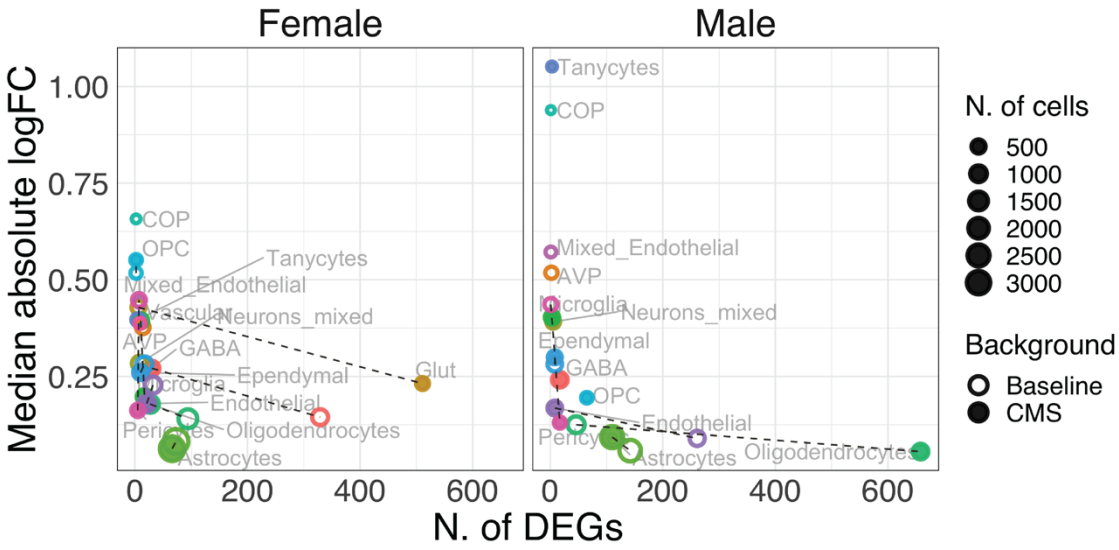


Figure 4.40: Spatial Visualisation of ARS Responses. Visual representation of the ARS response in baseline and CMS conditions for each cell clusters. Each cell type is represented by its number of DEGs (x-axis) and the median absolute logFC (y-axis). Euclidean distance between conditions is shown by a black dashed line.

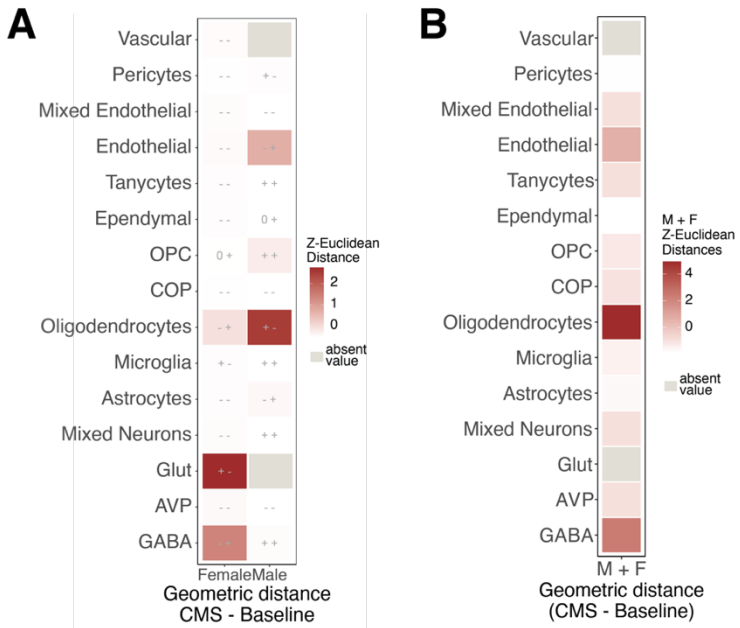


Figure 4.41: Euclidean Distances per Cell Types. (**A**) Z-scored Euclidean distances for each cell type and sex, showing sex- and cell-type-specific changes. Signs inside squares represent directionality of the changes for number of DEGs (left) and median logFC (right). (**B**) Euclidean distances for each cell type summed between sexes. Oligodendrocytes are the most susceptible cell type across sexes.

4.7 Sex and Stress Modulate Stress Responsivity in Oligodendrocytes

Oligodendrocytes are dynamic glial cells that in recent years have been recognized as active players in stress-related disorders [261], making their sex-specific susceptibility an interesting finding in the context of psychiatric disorders. In contrast to cell types with low susceptibility (such as astrocytes, **Supplementary Figure 18,** *page 141*), the ARS response of oligodendrocytes showed limited overlap in DEGs between Baseline and CMS backgrounds for both sexes (**Figure 4.42A**, *page 69* and **Supplementary Figure 17A, B**, *page 141*). While their absolute overlap was limited, their SI were higher than expected due to striking difference in the total number of DEGs between the two conditions (**Supplementary Figure 17C**, *page 141* and method section "3.6.5 Stress Background Susceptibility Analysis", *page 32*). Female smallest response was less than a third of the other and male smallest response was 14 times smaller (**Figure 4.42A**, *page 69*).

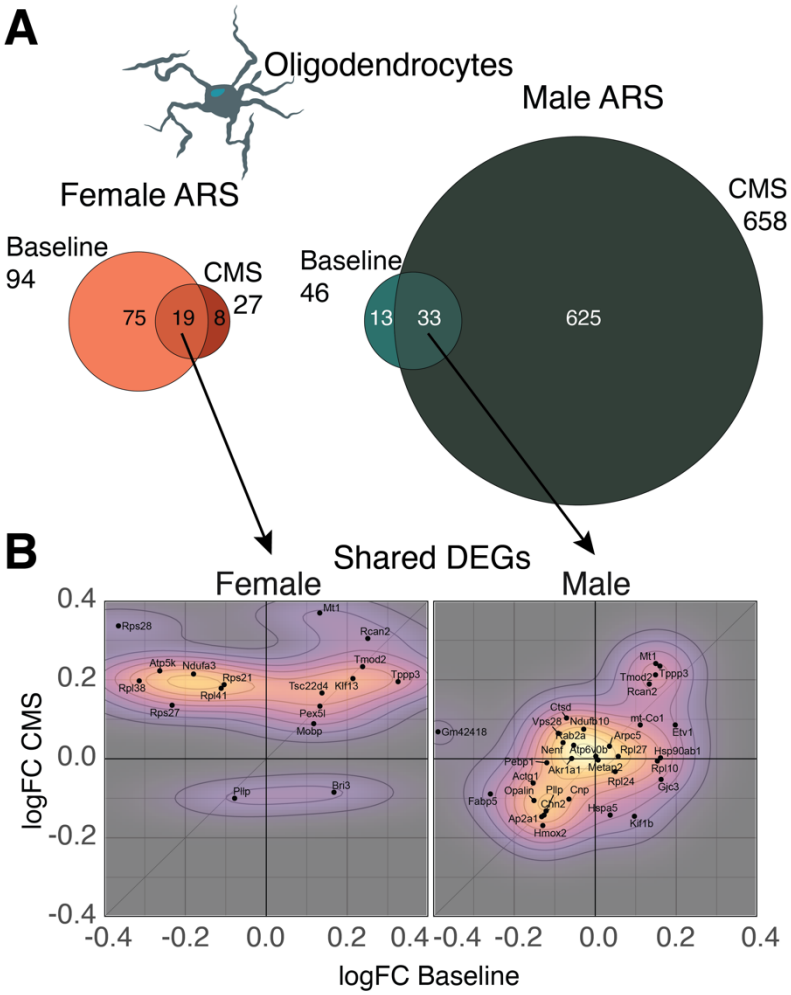


Figure 4.42: ARS Response of Oligodendrocytes. (**A**) Overlap between baseline and CMS ARS response for each sex. (**B**) Shared genes between baseline and CMS ARS responses in females and males represented by the logFC in each condition. Background colours show density distribution of genes.

Interestingly, as presented in the previous chapter, the SI already suggested the smallest response in the two sexes did not coincide with the same condition. In females, the biggest response to ARS was detected at Baseline (94 DEGs) while the smallest (27 DEGs) was the

response to ARS under CMS, indicating that CMS largely blunted the ARS response in female mice. On the other hand, male mice showed the opposite effect: 46 genes responded to ARS at Baseline, but 625 were identified after ARS under CMS background. While the shared DEGs for females were proportionally more, many of these genes were regulated in opposite directions; whereas the shared genes in the male samples were mostly consistent in direction (**Figure 4.42B**, *page 69*). We then checked if this characteristic was true for the whole transcriptomes or only limited to these shared DEGs with a RRHO analysis. We found a pattern similar to the ones observed for shared DEGs (**Figure 4.43**, *page 70*). Females indeed show two major groups of genes that were deregulated similarly: one upregulated in both stress backgrounds and one upregulated in baseline conditions but downregulated in CMS conditions. In males, only correlations between same-direction changes were identified, even if weaker than females. Overall oligodendrocytes displayed prominent sex differences in stress responsivity.

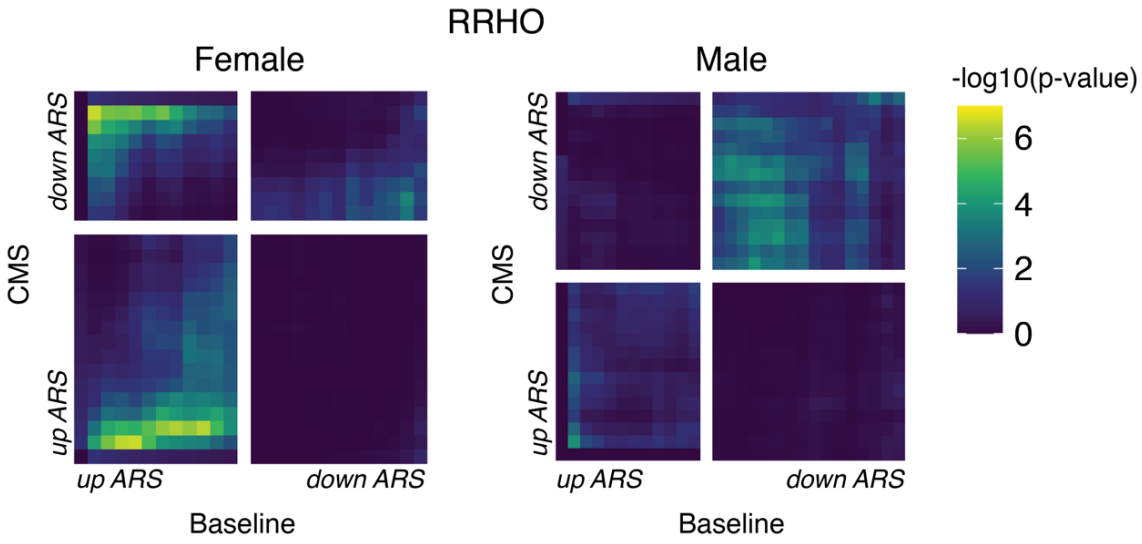


Figure 4.43: RRHO Analysis on the Differential Transcriptomes of Oligodendrocytes. RRHO analysis showed a different pattern of correlation between baseline ARS and CMS responses for female and male samples. Each differentially expressed gene is expressed by its p-value • sign of logFC and ranked along the x and y axis.

4.7.1 Stress Alters the Strength and Balance of Interactions Between Oligodendrocytes and Neurons

Aside from generating the myelin sheaths wrapped around neurons, oligodendrocytes also contribute to maintaining regional homeostasis, sensing the change in the environment, and bidirectionally exchanging information with neurons contributing to axonal maintenance and synaptic function [262], [263]. Because of their tight interaction with neurons, we investigated if the high susceptibility to stress background impacted anyhow on the relationship between these two cell types. To address this question, we built cell-cell interaction networks using the R package CCInx [196] to quantify ligand-receptor interactions as edge weights between oligodendrocytes and neurons. CCInx uses transcriptional information for each cell in the oligodendrocytes or neurons clusters to assess how strongly co-expressed pairs of receptor-ligands are in terms of edge weights. Because we were interested in assessing the bidirectional interactions between oligodendrocytes and neurons, we evaluate both possible types of pairs:

pairs in which oligodendrocytes contributed with a ligand interacting with a receptor in neurons (Oligo Ligand); pairs in which oligodendrocytes contributed with a receptor engaged by a ligand in neurons (Oligo Receptor). We calculated the edge weights for receptor-ligand pairs in each of the eight conditions (Female/Male, Baseline/CMS, Control/ARS) and then compared each pair of control-ARS samples to evaluate how much each gene pair changed. A permutation analysis was used to assess which of the receptor-ligands pairs significantly changed strength in response to ARS. We found that several receptor-ligand pairs were altered by ARS exposure in a way that seemed unique to sex (**Figure 4.44**, *page 71* and **Figure 4.45**, *page 72*). Importantly, we saw that deregulated pairs could either become weaker, stronger, be lost, or acquired after ARS (**Figure 4.45**, *page 72*).

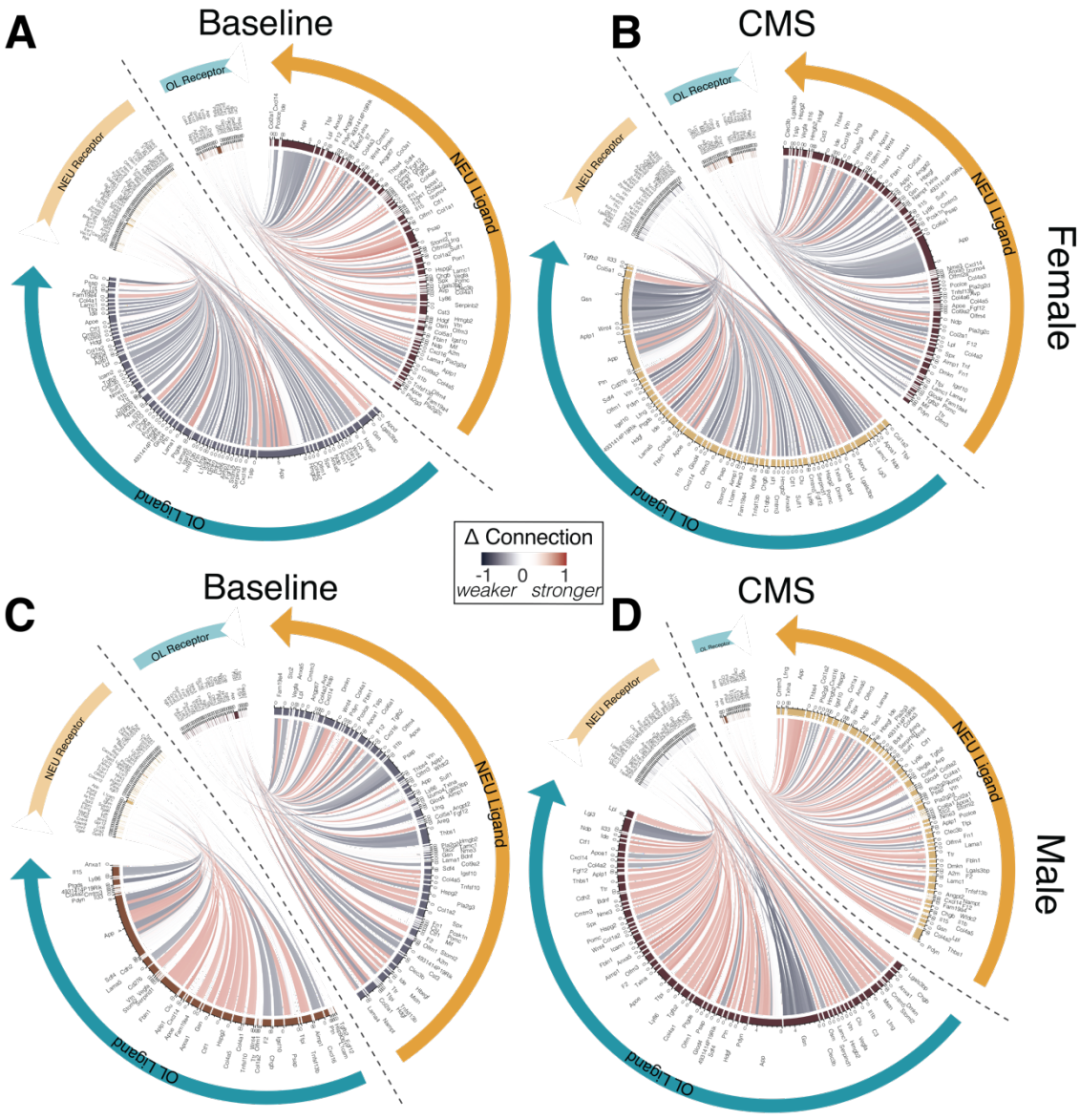


Figure 4.44: Receptor-Ligands Deregulated Pairs per Sex and Condition. Circle plots of receptors and ligands deregulated by ARS in (**A**) female at baseline, (**B**) female with CMS, (**C**) male at baseline, and (**D**) male with CMS from the cell-cell interaction analysis. Colours represent delta edge weights; chord widths are proportional to the representation of each gene.

Since the oligodendrocytes-neurons networks was indeed affected, we then explored if one direction of communication (OL Ligand or OL Receptor) was preferentially altered. ARS received under Baseline background caused changes in both directions to a similar extent (**Figure 4.46**, *page 72*). On the other hand, oligodendrocyte-neuron interactions after CMS ARS were affected unevenly in males, corroborating the idea of a male-specific susceptibility. The biggest contributors to these differences were the pairs unique to controls (**Supplementary Figure 19**, *page 142*).

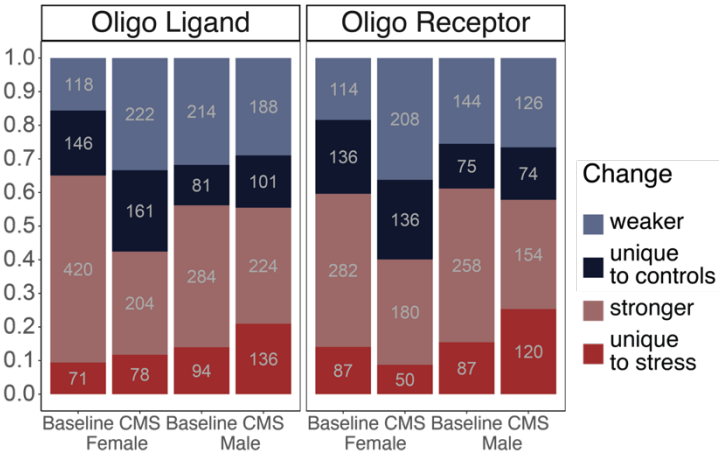


Figure 4.45: Numbers of Receptor-Ligand Pairs Significantly Deregulated by ARS. Distribution of receptor-ligand pairs significantly changed by ARS for each condition, sex, and direction of interaction. Oligo Ligand indicates pairs with the ligand in oligodendrocytes and the receptor in neurons, Oligo Receptor pairs with the ligand in neurons and the receptor in oligodendrocytes. For all conditions, four types of changes were observed, which however differ between sexes.

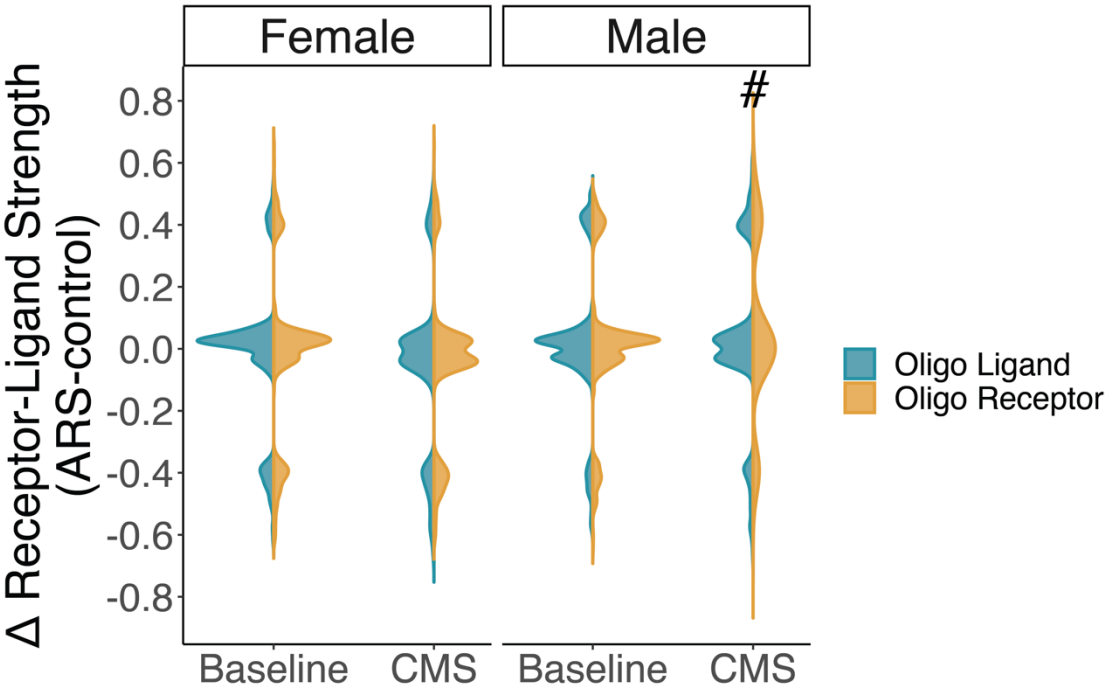


Figure 4.46: Distributions of the Change in Edge Weights for the Significantly Deregulated Receptor-Ligand Pairs. Distribution of the change in edge weights (ARS - control) for each condition and sex compared between the Oligo Ligand (blue) and Oligo Receptor (yellow) directions. All conditions exception made for Male CMS showed similar changes in both direction (Kruskal-Wallis with Dunn's test, p = 0.0761).

To understand if this unevenness impacted on the balance in the directionality of communication, we looked directly at the distribution of strength of all receptor-ligand pairs in each state (Control, Baseline ARS, and CMS ARS) and compared the distribution of the two directions (**Figure 4.47**, *page 73*). In control conditions, outputs from oligodendrocytes to neurons (Oligo Ligand) were stronger than inputs (Oligo Receptor). After ARS, this relationship still existed in Baseline background, but was lost with the CMS background, due to an overall decrease in ligand strength of the output direction (Oligo Ligand). This suggests that the exposure to ARS after CMS preferentially weaken the output direction from oligodendrocytes to neurons, possibly causing an imbalance in communication with neighbouring neurons.

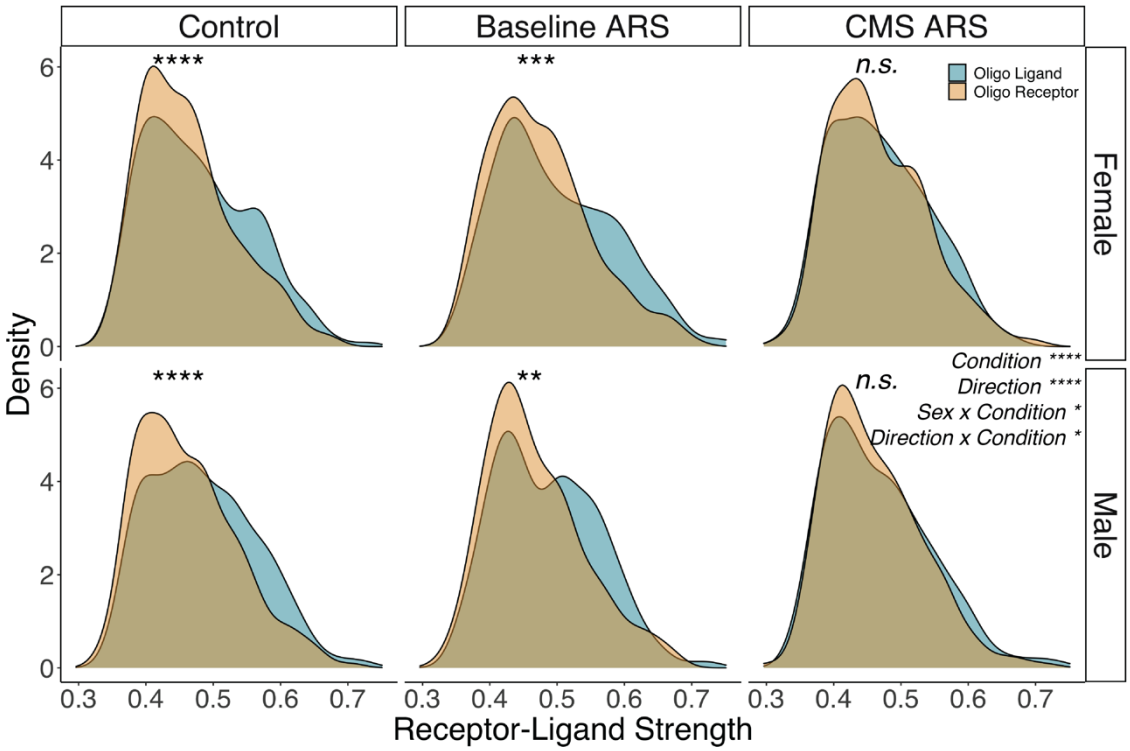


Figure 4.47: Distributions of Edge Weight for Significantly Deregulated Receptor-Ligand Pairs. Distribution of edge weight for each receptor-ligand pair within each condition (Control includes both backgrounds collapsed). After the combination of chronic and acute stress, Oligo Ligand pairs with high strength are selectively lost. Three-way ANOVA, pairwise comparisons, Tukey's post-hoc corrected.

4.7.2 Male Oligodendrocytes After CMS ARS Are Shifted Towards Immature Stages

Since the interactions between neurons and oligodendrocytes change along their developmental trajectory [264] and oligodendrocytes actively proliferate and mature throughout the adult life [265], we hypothesized that stress exposure might affect the developmental state of oligodendrocytes in a sex-specific way. In an effort to explore this further, we performed a pseudotime trajectory analysis on all clusters belonging to the oligodendrocyte lineage (OPCs, COPs, and three mature oligodendrocytes clusters, **Figure 4.48A**, *page 74*). The isolated clusters were first re-clustered (**Figure 4.48B**, *page 74*), before importing them in monocle3 for pseudotime computation [200]–[202]. The re-clustering allowed to obtain a better spatial

resolution of cell identities and developmental progression, showing a possible continuity between clusters.

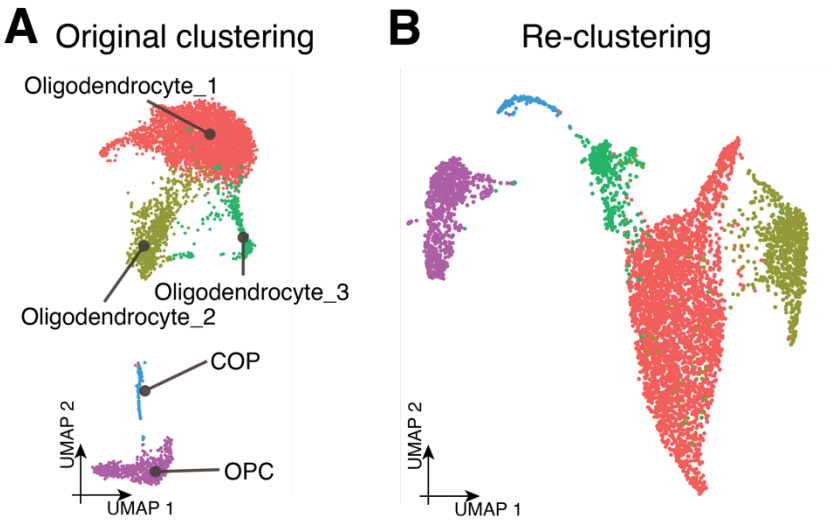


Figure 4.48: Clustering of Oligodendrocytes for Pseudotime Analysis. Clusters belonging to the oligodendrocyte's lineage (Oligodendrocytes, COP, OPC) were re-clustered for pseudotime analysis. (**A**) UMAP plot of the original clusters showing three mature, one committed progenitor and one progenitor clusters. (**B**) UMAP plot of the isolated oligodendrocytes cells re-clustered before pseudotime analysis.

For each cell, we then computed a pseudotime value which ranged from 0 (immature) to 30 (mature), which successfully ordered cells from OPCs to COPs and then to mature oligodendrocytes (**Figure 4.49**, *page 74*).

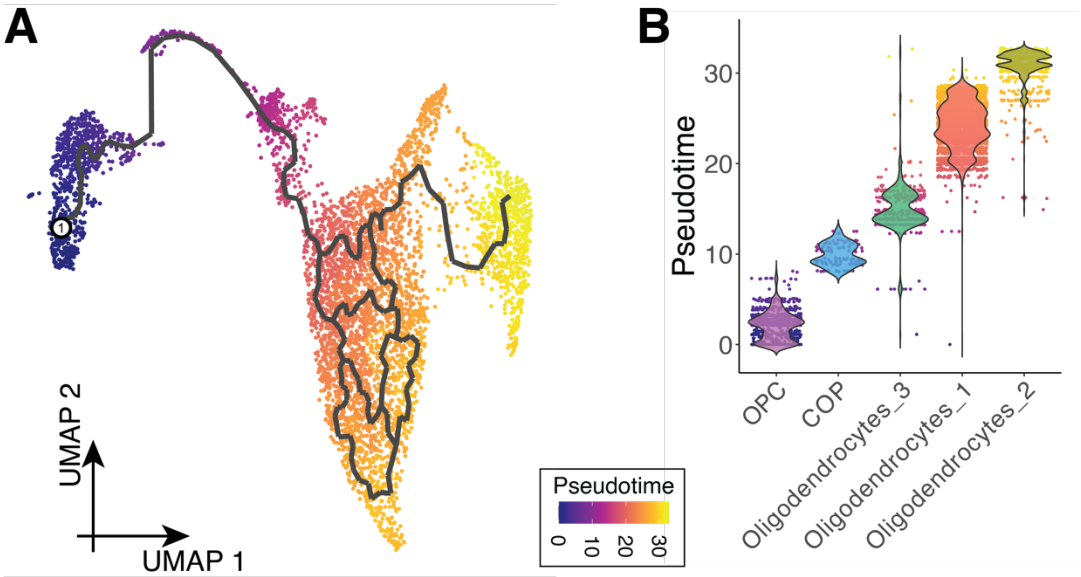


Figure 4.49: Pseudotime Analysis. Pseudotime analysis assigned pseudotime values to each cell. (**A**) UMAP plot of re-clustered oligodendrocytes cells coloured by pseudotime values. Circled 1 indicates the root for the pseudotime calculation. (**B**) Pseudotime values for each cluster. Analysis successfully ordered immature (OPC cluster) to mature cells (Oligodendrocytes clusters).

Comparing the pseudotime distribution of control and CMS ARS cells showed a significant shift of male cells toward more immature stages: 50% of all male cells were contained in a smaller

pseudotime interval than the control one (1.6 points less, 90% smaller) (**Figure 4.50,** *page 75*). Females, on the other hand, did not show any significant differences, nor did any sexes after Baseline ARS (**Figure 4.51**, *page 75*). All in all, the pseudotime analysis suggests that exposure to a combination of chronic and acute stress causes a shift in the maturation state of male oligodendrocytes in the PVN.

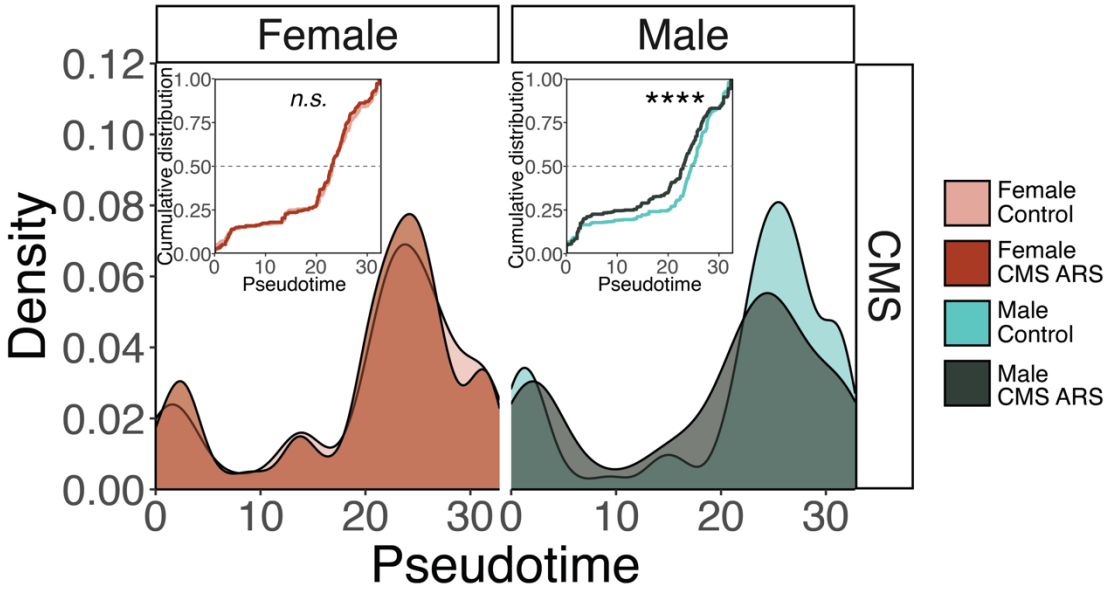


Figure 4.50: Distribution of Male and Female Oligodendrocytes Cells Along the Pseudotime for CMS Background. Pseudotime analysis reveals a shift toward more immature stages in male cells under CMS background. Inset graphs show cumulative curves for cell distributions (Kolmogorov-Smirnov test).

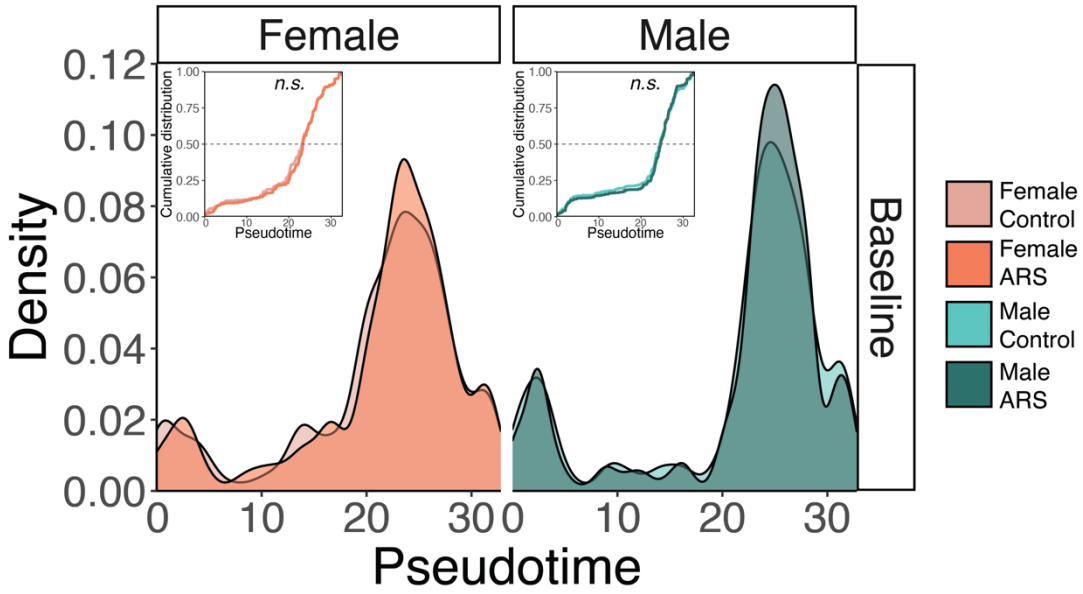


Figure 4.51: Distribution of Male and Female Oligodendrocytes Cells Along the Pseudotime for Baseline Background. Pseudotime analysis did not detect any difference in cell distribution along the maturation stages. Inset graphs show cumulative curves for cell distributions (Kolmogorov-Smirnov test).

4.7.3 Stress Exposure Impacts on the Morphology of Oligodendrocytes in a Sex-Specific Way

Along their development from progenitor to mature cells, grey matter oligodendrocytes generate several branches and ramification to make contacts with the surrounding cells [265], [266]. Considering that our bioinformatical approaches suggested that after CMS male oligodendrocytes showed a more immature profile and a different interaction with neurons, we hypothesised that these would reflect in possible morphological changes. In order to characterize the morphology of oligodendrocytes in the PVN, we labelled the cell body and the projections of mature oligodendrocytes by immunostaining for the protein Tmem10 [267] and confirmed the identity of these cells with the marker Olig2, a pan-oligodendrocyte marker [268] (**Figure 4.52**, *page 76*).

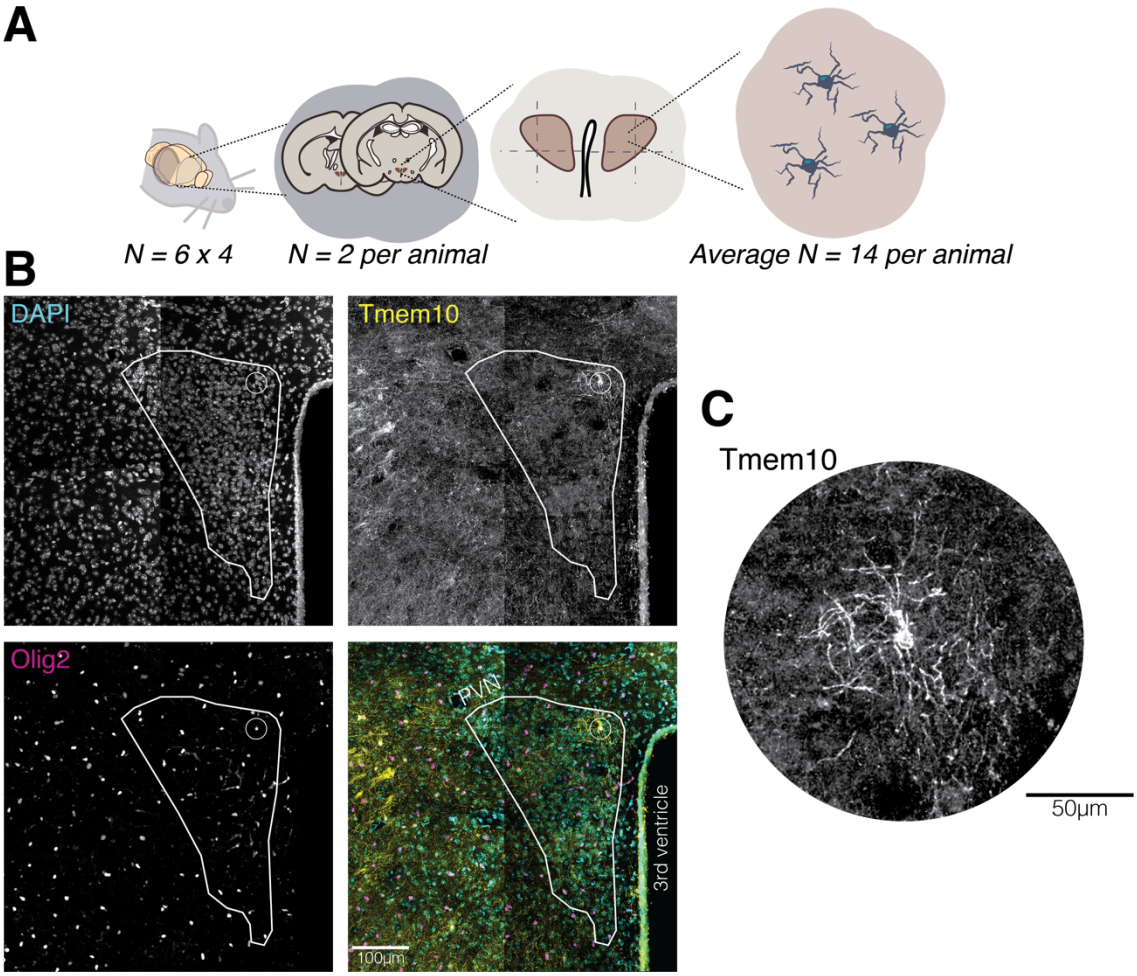


Figure 4.52: In Vivo Morphology of Oligodendrocytes. Immunofluorescence was used to observe the *in vivo* morphology of oligodendrocytes in the PVN. (**A**) Schematic representation of experimental design. Two slices per animal for an average of 14 cells per animal and 6 animal per condition were used. (**B**) Representative tiled images of oligodendrocytes traced in the PVN. Cells were stained for *Tmem10*, *Olig2*, and DAPI. (**C**) Example of Tmem10 signal used for tracing.

We then traced the cell morphology in animals after CMS ARS or controls. We did not observe any differences in soma size between sexes or condition (**Figure 4.53A**, *page 77*), however we did observe that female cells had higher total length of processes (**Figure 4.53B**, *page 77*).

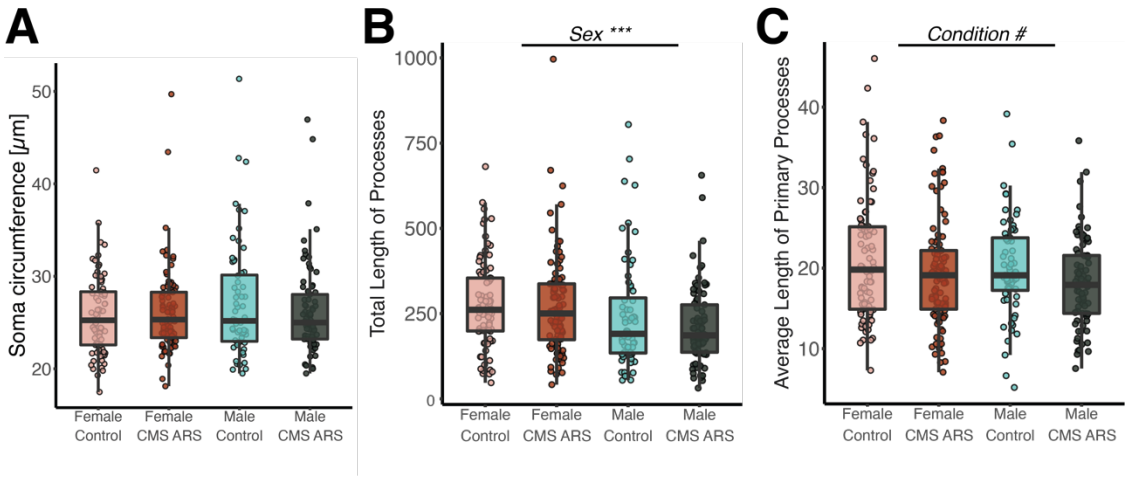


Figure 4.53: Complexity Analysis on PVN Oligodendrocytes. Complexity analysis on the male and female traced oligodendrocytes in the PVN revealed no differences in (**A**) soma size, but a sex-effect for (**B**) the total length of processes, since female cells show on average longer processes. (**C**) Stress exposure mildly reduced the average length of primary processes. Linear nested mixed-effect models. N cells: F CMS = 97, F ctrl = 82, M CMS = 81, M ctrl = 64.

We also observed a statistical trend for a condition effect on the average length of primary processes (**Figure 4.53,** *page 77*), since stress cells tended to have shorter primary processes on average. This suggests that CMS exposure can indeed altered cell morphology. None of the other parameters measured showed any statistically significant effect of sex or condition (**Figure 4.54**, *page 78*).

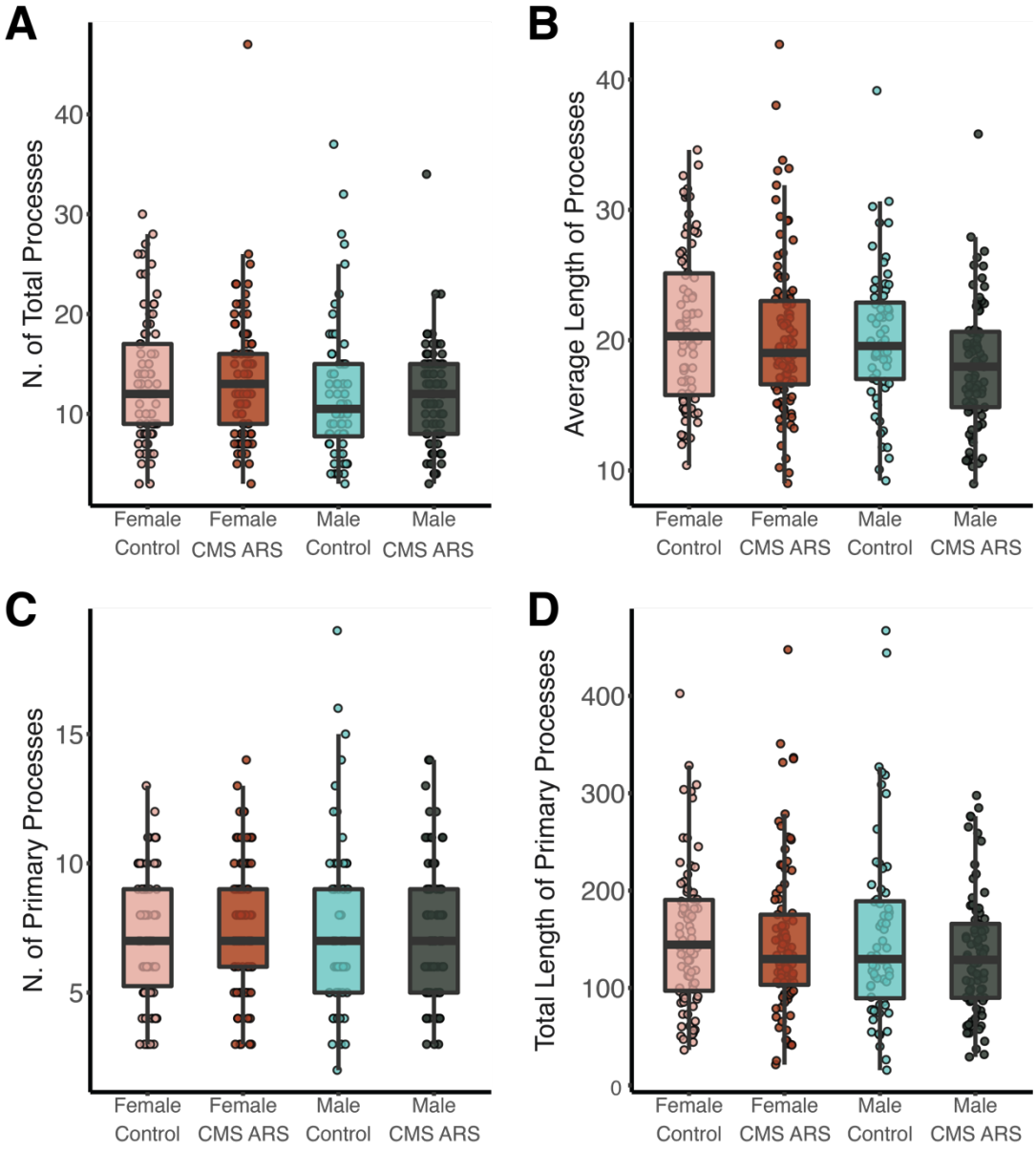


Figure 4.54: Complexity Analysis on PVN Oligodendrocytes. Complexity analysis on the male and female traced oligodendrocytes in the PVN revealed no differences in the: (**A**) total number of processes, (**B**) average length of processes, (**C**) number of primary processes, and (**D**) total length of primary processes. Linear nested mixed-effect models. N cells: F CMS = 97, F ctrl = 82, M CMS = 81, M ctrl = 64.

We further explored the ramification structure of these cells with a Sholl analysis (**Figure 4.55A-B**, *page 79*) and identified a significant interaction between sex and condition. Male oligodendrocytes after CMS ARS appeared less complex with significant less ramified branches. The Sholl analysis also confirmed the existence of sex dimorphism in the size of oligodendrocytes, since female cells had on average approximately 6 μm longer maximal extensions (max radius for female control 42.11 μm (± 4.86), CMS ARS 38.20 μm (± 3.94); male control 35.15 μm (± 5.85), CMS ARS 33.91 μm (± 5.69)) (**Figure 4.55C**, *page 79*). Maximal extension was also mildly affected by stress exposure (control 38.63 ± 6.29 μm, CMS ARS 36.06 ± 5.17 μm) (**Figure 4.55C**, *page 79*).

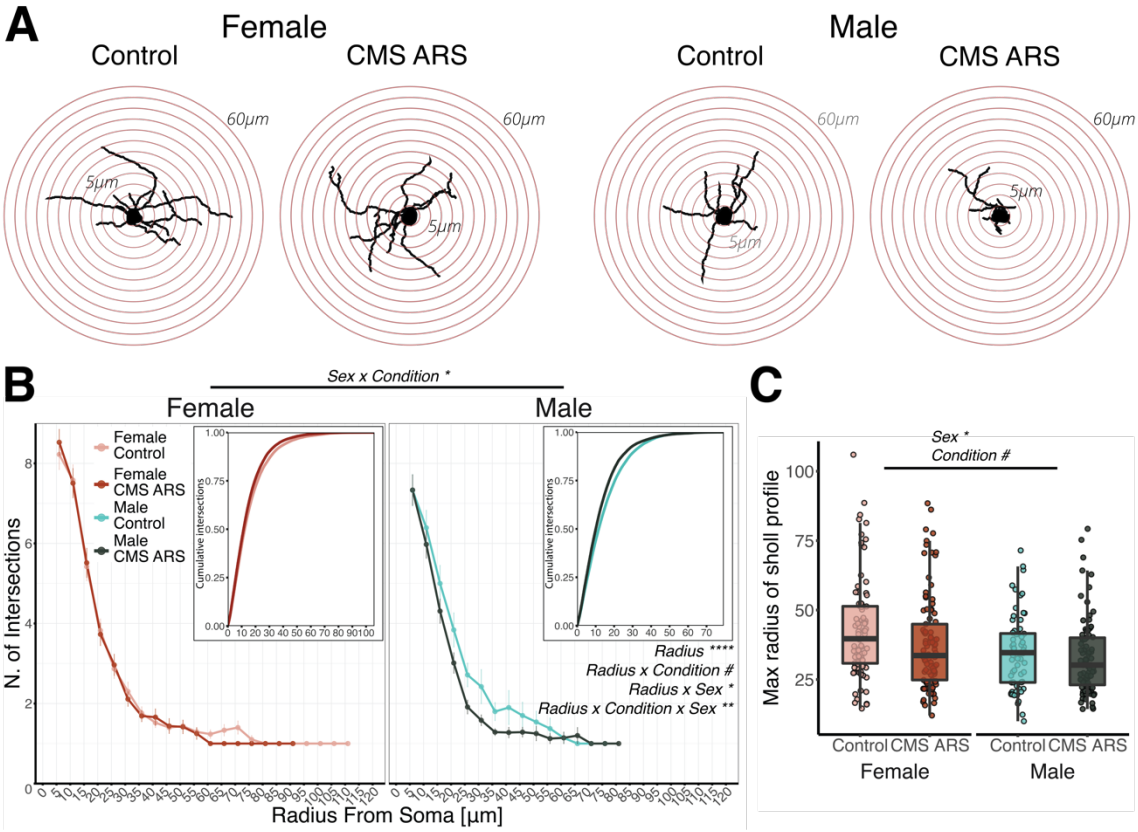


Figure 4.55: Sholl Analysis on PVN Oligodendrocytes. Sholl analysis was performed with a 5μm radius step to characterize the ramification of cells showing sex and sex x condition effects. (**A**) Representative skeletons of male and female oligodendrocytes and sholl steps. (**B**) Sholl analysis on branching of female (left panel) and male (right panel) oligodendrocytes shows a sex x condition effect on cell (linear nested mixed-effect model). Inset plots show cumulative distribution of intersections. (**C**) Maximum radius in the Sholl analysis. Female oligodendrocytes extended further from the soma, while stress exposure mildly reduced this parameter. N of traced cells: F CMS = 97, F ctrl = 82, M CMS = 81, M ctrl = 64.

While we saw clear difference in the intersections and the maximal extensions of these cells, the calculation of the area under the curve (AOC) of the Sholl analysis did not show any significant difference (**Figure 4.56**, *page 79*).

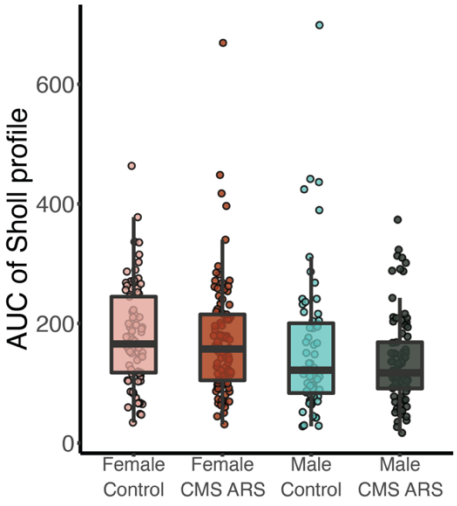


Figure 4.56: Area Under the Curve from Sholl Analysis on PVN Oligodendrocytes. Area under the curve (AUC) from Sholl analysis showing no significant differences. Linear nested mixed-effect models. N cells: F CMS = 97, F ctrl = 82, M CMS = 81, M ctrl = 64.

Altogether our results combined demonstrated evidence of a sex-specific transcriptional response to stress in the PVN and identified multiple cell types contributing to this dimorphism. In addition, we showed that these differences can reflect on cell morphology *in vivo*, providing the valuable example of oligodendrocytes.

4.8 Data Availability

To ensure that our dataset could contribute to the work of other researchers interested in sex differences and/or stress, we opted for making our data available online. ScRNA-seq data can be quite inaccessible, however, to researcher without bioinformatics and coding resources. To allow therefore for our dataset to be as accessible as possible, we generate an online web interface that allows to query specific information inside our already processed dataset. To do so, we created a shiny app with the help of the R package ShinyCell v. 2.1.0 [215]. Shiny apps are R-based apps that allows to create an interactive interface easy to personalize and upload online. ShinyCell has been specifically developed for sharing scRNA-seq data, which allowed us to easy upload our dataset in a robust and well-designed structure without the need of building the whole app from scratch. SchinyCell relies on the standard R packages shiny, shinyhelper [216], [217] and is an open-source code which also enabled us to make modifications to the layout and the interface as we pleased, including adapting the colour scheme to match our lab website (https://alonchenlab.com/). The app we built contains an initial description of the dataset (**Figure 4.57**, *page 80*) and several tabs that allow to query the full clustered dataset described in this thesis.

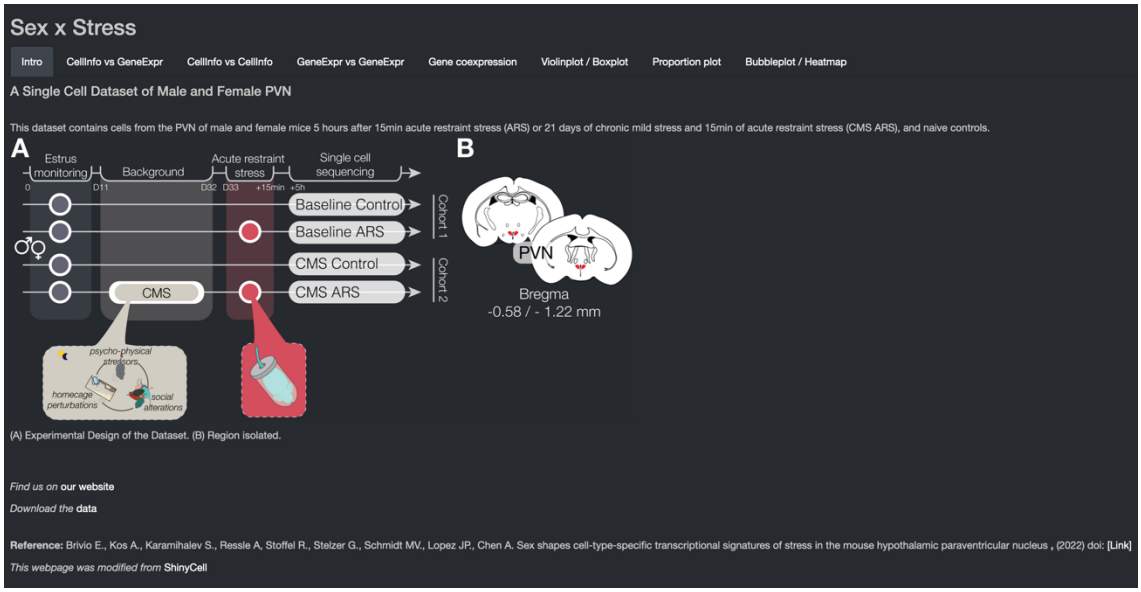


Figure 4.57: Homepage of the Interactive Web Interface. ShinyCell was used to develop a web interactive app containing the processed and cluster scRNA-seq dataset.

Among the other available functionality, researchers can also explore the clustering and query for specific genes of interest (**Figure 4.58**, *page 81*), and look for gene co-expression patterns (**Figure 4.59**, *page 81*). The app also allows for creating heatmaps, violin plots of the queries of interest and cell distribution plots.

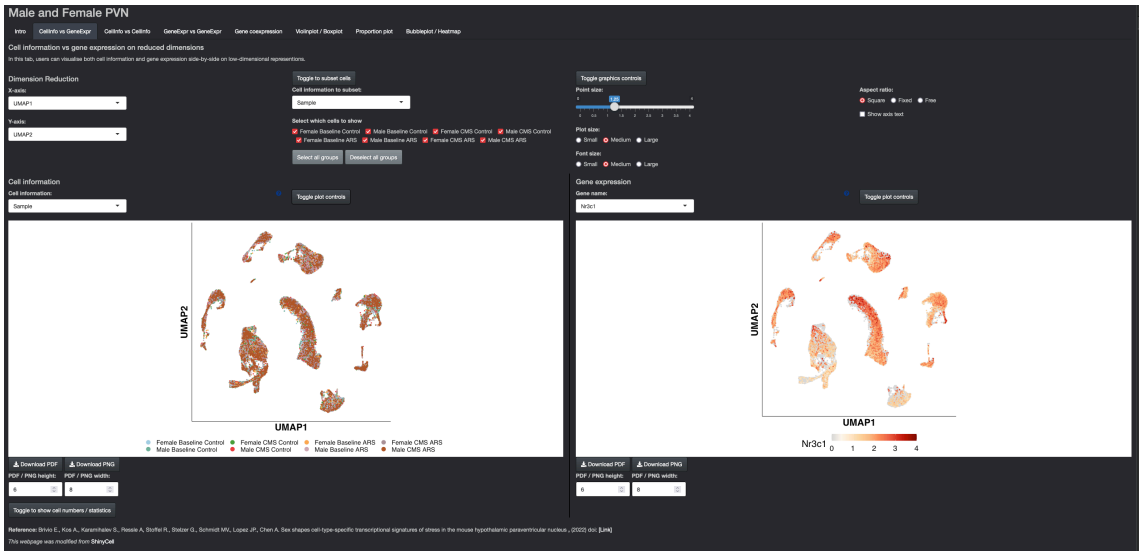


Figure 4.58: Example of a Tab within the Interactive Web Interface. The first tab within the interactive app allows explore the clustering by any variable of interest (left side) and query for any gene of interest (right side).

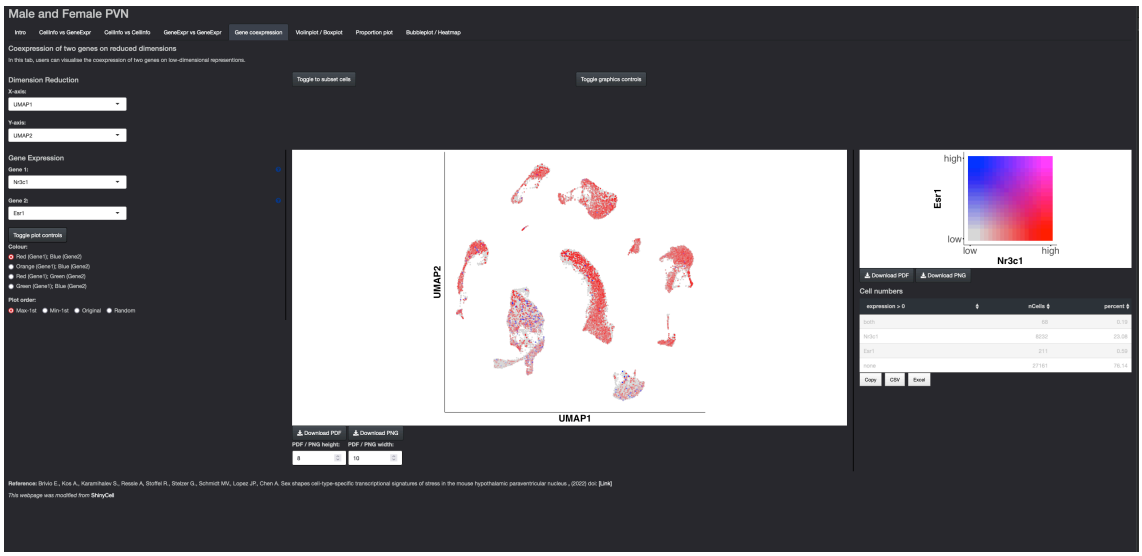


Figure 4.59: Example of a Tab within the Interactive Web Interface. The app allows for the exploration of gene co-expression patterns.

The app containing the male-female dataset will be available online upon publication of the data in a peer-reviewed journal. We hope that other researcher and their research will benefit from accessing our dataset.

5 | Discussion

Sex biases in prevalence, progression, and treatment of psychiatric disorders are commonly observed in clinical settings ("1.1 Psychiatric Disorders and Sex Dimorphism", *page 3*). The development of several of these disorders is heavily influence by stress exposure, an environmental factor processed by the stress system which not surprisingly also presents several sex differences in its activation, and cellular and molecular composition ("1.2 The Stress System and the Stress Response", *page 5*, and "1.3 Sex Dimorphism in the Stress System", *page 8*). Recent studies have suggested that some of the molecular differences observed after stress could arise by a different cell-type contribution in males and females ("1.4 Single Cell Transcriptomics for the Investigation of the Brain", *page 14*). However, the lack of single-cell resolution studies delayed the ability directly verify this hypothesis.

In this thesis, we generated the first scRNA-seq dataset to specifically explore adult sex differences in response to stress in the PVN, the central coordinator of the HPA axis. We showed that different cell types in the PVN express a different degree of sex differences in their transcriptome. These cell-type-specific transcriptomes change in response to acute stress in a substantially different way between sexes. Additionally, previous stress exposure modulates these responses in a sex- and cell-type-dependent way in both neuronal and non-neuronal cell types. We used this dataset to identify cell populations worthy of further investigation for exploring sex differences in stress. Vasopressin and tanycytes showed demarked dimorphisms in cell distribution; GABAergic neurons were especially strong responders to acute stress, selectively in females, possibly because of a direct action of circulating estrogen; Oligodendrocytes had the most abundant transcriptional differences between controls and were the most affected by the interaction between sex and history of stress. Finally, to demonstrate the possible impact of our dataset, we provided a deep characterization of the oligodendrocytes. We established that the transcriptional response after stress can be the reflection of changes in cell development and interaction with surrounding cell types such as neurons. Our bioinformatic approach further led us to identify an *in vivo* morphological defect in these oligodendrocytes. In conclusion, we believe this dataset is providing several new directions for exploring sex differences in stress. The most relevant of them are discussed in the next chapters in combination with their limitations.

5.1 Stressed Behavioural Phenotype

The main goal of our project was to obtain a reliable dataset to compare the stress response in males and females. In order to achieve this, we carefully selected stressors that would be administered without biases to both sexes. Our choices of acute restraint stress and chronic mild stress were successful and elicited an acute and a chronic stress state in both sexes. Indeed, ARS animals showed strong CORT responses and CMS animals showed a classical reduced bodyweight gain and deteriorated coat state. While these parameters clearly showed us that the stressors were effective, CMS mice did not show any alteration in thymus size or in basal CORT levels and only a moderate enlargement (~15%) in adrenal size. We did not observe any increased basal CORT levels in CMS animals neither when we measured it in the afternoon (**Figure 4.4**, *page 40*), nor in the morning (Basal CORT in **Figure 4.18**, *page 50*).

Strong changes in these physiological parameters are generally expected when using strong chronic stress paradigms such as CSDS and CVS [186], [269], [270], however CMS is specifically designed to apply just a mild stress pressure on the individuals, resembling a more naturalistic set-up [271]. Other studies before us often did no observe any thymus size change and small adrenal size changes [272], and it is not uncommon for CMS to not show basal changes in CORT and organ size [235]. We also did not observe sex differences in the susceptibility to these stress paradigms, while some works before us did [273], [274]. On the other hand, we did saw bigger organ size and higher basal CORT levels in females, a well-known physiological characteristics of females in comparison with males [44], [90].

Interestingly, while exposure to CMS did not change basal CORT levels, it seemed to blunt the CORT response to ARS (**Figure 4.18**, *page 50*). While we had no expectations regarding change in CORT response with or without a history of chronic stress, we are not the first to observe this effect of CMS over CORT response elicited by acute stress [275]. Differently, Borrow et al. detected this phenomenon when exposing mice to CMS and ARS but only in the afternoon, and not in the morning when CORT levels are higher [275]. While we cannot draw a direct relationship between PVN activation levels and downstream CORT amount produced, it is interesting to note that similar effects have been observed when evaluating PVN activation through cFOS measurements [145]. Elevated platform stress for instance, has been shown to increase cFOS levels in the PVN. When male mice were exposed to chronic restraint stress right before the EPM, the level of PVN activation was still increased by EPM but to a lesser extent. A resting time between the two stressors in females, on the other hand, led to a stronger PVN activation [145], suggesting the existence of complex sex-specific mechanisms we are still far from understand. More basic research is needed to understand exactly how different stressors can impact on the HPA activation, which of these processes could be indeed maladaptive and how it relates to the downstream release of CORT. Nonetheless, the CORT response to negative emotions in depressed patients is also blunted in a way similar to our stress models [108], [109], suggesting these could be valuable models for also getting more insight on psychopathologies.

In addition to CORT measurement, the overall behavioural assessment was successful at confirming the impact of CMS on the mice wellbeing. The use of combined scores allowed us to describe a complex stress phenotype in both sexes. Nevertheless, often individual tests did not detect any significant differences, For example, while anhedonia is classically described as one of the behavioural alterations in response to stress [235], [237], we did not detect any reduction in sucrose preference in CMS mice. Extensive reports in literature have already highlighted however the inconsistency of this test supporting the idea it might just be a lack of resolution of the test itself [276]. In addition, females have been often described as more susceptible to stress in general, also from a behavioural point of view [277]. Despite this, we did not find any sex differences in the stress-dependent behavioural changes, while we did observe the expected basal sex differences such as increased locomotion and exploration in females [235], [242]. It is important to note that current literature provides many contradicting results regarding sex differences in the stressed behavioural phenotype [235]. Due to the lack of standardisation in the development of chronic stress paradigm in both sexes and the fact that many factors can alter the behavioural outcome, from the strain of the mice [278], to the housing strategy and the testing strategy [11], [242], it is often difficult to directly compare results across different studies. A better standardization and the introduction of more robust behavioural assessment is a strong need for

the field to improve upon understanding different vulnerability to stress between males and females [279]. Overall, despite we are missing some of the expected stress-related changed (e.g., thymus size change, decreased sucrose preference, passive coping in TST), we have clear evidence that animals exposed to CMS are exposed to a history of chronic stress as much male and female mice exposed to ARS perceive the stressor in an equally stressful way.

5.2 Basal Sex Dimorphism

Previous work at the single cell resolution have shown that the brain has a certain extent of persistent sex dimorphism in numerous brain areas, including the one involved in the stress system such as amygdala and pituitary [44], [138], [250], [280], [281]. Our study also identified several basal sex differences in cell distribution and gene expression of the PVN. Especially we found some mild sex dimorphisms in cell distribution in vasopressin neurons and tanycytes and transcriptional differences in other several cell types, in particular oligodendrocytes.

5.2.1 Vasopressin Neurons

Vasopressin neurons are GABAergic neurons, resident in the PVN and several other brain regions, which are characterized by the production of the neuropeptide AVP [248], [282]. AVP is an important contributor to the stress response regulation [283] and it is often regulated after stress exposure.

The vasopressin system has been shown to be highly dimorphic in other regions such as the bed nucleus of the stria terminalis or the MeA, in terms of number of cells or basal expression levels of the peptide [284]–[286]. Information on the PVN, on the other hand, have been less concordant and uniform. In our data, we found good evidence that also the PVN shows dimorphism in the AVP system. We in fact detected more female vasopressin neurons than males. Interestingly this neuronal subtype was inversely enriched in male cells in both stress conditions. Female AVP neurons in the hypothalamus have been described to be smaller than males [287], thus we could expect that smaller cells would be compensated by having more of them to fill the same size region. However, on a previous study with an *in situ* hybridisation approach, Borrow et al. did not find any differences in the total number of PVN AVP neurons [144]. Still, authors described a strong reduction in AVP granules selectively in females after stress exposure (in accordance with [275], [288]). Importantly, it needs to be noted that all their controls were exposed to a series of behavioural tests before sacrifice and therefore could have developed a mild stressed phenotype. If so, a female-specific decrease in AVP due to this mild stressor could have concealed and initial higher basal levels in females. This effect, however, would not be present in our dataset since our controls were not exposed to any stressors. In addition, a female-selective reduction in the AVP gene expression after stress could explain why we observed a “loss” of female AVP-positive cells after stress. If AVP expression levels were to decrease below detectability of scRNA-seq, cells would not be categorized as AVP-neurons anymore, hence their reduction in number. Given the fact that differential gene expression was conducted only between same cell types, this change in identity would also prevent us from observing any change in *Avp* expression levels. Finally, we need to mention that other studies which lack single cell resolution and look at gene expression changes with bulk qPCR did not detect any changes in *Avp* after CSV [142], suggesting that a lack of single cell resolution might make these small changes difficult to detect.

Overall, our results support the idea that the male and female brains might have small but significant cellular compositions in vasopressin neurons and that vasopressin expression levels could be stress-sensitive in a sex-specific way.

5.2.2 Tanycytes

Aside from vasopressin neurons, a second cell type was found mildly unbalanced between male and female control cells: tanycytes. Tanycytes are the specialized ependymal population that lines the third ventricle, monitor the CSF and convey information from the neuroendocrine nuclei to the CSF and the way back [251]. Interestingly, several subtypes of tanycytes different in morphology, spatial positioning and function have been described and characterized in the latest years [251]. These in-depth characterization also led to the discovery that tanycytes have more functions than previously thought, including retaining a degree of staminality that gives them proliferation and differentiation abilities [289], [290].

Our dataset showed enrichment for female cells in controls, which was not detected after exposure to CMS and ARS. Before us, some types of stress, such as dietary stress, have been shown to impact on the stem cell-like abilities of tanycytes in the median eminence of the hypothalamus in a sex-specific way [291]. Accordingly, the change in female/male cell ratios we observed across conditions could be the result of the stressors acting on either proliferation or survival in a similar way to dietary stress. The further analysis on the tanycytes subtypes indeed pointed at the fact that these unbalanced is mostly contributed by the a subtype characterised by the expression of several cell cycle TF such as Krüppel-like factors (*Klf2*, *Klf4*) [252], [253], which probably coincide to the proliferative α2 subtype. Our results suggest that the female PVN has a bigger reservoir of proliferative tanycytes that can get potentially activated in condition of stress. Unfortunately, the literature available about proliferation in tanycytes in general but especially about sex differences in tanycytes is still scarce. Further exploration of these factors could enrich our understanding of stress response and cell regeneration after stress exposure.

5.2.3 Transcription and Oligodendrocytes

In addition to differences in cell distributions, basal differential gene expression between male and female cells was also present in some populations, in particular oligodendrocytes, astrocytes, and ependymal cells. While we did not identify any differences in the gene markers used for assigning cell identities, other genes had stronger or weaker expression when comparing the two sexes. Importantly, the reliability and effectiveness of our dataset is proved by the identification of *Xist* as a DEGs in all cell types; *Xist* is the gene responsible for the X inactivation process and therefore uniquely expressed in females [258]. Transcriptional differences between male and female brains had been already extensively described in several research models, including humans, mice, rats, macaque, and dogs and several organs including the brains [292]–[295]. Our study further showed that the extent of these differences in the brain, or at least the PVN, depends on the cell type investigated: similarly to what has been shown before for the heart, adrenal glands, and immune cells to cite some [296]–[298]. These different transcriptional landscapes can confer more or less molecular flexibility of cell types to respond to stimuli such as stressors on a sex-specific manner. Additionally, it can influence the well-known sex differences in plasticity, brain connectivity and behavioural response, but more investigation is needed to connect gene

expression output differences. However, in our dataset oligodendrocytes stood up as the most sex-different cell types and the cells that were the influenced by chronic stress in a sex-biased way. In control cells, we identified several genes involved in the metabolic processes, and cell stress response system to be basally expressed differently in males and females and we ultimately described a strong difference in stress response as well. It is therefore likely that these two pieces of evidence are connected. In support of this theory, among the sex-biased genes we also found enrichment for myelin-related pathways, while showing morphological differences between male and female mature oligodendrocytes in the PVN. This indeed suggest that the transcriptional basal differences can have a direct impact on how male and female cells are and possibly react. The additional findings about oligodendrocytes are further discussed in the next chapters.

5.3 Transcriptional Response to ARS and Sex

In addition to basal sex differences, we thoroughly characterized the response to ARS under different stress conditions, identifying extended sex differences in both neuronal and non-neuronal stress-responsive cells. We first showed that females had an overall more pronounced transcriptional response to ARS, showing a higher number of total DEGs. This result does not come as a surprise: several studies using bulk transcriptomics have found females to have higher number of DEGs in response to different stressors [138], [140], [143], [146], [148], [299], [300]. It is still not clear what mechanisms are responsible for this bigger response in females and importantly if this difference is associated to any specific biological meaning. However, we also found that more cell types showed any DEGs in females than in males, suggesting that the higher number of DEGs could be, at least partially, the result from a wider involvement of cell types.

About these differences, the biggest contributors in females were the GABAergic neurons, while in male the endothelial cells. Endothelial cells have been shown to be among the most responsive cells in males also after chronic social defeat [186] and many pathways found altered in the cortex of MDD patients can be reconducted to endothelial genes [146]. While we and others did not identify extensive basal sex differences in endothelial cells [301], the different gene expression regulation in response to stress could be regulating activity and functionality of the blood brain barrier (BBB), especially under a stress condition. Endothelial cells are in charge of maintaining the BBB integrity and regulate the bidirectional exchange of nutrients and transmitters with the blood [302]. Supporting this hypothesis, we found several transporters among the males DEGs, such as *Slco1c1*, *Slc50a1*, *Slc38a5*, *Slc25a5*, and junction components such as *Cldn5*, *Tjp1* and *Pecam1,* deregulated by ARS. The lack of these changes in females could signify that a different regulation of import and export of metabolites (such as sugar, amino acids) and hormones, and tightness of the barrier could generate two different sex-specific environments for the endothelial and all brain cells after stress. It has also been previously shown that the cerebral vasculature of males and females differs in functionality, mostly by sex steroids action [308], but how different the role of male and female endothelial cells in psychiatric disorders is less characterized. Even so, BBB integrity and functionality has been shown to be altered in sex-biased psychiatric disorders such as schizophrenia [303], depression [304]–[306] and ASD [307]. More work is needed to understand why these mechanisms are restricted to one sex and if they give rise to sex-specific microenvironmental differences in psychiatric disorders.

Regarding sex-restricted mechanisms, GABAergic neurons in turn showed a highly female-specific response to ARS, completely absent in males. This is in contrast with results obtained with scRNA-seq in the PVN after CSDS from our lab, in which we did detect DEGs in the GABAergic population [186]. While we cannot exclude that we are just lacking resolution for detecting a response in males, it is very probable that GABAergic neurons might just be more responsive in females and show a transcriptional response already after an acute challenge, while they get activated in males only after a strong chronic stress such as CSDS. We indeed detected DEGs in GABAergic neurons also in male in the animals precedently primed with CMS.

In addition, we also confirmed that a history of stress (in the form of CMS) has an impact on how cells will respond to a subsequent novel stressor. This impact is more or less pronounced depending on the specific cell type and the sex of the individual, and is independent on the cell being a neuronal, glial, or stromal type. Considerably more cell types in males than females changed their transcriptome after ARS if previously exposed to CMS. Oppositely, female cells mostly had reduced number of DEGs and less cell types responding after CMS ARS. This is in apparent contrast with clinical data that suggests that females have enhanced sensitivity to chronic stress and are more prone to develop maladaptive stress responses thereafter. However, preclinical research have clearly shown that female rodents are indeed more susceptible to chronic paradigms, but they also develop better adaptive responses to acute challenges after exposure to CMS [274], supporting our results. It is important to note, however, that we cannot discriminate which of the changes of the ARS response after CMS could be deemed maladaptive and which could be just a different why of adaptive coping given a different basal state (a CMS or not CMS state). Deeper analyses on the genes and pathways actively altered by the CMS exposure could elucidate these factors and possibly help identify which processes could be advantageous to enhance and which to prevent in each sex.

Moreover, we have to recognize that the use of – as conventional as they are – arbitrary significance thresholds to determine the presence or absence of a deregulation in gene expression can sometimes limit direct comparisons between different analysis. DEGs not shared between ARS and CMS ARS could be easily regulated in a similar manner across conditions, but fail to meet the significance threshold for a statistical rather than a biological reason. Studying these regulations with wider approaches that take in consideration the whole transcriptome regardless of significance thresholds, such as the GSEA and RRHO approaches that we integrated in our analyses as well, can increase the understanding of the gene expression regulation at a macroscopical scale. For example, male glutamatergic neurons lack completely DEGs in males not allowing meaningful sex or condition comparisons. In contrast, the RRHO approach, which takes the significance in consideration but is not bound by a hard threshold, allowed us to identify that female and male glutamatergic neurons show profoundly different gene expression changes in relations to their history of chronic stress. Other approaches that also analyse the whole transcriptome and detect small changes negligible in individual gene but noteworthy if shared by several could also overcome these limitations. Especially gene networks analysis that look at co-expression patterns of networks of genes and their perturbation would nicely complement our analysis. This bioinformatic approach has been already very successfully applied to bulk transcriptomic studies to identify complex pattern of gene expression across stress conditions or between sexes [146], [150]. Transposition of similar networks to scRNA-seq dataset is a process still in the making that has the potential to dramatically improve the current

understanding of gene expression regulation across cell types and could be applied in the future to better characterise the gene expression adaptation in response to the change in stress background.

Nevertheless, the simpler approach of using DEGs was still successful of identifying important cell-specific changes in sex-specific manners.

5.4 GABAergic Neurons and Estrogen

We identified several sex differences in the DEGs after ARS for several cell populations. Among them, the GABAergic neurons had the highest number of deregulated genes fully restricted to females. The DEGs included steroid hormones chaperons (e.g., *Hsp90aa1*, *Hsp90ab1*, *Dnaja1),* genes associated to synaptic release (e.g., *Snap47*, *Nrnx1*, *Nrnx3*, *Rab3a*, and *Syt1*) and GABA cycle (*Slc32a1*, *Slc6a1, Gabrb1*, *Gabrb2*), suggesting that female GABAergic neurons possibly respond to stress-dependent increased circulating steroid hormones such as CORT and regulate cell activity in a way males do not. Accordingly, GABAergic neurons have been shown to transcriptionally respond to stress differently between males and females also in other regions such as the prefrontal cortex [309]. Knowing that the functionality of GABAergic neurons has also been long associated with regulation of PVN activity [249], and that the female HPA axis is hyperresponsive to acute challenges [87], [112], our results provide a possible molecular link between these two processes. In support of this idea, these DEGs were enriched for genes interacting with the estrogen receptor α (*Esr1*). Estrogen receptors (ERs), the nuclear receptor family activated by estrogens, are known in fact to influence the activity of the HPA axis [112]. Expression levels and activity of ERα has also been connected to resiliency to social defeat stress [100] and its overexpression can confers resiliency through sex-specific downstream molecular mechanisms [310]. While we did not find a direct change in *Esr1* expression, we did find evidence for its involvement in the female-specific ARS response. Further studies exploring the activity of this receptor (either bioinformatically-predicted activity with software like DoRothEA [311]–[313], or indirect activity with activity-based assays and evaluation of its translocation) will be important to quantify the extent of its role. Removal of circulating estrogens normally eliminates the hyperresponsivity of the HPA axis and the high levels of CORT [87]. In our set-up, ovariectomy also eliminates the GABAergic response, strengthening the possibility of a correlation between the two processes. Our data suggests that such hormones could also coordinate the activation of subpopulation of neurons in response to stress and therefore ultimately regulate the differential activation of HPA axis to stress.

Notably, the combination of CMS and ARS identified an additional interesting pattern of activation and sensitivity to the stress background within the female GABAergic-glutamatergic circuitry. GABAergic cells were the strongest responders under baseline conditions in females but lost most of this response under CMS. In contrast glutamatergic cells had a limited response to ARS which was increased when combined with CMS. Alterations in balance between glutamate and GABA are of particular importance due to their association to many psychiatric conditions. Our results further suggested that sex might enhance or attenuate the system balance in response to stress, in accordance with results from previous work in the cortex [314]. Importantly, the GABA-glutamate system is an important target for classic and rapid antidepressants [314] and indeed

rapid antidepressant such as ketamine have recently been confirmed to act in a sex-specific way [37]. Our dataset can be used to identify further subtypes of glutamatergic and GABAergic neurons that are either susceptible or not to stress to identify new targets for translational research. Future studies could try to dissect the identity of these subgroups, their relative abundance between the sexes, and any sex-bias in their molecular landscape that could lead to a different functionality of the circuitry under stress for males and females. Additionally, we would benefit from more in-depth knowledge about their synaptic organization, activity, and remodelling after stress in the context of the sex-biased HPA activation. Unfortunately, despite the high number of cells present in our dataset, we do not have enough resolution to dissect all known transcriptional subtypes of GABAergic and especially the rarer hypothalamic glutamatergic neurons. The field would also benefit from additional scRNA-seq datasets of GABAergic and glutamatergic neurons with male and female samples for a better resolved clustering and resolution in gene expression. Altogether future studies in these directions could provide new insights into how sex influences the GABA-glutamate system and lead to new and more effective drugs designed to target men and women differently.

5.5 Oligodendrocytes in the Grey Matter

The dataset presented in this thesis provides by bioinformatic means many new directions to explore sex differences in stress. To consolidate the relevance and potential of our dataset, we further validated and characterized the cell population whose ARS response was the most affected by a history of chronic stress: the oligodendrocytes.

While recent studies have described how oligodendrocytes are sexually dimorphic cells in terms of morphology, proliferation, and survival [315]–[318], to the best of our knowledge we are the first study to identify sex differences in stress reactivity. Our data first support the idea that male and female oligodendrocytes of the PVN have an intrinsic different molecular basal state in several relevant pathways of stress coping and cell functionality, already discussed in ”5.2.3 Transcription and Oligodendrocytes”, *page 86*). Secondly, our data also suggest that male but not female oligodendrocytes are strongly affected by exposure to chronic stress, which modulates their transcriptomic response to stress, determines a morphological defect, and changes their interaction with surrounding neurons (**Figure 5.1**, *page 91*). Interestingly, our data complements recent transcriptomic findings suggesting an opposite effect of MDD on men and women oligodendrocytes [148], as well as single-nuclei RNA-seq findings which identified a change in interaction between neurons and oligodendrocyte progenitors in *post-mortem* brains of male depressed patients [198]. This study did not include female patients, it is therefore unsure if their results apply to women as well or if they identified another male specific effect.

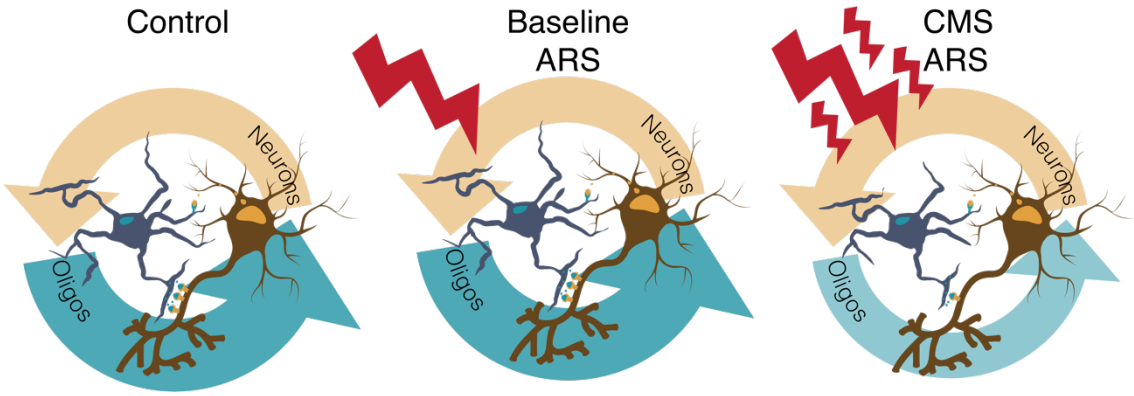


Figure 5.1: Summary of the Male Phenotype of Oligodendrocytes. Male oligodendrocytes show a distinctive sensitivity to stress. In control conditions and after ARS, oligodendrocytes show more output towards neurons than inputs. If ARS is received after CMS, however, these imbalance in interaction is lost and oligodendrocytes show a more immature morphology.

We do not fully understand why male and female oligodendrocytes show a different sensitivity to stress, but basal sex differences in gene expression suggest that these cells might be equipped with a different machinery to respond to cell stress. Moreover elevated corticosterone levels, a hallmark of stress exposure, have been shown to modulate proliferation and maturation of oligodendrocytes [319]. The effect of sex on corticosterone sensitivity and on proliferation or cell death resilience under stress are therefore important aspects still to be characterised. These future studies could lead to the identification of new candidate genes that regulate oligodendrocytes survival in a sex-specific manner. In addition these studies could also identify valuable new drug targets for not only stress-related psychiatric disorders [261], but also other disorders in which the involvement of oligodendrocytes has been described with a strong sex bias such as multiple sclerosis [320].

5.6 Limitations

We already discussed some limitations in relation to the results discussed so far. This chapter addresses some additional and more aspects worth of be discussed.

The primary goal of the project was to characterise stress changes in both sexes. To achieve this, we made sure to handle both sexes in the most similar way (e.g., handling males while collecting vaginal samples of females), maintaining all same experimental timelines, and processing samples as close in time as possible. Unfortunately, single cell suspension required long processing time and precisely timed five hours after ARS, making it hardly possible to process all samples in parallel. We opted for minimizing confounding factors in the stress analysis maintaining control and stress for each background always on the same day for a robust differential analysis. As a consequence, male and female samples were loaded on the 10x Chromium controller on two consecutive days, but not on the same run. Despite not being able to generate single cell suspensions of male and female samples on the same day, we did process the samples, generate libraries, and sequence them as single cohorts. Because of these attentions and having actual biological replicates for the control conditions, we believe that just minimal bias could be confounding the basal sex differences.

Secondly, our ambitious goal of characterising a very small and precise regions such as the PVN came with the caveat of little amount of tissue and ultimately cells for sequencing. For this reason, we pooled multiple animals in each preparation to obtain enough starting material to meet technical requirements for scRNA-seq. This ultimately prevented us from collecting biological replicates for each of our conditions. The presence of multiple individuals in each sample, however, increased biological variability and reduced dissection and technical variability, compensating – at least partially – for the lack of replicates. Importantly, because controls in both cohorts did not experience any stress, we did obtain biological duplicates for the control condition. While this design strengthens our confidence in the basal stress differences, it also meant not having controls who experienced CMS without ARS. This prevented us from finely dissect if any of the ARS signature observed under CMS conditions are a persistent effect of CMS exposure instead of a direct consequence of ARS.

Thirdly, because of the precise timeline of 21+1 days of CMS ARS, we could not easily select for a specific estrus cycle for the females at sacrifice. While this could be seen as a disadvantage for many, pooling five individuals at different phases of their estrus cycle possibly paints a more representative picture of the whole population of cycling female.

Despite the limitations here discussed, the high number of cells, and the rigorousness in experimental design make our dataset a valuable new resource for the researchers interested in approaching sex as a biological variable in stress.

5.7 Future directions

What we are providing within the framework of this thesis is a new rich dataset of thousands of single cells in which several new research directions emerged.

Follow-up research should be concerned with validating these bioinformatics results, evaluating how specific they are to the PVN and better dissecting the origin of these sex difference. It is, in fact, well known that stress type and timing, and the region of interest have a strong influence on the molecular mechanisms [23].

Basal sex differences between unstressed male and female PVN can be further validated by evaluating the number of AVP neurons present in the PVN by RNA or protein staining such as *in situ hybridisation* (ISH) or immunofluorescence. Alternatively, RNAscope provides a new high-resolution ISH that significantly improve upon limitations of previous approaches and might help settle about discrepancies in literature.

Our results also suggest a different potential for proliferation and regeneration of male and female tanycytes. These characteristics could be further studied by evaluation of proliferative markers. Equally, approaches such as Bromodeoxyuridine (BrdU) administration, which allow to estimate the cells cycling *in vivo* could also foster new interesting line of research about sex differences in adult neurodevelopment. If these studies confirm a different ability of males and females to regenerate cells in basal or stress conditions, they could revolutionize our understanding of resilience and susceptibility in the brain.

OVX animals showed that part of the differences between the sexes in basal brain anatomy and stress response are dependent on circulating gonadal hormones. Additional mechanistic

experiments, for example re-supplementing estrogens to OVX animals or directly manipulating hormonal levels in males through orchidectomy, are needed to consolidate our results. Additionally, the Four Core Genotypes mouse line in which chromosomal sex and sex hormone production are genetically independent could help in the characterization of the individual contributions of sex chromosome, developmental sex hormones, adult sex hormones to the architectural and activational differences we observed [321].

Finally, our results highlight the oligodendrocytes as a sexually dimorphic cell type with high susceptibility to stress. In vitro models of oligodendrocytes have been successful in the past in identifying some sex differences [315]–[318] and could a valuable approach to study the molecular mechanisms behind sex dimorphisms in stress. For example, it could be used to evaluate the role of CORT in the stress-related sex differences we described. Additionally, it will be important to further validate the deregulation of the main DEGs found to be sex-specifically changed after stress. An overlap analysis between these genes and the pairs of receptor ligands changed by stress could also help identifying molecular pathways that work differently in male and female oligodendrocytes. We also suggest that the origin for some of these changes could be found in a sex-different regulation of the developmental trajectories by stress. Complementary studies looking into master transcription factors that regulate these processes and manipulation of such in a sex-specific way could identify new targets for regulating oligodendrocytes developmental patterns, not only in the context of stress but also in broader contexts such as myelin disorders.

All in all, our results provide numerous ideas for investigating the brain of male and female mice, better understanding the impact of stress, and suggest several new directions for future studies.

5.8 Conclusions

Mental disorders are closely associated to stress exposure and stress adaptation processes; mechanisms that have been shown to be extensively sexually dimorphic. The PVN is the central coordinator of the stress response, therefore a pivotal region to characterise to understand sex differences in stress and stress-related psychiatric disorders. Early work on the hypothalamus have identified small sex differences in gene expression of key genes, but almost no studies have tried to understand the cellular contribution to these differences. Previous hypothalamic scRNA-seq datasets using male and female samples have mostly focused on the anatomical characterisation of the broad hypothalamic region [166], [322], and often with a selective focus on neuronal populations [322], [323]. Even fewer single cell transcriptomic studies have studied the effects of stress in males and females: they used designs restricted to single manipulations, namely diet stress in the arcuate and median eminence [169] and formalin stress in the whole hypothalamus [322].

In contrast, our data provides an unbiased dataset that covers neuronal, glial, and stromal cell types selectively of the PVN, instead of the broad hypothalamic region. As a result, our dataset allows the exploration of multiple stress combinations in neuronal and non-neuronal populations of adult male and female mice. When combined with other recent studies [166], [323], our results provide further molecular and cellular characterization of the hypothalamus and identify several cell types as priorities to explore sex differences in the context of stress. Further investigation of

how many of these differences exist also in the human brain could provide new insights in how we understand the origin of the sex differences in brain structure and function [324].

Our results support the implementation of more single cell resolution studies in females and males and help in the identification of new directions to dissect the molecular processes driving sex differences in normal physiology and stress response. We provide new directions to explore how sex influences cell composition and activation of the PVN and HPA axis for a better understanding of the mechanisms behind cell-type susceptibility to stress and the identification of cell targets for sex-personalized medicine in stress-related psychiatric disorders.

6 | Bibliography

[1] K. A. Liu and N. A. Dipietro Mager, “Women’s involvement in clinical trials: Historical perspective and future implications,” *Pharm. Pract. (Granada).*, vol. 14, no. 1, pp. 1–9, 2016, doi: 10.18549/PharmPract.2016.01.708.

[2] R. N. Hughes, “Sex does matter: comments on the prevalence of male-only investigations of drug effects on rodent behaviour,” *Behav. Pharmacol.*, vol. 18, no. 7, pp. 583–589, Nov. 2007, doi: 10.1097/FBP.0b013e3282eff0e8.

[3] “NIH Policy and Guidelines on The Inclusion of Women and Minorities as Subjects in Clinical Research | grants.nih.gov,” 2001. Accessed: Jun. 22, 2019. [Online]. Available: https://grants.nih.gov/grants/funding/women_min/guidelines.htm

[4] L. R. Miller, C. Marks, J. B. Becker, P. D. Hurn, W. J. Chen, T. Woodruff, M. M. McCarthy, F. Sohrabji, L. Schiebinger, C. Lee Wetherington, S. Makris, A. P. Arnold, G. Einstein, V. M. Miller, K. Sandberg, S. Maier, T. L. Cornelison, and J. A. Clayton, “Considering sex as a biological variable in preclinical research,” *FASEB J.*, vol. 31, no. 1, pp. 29–34, 2017, doi: 10.1096/fj.201600781R.

[5] “NIH Policy on Sex as a Biological Variable | Office of Research on Women’s Health.” https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable (accessed May 26, 2019).

[6] “Accounting for sex and gender makes for better science,” *Nature*, vol. 588, no. 7837, pp. 196–196, Dec. 2020, doi: 10.1038/d41586-020-03459-y.

[7] D. A. Bangasser and B. Wicks, “Sex-specific mechanisms for responding to stress,” *J. Neurosci. Res.*, vol. 95, no. 1–2, pp. 75–82, Jan. 2017, doi: 10.1002/jnr.23812.

[8] M. M. McCarthy, “Sex differences in the developing brain as a source of inherent risk,” *Dialogues Clin. Neurosci.*, vol. 18, no. 4, pp. 361–372, 2016.

[9] Y. Itoh and A. P. Arnold, “Are females more variable than males in gene expression? Meta-analysis of microarray datasets,” *Biol. Sex Differ.*, vol. 6, no. 1, p. 18, Dec. 2015, doi: 10.1186/s13293-015-0036-8.

[10] J. B. Becker, B. J. Prendergast, and J. W. Liang, “Female rats are not more variable than male rats: a meta-analysis of neuroscience studies,” *Biol. Sex Differ.*, vol. 7, no. 1, p. 34, Dec. 2016, doi: 10.1186/s13293-016-0087-5.

[11] B. J. Prendergast, K. G. Onishi, and I. Zucker, “Female mice liberated for inclusion in neuroscience and biomedical research,” *Neurosci. Biobehav. Rev.*, vol. 40, pp. 1–5, Mar. 2014, doi: 10.1016/j.neubiorev.2014.01.001.

[12] A. K. Beery, “Inclusion of females does not increase variability in rodent research studies,” *Curr. Opin. Behav. Sci.*, vol. 23, pp. 143–149, Oct. 2018, doi: 10.1016/j.cobeha.2018.06.016.

[13] R. M. Shansky, “Are hormones a ‘female problem’ for animal research?,” *Science (80-.).*, vol. 364, no. 6443, pp. 825–826, May 2019, doi: 10.1126/science.aaw7570.

[14] S. Dattani, H. Ritchie, and M. Roser, “Mental Health,” *online at OurWorldInData.org*, 2021. https://ourworldindata.org/mental-health

[15] F. Jacobi, M. Höfler, J. Siegert, S. Mack, A. Gerschler, L. Scholl, M. A. Busch, U. Hapke, U. Maske, I. Seiffert, W. Gaebel, W. Maier, M. Wagner, J. Zielasek, and H.-U. Wittchen, “Twelve-month prevalence, comorbidity and correlates of mental disorders in Germany: the Mental Health Module of the German Health Interview and Examination Survey for Adults (DEGS1-MH),” *Int. J. Methods Psychiatr. Res.*, vol. 23, no. 3, pp. 304–319, Sep. 2014, doi: 10.1002/mpr.1439.

[16] H. U. Wittchen, F. Jacobi, J. Rehm, A. Gustavsson, M. Svensson, B. Jönsson, J. Olesen, C. Allgulander, J. Alonso, C. Faravelli, L. Fratiglioni, P. Jennum, R. Lieb, A. Maercker, J.

van Os, M. Preisig, L. Salvador-Carulla, R. Simon, and H.-C. Steinhausen, “The size and burden of mental disorders and other disorders of the brain in Europe 2010,” *Eur. Neuropsychopharmacol.*, vol. 21, no. 9, pp. 655–679, Sep. 2011, doi: 10.1016/j.euroneuro.2011.07.018.

[17] GBD 2019 Mental Disorders Collaborators, “Global, regional, and national burden of 12 mental disorders in 204 countries and territories , 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019,” 2019. Accessed: Oct. 28, 2021. [Online]. Available: http://www.healthdata.org/research-article/global-regional-and-national-burden-12-mental-disorders-204-countries-and

[18] M. GBD 2016 Causes of Death Collaborators, A. A. Abajobir, C. Abbafati, K. M. Abbas, F. Abd-Allah, S. F. Abera, V. Aboyans, O. Adetokunboh, A. Afshin, A. Agrawal, A. Ahmadi, M. B. Ahmed, A. N. Aichour, M. T. E. Aichour, I. Aichour, S. Aiyar, F. Alahdab, Z. Al-Aly, K. Alam, N. Alam, T. Alam, K. A. Alene, A. Al-Eyadhy, S. D. Ali, R. Alizadeh-Navaei, J. M. Alkaabi, A. Alkerwi, F. Alla, P. Allebeck, C. Allen, R. Al-Raddadi, U. Alsharif, K. A. Altirkawi, N. Alvis-Guzman, A. T. Amare, E. Amini, W. Ammar, Y. A. Amoako, N. Anber, H. H. Andersen, C. L. Andrei, S. Androudi, H. Ansari, C. A. T. Antonio, P. Anwari, J. Ärnlöv, M. Arora, A. Artaman, K. K. Aryal, H. Asayesh, S. W. Asgedom, T. M. Atey, L. Avila-Burgos, E. F. G. Avokpaho, A. Awasthi, T. K. Babalola, U. Bacha, K. Balakrishnan, A. Barac, M. A. Barboza, S. L. Barker-Collo, S. Barquera, L. Barregard, L. H. Barrero, B. T. Baune, N. Bedi, E. Beghi, Y. Béjot, B. B. Bekele, M. L. Bell, J. R. Bennett, I. M. Bensenor, A. Berhane, E. Bernabé, B. D. Betsu, M. Beuran, S. Bhatt, S. Biadgilign, K. Bienhoff, B. Bikbov, D. Bisanzio, R. R. A. Bourne, N. J. K. Breitborde, L. N. B. Bulto, B. R. Bumgarner, Z. A. Butt, L. Cahuana-Hurtado, E. Cameron, J. C. Campuzano, J. Car, R. Cárdenas, J. J. Carrero, A. Carter, D. C. Casey, C. A. Castañeda-Orjuela, F. Catalá-López, F. J. Charlson, C. E. Chibueze, O. Chimed-Ochir, V. H. Chisumpa, A. A. Chitheer, D. J. Christopher, L. G. Ciobanu, M. Cirillo, A. J. Cohen, D. Colombara, C. Cooper, B. C. Cowie, M. H. Criqui, L. Dandona, R. Dandona, P. I. Dargan, J. das Neves, D. V Davitoiu, K. Davletov, B. de Courten, B. K. Defo, L. Degenhardt, S. Deiparine, K. Deribe, A. Deribew, S. Dey, D. Dicker, E. L. Ding, S. Djalalinia, H. P. Do, D. T. Doku, D. Douwes-Schultz, T. R. Driscoll, M. Dubey, B. B. Duncan, M. Echko, Z. Z. El-Khatib, C. L. Ellingsen, A. Enayati, S. P. Ermakov, H. E. Erskine, S. Eskandarieh, A. Esteghamati, K. Estep, C. S. e S. Farinha, A. Faro, F. Farzadfar, V. L. Feigin, S.-M. Fereshtehnejad, J. C. Fernandes, A. J. Ferrari, T. R. Feyissa, I. Filip, S. Finegold, F. Fischer, C. Fitzmaurice, A. D. Flaxman, N. Foigt, T. Frank, M. Fraser, N. Fullman, T. Fürst, J. M. Furtado, E. Gakidou, A. L. Garcia-Basteiro, T. Gebre, G. B. Gebregergs, T. T. Gebrehiwot, D. Y. Gebremichael, J. M. Geleijnse, R. Genova-Maleras, H. A. Gesesew, P. W. Gething, R. F. Gillum, A. Z. Giref, M. Giroud, G. Giussani, W. W. Godwin, A. L. Gold, E. M. Goldberg, P. N. Gona, S. V. Gopalani, H. N. Gouda, A. C. Goulart, M. Griswold, R. Gupta, T. Gupta, V. Gupta, P. C. Gupta, J. A. Haagsma, N. Hafezi-Nejad, A. D. Hailu, G. B. Hailu, R. R. Hamadeh, M. T. Hambisa, S. Hamidi, M. Hammami, J. Hancock, A. J. Handal, G. J. Hankey, Y. Hao, H. L. Harb, H. A. Hareri, M. S. Hassanvand, R. Havmoeller, S. I. Hay, F. He, M. T. Hedayati, N. J. Henry, I. B. Heredia-Pi, C. Herteliu, H. W. Hoek, M. Horino, N. Horita, H. D. Hosgood, S. Hostiuc, P. J. Hotez, D. G. Hoy, C. Huynh, K. M. Iburg, C. Ikeda, B. V. Ileanu, A. A. Irenso, C. M. S. Irvine, S. M. S. Islam, K. H. Jacobsen, N. Jahanmehr, M. B. Jakovljevic, M. Javanbakht, S. P. Jayaraman, P. Jeemon, V. Jha, D. John, C. O. Johnson, S. C. Johnson, J. B. Jonas, M. Jürisson, Z. Kabir, R. Kadel, A. Kahsay, R. Kamal, A. Karch, S. M. Karimi, C. Karimkhani, A. Kasaeian, N. A. Kassaw, N. J. Kassebaum, S. V. Katikireddi, N. Kawakami, P. N. Keiyoro, L. Kemmer, C. N. Kesavachandran, Y. S. Khader, E. A. Khan, Y.-H. Khang, A. T. A. Khoja, M. H. Khosravi, A. Khosravi, J. Khubchandani, A. A. Kiadaliri, C. Kieling, D. Kievlan, Y. J. Kim, D. Kim, R. W. Kimokoti, Y. Kinfu, N. Kissoon, M. Kivimaki, A. K. Knudsen, J. A. Kopec, S. Kosen, P. A. Koul, A. Koyanagi, X. R. Kulikoff, G. A. Kumar, P. Kumar, M. Kutz, H. H. Kyu, D. K. Lal, R. Lalloo, T. L. N. Lambert, Q. Lan, V. C. Lansingh, A. Larsson, P. H. Lee, J. Leigh, J. Leung, M. Levi, Y. Li, D. L. Kappe, X. Liang, M. L. Liben, S. S. Lim, P. Y. Liu, A. Liu, Y. Liu, R. Lodha, G. Logroscino, S. Lorkowski, P. A. Lotufo, R. Lozano, T. C. D. Lucas, S. Ma, E. R. K. Macarayan, E. R. Maddison, M. M. A. El Razek, M. Majdan, R. Majdzadeh, A. Majeed, R. Malekzadeh, R. Malhotra, D. C. Malta, H. Manguerra, T. Manyazewal, C. C. Mapoma, L. B. Marczak, D. Markos, J. Martinez-Raga,

F. R. Martins-Melo, I. Martopullo, C. McAlinden, M. McGaughey, J. J. McGrath, S. Mehata, T. Meier, K. G. Meles, P. Memiah, Z. A. Memish, M. M. Mengesha, D. T. Mengistu, B. G. Menota, G. A. Mensah, T. J. Meretoja, A. Meretoja, A. Millear, T. R. Miller, S. Minnig, M. Mirarefin, E. M. Mirrakhimov, A. Misganaw, S. R. Mishra, I. A. Mohamed, K. A. Mohammad, A. Mohammadi, S. Mohammed, A. H. Mokdad, G. L. D. Mola, S. K. Mollenkopf, M. Molokhia, L. Monasta, J. C. Montañez, M. Montico, M. D. Mooney, M. Moradi-Lakeh, P. Moraga, L. Morawska, C. Morozoff, S. D. Morrison, C. Mountjoy-Venning, K. B. Mruts, K. Muller, G. V. S. Murthy, K. I. Musa, J. B. Nachega, A. Naheed, L. Naldi, V. Nangia, B. R. Nascimento, J. T. Nasher, G. Natarajan, I. Negoi, J. W. Ngunjiri, C. T. Nguyen, Q. Le Nguyen, T. H. Nguyen, G. Nguyen, M. Nguyen, E. Nichols, D. N. A. Ningrum, V. M. Nong, J. J. N. Noubiap, F. A. Ogbo, I.-H. Oh, A. Okoro, A. T. Olagunju, H. E. Olsen, B. O. Olusanya, J. O. Olusanya, K. Ong, J. N. Opio, E. Oren, A. Ortiz, M. Osman, E. Ota, M. PA, R. E. Pacella, S. Pakhale, A. Pana, B. K. Panda, S. Panda-Jonas, C. Papachristou, E.-K. Park, S. B. Patten, G. C. Patton, D. Paudel, K. Paulson, D. M. Pereira, F. Perez-Ruiz, N. Perico, A. Pervaiz, M. Petzold, M. R. Phillips, D. M. Pigott, C. Pinho, D. Plass, M. A. Pletcher, S. Polinder, M. J. Postma, F. Pourmalek, C. Purcell, M. Qorbani, B. P. A. Quintanilla, A. Radfar, A. Rafay, V. Rahimi-Movaghar, M. H. U. Rahman, M. Rahman, R. K. Rai, C. L. Ranabhat, Z. Rankin, P. C. Rao, G. K. Rath, S. Rawaf, S. E. Ray, J. Rehm, R. C. Reiner, M. B. Reitsma, G. Remuzzi, S. Rezaei, M. S. Rezai, M. B. Rokni, L. Ronfani, G. Roshandel, G. A. Roth, D. Rothenbacher, G. M. Ruhago, R. SA, S. Saadat, P. S. Sachdev, N. Sadat, M. Safdarian, S. Safi, S. Safiri, R. Sagar, R. Sahathevan, J. Salama, P. Salamati, J. A. Salomon, A. M. Samy, J. R. Sanabria, M. D. Sanchez-Niño, D. Santomauro, I. S. Santos, M. M. S. Milicevic, B. Sartorius, M. Satpathy, M. I. Schmidt, I. J. C. Schneider, S. Schulhofer-Wohl, A. E. Schutte, D. C. Schwebel, F. Schwendicke, S. G. Sepanlou, E. E. Servan-Mori, K. A. Shackelford, S. Shahraz, M. A. Shaikh, M. Shamsipour, M. Shamsizadeh, J. Sharma, R. Sharma, J. She, S. Sheikhbahaei, M. Shey, P. Shi, C. Shields, M. Shigematsu, R. Shiri, S. Shirude, I. Shiue, H. Shoman, M. G. Shrime, I. D. Sigfusdottir, N. Silpakit, J. P. Silva, J. A. Singh, A. Singh, E. Skiadaresi, A. Sligar, D. L. Smith, A. Smith, M. Smith, B. H. A. Sobaih, S. Soneji, R. J. D. Sorensen, J. B. Soriano, C. T. Sreeramareddy, V. Srinivasan, J. D. Stanaway, V. Stathopoulou, N. Steel, D. J. Stein, C. Steiner, S. Steinke, M. A. Stokes, M. Strong, B. Strub, M. Subart, M. B. Sufiyan, B. F. Sunguya, P. J. Sur, S. Swaminathan, B. L. Sykes, R. Tabarés-Seisdedos, S. K. Tadakamadla, K. Takahashi, J. S. Takala, R. T. Talongwa, M. R. Tarawneh, M. Tavakkoli, N. Taveira, T. K. Tegegne, A. Tehrani-Banihashemi, M.-H. Temsah, A. S. Terkawi, J. S. Thakur, O. Thamsuwan, K. R. Thankappan, K. E. Thomas, A. H. Thompson, A. J. Thomson, A. G. Thrift, R. Tobe-Gai, R. Topor-Madry, A. Torre, M. Tortajada, J. A. Towbin, B. X. Tran, C. Troeger, T. Truelsen, D. Tsoi, E. M. Tuzcu, S. Tyrovolas, K. N. Ukwaja, E. A. Undurraga, R. Updike, O. A. Uthman, B. S. C. Uzochukwu, J. F. M. van Boven, T. Vasankari, N. Venketasubramanian, F. S. Violante, V. V. Vlassov, S. E. Vollset, T. Vos, T. Wakayo, M. T. Wallin, Y.-P. Wang, E. Weiderpass, R. G. Weintraub, D. J. Weiss, A. Werdecker, R. Westerman, B. Whetter, H. A. Whiteford, T. Wijeratne, C. S. Wiysonge, B. G. Woldeyes, C. D. A. Wolfe, R. Woodbrook, A. Workicho, D. Xavier, Q. Xiao, G. Xu, M. Yaghoubi, B. Yakob, Y. Yano, M. Yaseri, H. H. Yimam, N. Yonemoto, S.-J. Yoon, M. Yotebieng, M. Z. Younis, Z. Zaidi, M. E. S. Zaki, E. A. Zegeye, Z. M. Zenebe, T. A. Zerfu, A. L. Zhang, X. Zhang, B. Zipkin, S. Zodpey, A. D. Lopez, and C. J. L. Murray, “Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016.,” *Lancet (London, England)*, vol. 390, no. 10100, pp. 1151–1210, Sep. 2017, doi: 10.1016/S0140-6736(17)32152-9.

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Mooney, M. Moradi-Lakeh, P. Moraga, L. Morawska, C. Morozoff, S. D. Morrison, C. Mountjoy-Venning, K. B. Mruts, K. Muller, G. V. S. Murthy, K. I. Musa, J. B. Nachega, A. Naheed, L. Naldi, V. Nangia, B. R. Nascimento, J. T. Nasher, G. Natarajan, I. Negoi, J. W. Ngunjiri, C. T. Nguyen, Q. Le Nguyen, T. H. Nguyen, G. Nguyen, M. Nguyen, E. Nichols, D. N. A. Ningrum, V. M. Nong, J. J. N. Noubiap, F. A. Ogbo, I.-H. Oh, A. Okoro, A. T. Olagunju, H. E. Olsen, B. O. Olusanya, J. O. Olusanya, K. Ong, J. N. Opio, E. Oren, A. Ortiz, M. Osman, E. Ota, M. PA, R. E. Pacella, S. Pakhale, A. Pana, B. K. Panda, S. Panda-Jonas, C. Papachristou, E.-K. Park, S. B. Patten, G. C. Patton, D. Paudel, K. Paulson, D. M. Pereira, F. Perez-Ruiz, N. Perico, A. Pervaiz, M. Petzold, M. R. Phillips, D. M. Pigott, C. Pinho, D. Plass, M. A. Pletcher, S. Polinder, M. J. Postma, F. Pourmalek, C. Purcell, M. Qorbani, B. P. A. Quintanilla, A. Radfar, A. Rafay, V. Rahimi-Movaghar, M. H. U. Rahman, M. Rahman, R. K. Rai, C. 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Ray, J. Rehm, R. C. Reiner, M. B. Reitsma, G. Remuzzi, S. Rezaei, M. S. Rezai, M. B. Rokni, L. Ronfani, G. Roshandel, G. A. Roth, D. Rothenbacher, G. M. Ruhago, R. SA, S. Saadat, P. S. Sachdev, N. Sadat, M. Safdarian, S. Safi, S. Safiri, R. Sagar, R. Sahathevan, J. Salama, P. Salamati, J. A. Salomon, A. M. Samy, J. R. Sanabria, M. D. Sanchez-Niño, D. Santomauro, I. S. Santos, M. M. Santric Milicevic, B. Sartorius, M. Satpathy, M. I. Schmidt, I. J. C. Schneider, S. Schulhofer-Wohl, A. E. Schutte, D. C. Schwebel, F. Schwendicke, S. G. Sepanlou, E. E. Servan-Mori, K. A. Shackelford, S. Shahraz, M. A. Shaikh, M. Shamsipour, M. Shamsizadeh, J. Sharma, R. Sharma, J. She, S. Sheikhbahaei, M. Shey, P. Shi, C. Shields, M. Shigematsu, R. Shiri, S. Shirude, I. Shiue, H. Shoman, M. G. Shrime, I. D. Sigfusdottir, N. Silpakit, J. P. Silva, J. A. Singh, A. Singh, E. Skiadaresi, A. Sligar, D. L. Smith, A. Smith, M. Smith, B. H. A. Sobaih, S. Soneji, R. J. D. Sorensen, J. B. Soriano, C. T. Sreeramareddy, V. Srinivasan, J. D. Stanaway, V. Stathopoulou, N. Steel, D. J. Stein, C. Steiner, S. Steinke, M. A. Stokes, M. Strong, B. Strub, M. Subart, M. B. Sufiyan, B. F. Sunguya, P. J. Sur, S. Swaminathan, B. L. Sykes, R. Tabarés-Seisdedos, S. K. Tadakamadla, K. Takahashi, J. S. Takala, R. T. Talongwa, M. R. Tarawneh, M. Tavakkoli, N. Taveira, T. K. Tegegne, A. Tehrani-Banihashemi, M.-H. Temsah, A. S. Terkawi, J. S. Thakur, O. Thamsuwan, K. R. Thankappan, K. E. Thomas, A. H. Thompson, A. J. Thomson, A. G. Thrift, R. Tobe-Gai, R. Topor-Madry, A. Torre, M. Tortajada, J. A. Towbin, B. X. Tran, C. Troeger, T. Truelsen, D. Tsoi, E. M. Tuzcu, S. Tyrovolas, K. N. Ukwaja, E. A. Undurraga, R. Updike, O. A. Uthman, B. S. C. Uzochukwu, J. F. M. van Boven, T. Vasankari, N. Venketasubramanian, F. S. Violante, V. V. Vlassov, S. E. Vollset, T. Vos, T. Wakayo, M. T. Wallin, Y.-P. Wang, E. Weiderpass, R. G. Weintraub, D. J. Weiss, A. Werdecker, R. Westerman, B. Whetter, H. A. Whiteford, T. Wijeratne, C. S. Wiysonge, B. G. Woldeyes, C. D. A. Wolfe, R. Woodbrook, A. Workicho, D. Xavier, Q. Xiao, G. Xu, M. Yaghoubi, B. Yakob, Y. Yano, M. Yaseri, H. H. Yimam, N. Yonemoto, S.-J. Yoon, M. Yotebieng, M. Z. Younis, Z. Zaidi, M. E. S. Zaki, E. A. Zegeye, Z. M. Zenebe, T. A. Zerfu, A. L. Zhang, X. Zhang, B. Zipkin, S. Zodpey, A. D. Lopez, and C. J. L. Murray, “Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016,” *Lancet*, vol. 390, no. 10100, pp. 1151–1210, Sep. 2017, doi: 10.1016/S0140-6736(17)32152-9.

[20] World Health Organization, “Depression and Other Common Mental Disorders: Global Health Estimates,” World Health Organization, Geneva, 2017. Accessed: Oct. 18, 2017. [Online]. Available: http://www.who.int/mental_health/management/depression/prevalence_global_health_estimates/en/

[21] D. Vigo, G. Thornicroft, and R. Atun, “Estimating the true global burden of mental illness,” *The Lancet Psychiatry*, vol. 3, no. 2, pp. 171–178, Feb. 2016, doi: 10.1016/S2215-0366(15)00505-2.

[22] T. Vos, A. A. Abajobir, C. Abbafati, K. M. Abbas, K. H. Abate, F. Abd-Allah, A. M. Abdulle, T. A. Abebo, S. F. Abera, V. Aboyans, L. J. Abu-Raddad, I. N. Ackerman, A. A. Adamu, O. Adetokunboh, M. Afarideh, A. Afshin, S. K. Agarwal, R. Aggarwal, A. Agrawal, S. Agrawal, A. Ahmad Kiadaliri, H. Ahmadieh, M. B. Ahmed, A. N. Aichour, I. Aichour, M. T. E. Aichour, S. Aiyar, R. O. Akinyemi, N. Akseer, F. H. Al Lami, F. Alahdab, Z. Al-Aly, K. Alam, N. Alam, T. Alam, D. Alasfoor, K. A. Alene, R. Ali, R. Alizadeh-Navaei, A. Alkerwi, F. Alla, P. Allebeck, C. Allen, F. Al-Maskari, R. Al-Raddadi, U. Alsharif, S. Alsowaidi, K. A. Altirkawi, A. T. Amare, E. Amini, W. Ammar, Y. A. Amoako, H. H. Andersen, C. A. T. Antonio, P. Anwari, J. Ärnlöv, A. Artaman, K. K. Aryal, H. Asayesh, S. W. Asgedom, R. Assadi, T. M. Atey, N. T. Atnafu, S. R. Atre, L. Avila-Burgos, E. F. G. A. Avokpaho, A. Awasthi, B. P. Ayala Quintanilla, H. O. Ba Saleem, U. Bacha, A. Badawi, K. Balakrishnan, A. Banerjee, M. S. Bannick, A. Barac, R. M. Barber, S. L. Barker-Collo, T. Bärnighausen, S. Barquera, L. Barregard, L. H. Barrero, S. Basu, B. Battista, K. E. Battle, B. T. Baune, S. Bazargan-Hejazi, J. Beardsley, N. Bedi, E. Beghi, Y. Béjot, B. B. Bekele, M. L. Bell, D. A. Bennett, I. M. Bensenor, J. Benson, A. Berhane, D. F. Berhe, E. Bernabé, B. D. Betsu, M. Beuran, A. S. Beyene, N. Bhala, A. Bhansali, S. Bhatt, Z. A. Bhutta, S. Biadgilign, K. Bienhoff, B. Bikbov, C. Birungi, S. Biryukov, D. Bisanzio, H. M. Bizuayehu, D. J. Boneya,

S. Boufous, R. R. A. A. Bourne, A. Brazinova, T. S. Brugha, R. Buchbinder, L. N. B. Bulto, B. R. Bumgarner, Z. A. Butt, L. Cahuana-Hurtado, E. Cameron, M. Car, H. Carabin, J. R. Carapetis, R. Cárdenas, D. O. Carpenter, J. J. Carrero, A. Carter, F. Carvalho, D. C. Casey, V. Caso, C. A. Castañeda-Orjuela, C. D. Castle, F. Catalá-López, H.-Y. Y. Chang, J.-C. C. Chang, F. J. Charlson, H. Chen, M. Chibalabala, C. E. Chibueze, V. H. Chisumpa, A. A. Chitheer, D. J. Christopher, L. G. Ciobanu, M. Cirillo, D. Colombara, C. Cooper, P. A. Cortesi, M. H. Criqui, J. A. Crump, A. F. Dadi, K. Dalal, L. Dandona, R. Dandona, J. Das Neves, D. V. Davitoiu, B. De Courten, D. De Leo, L. Degenhardt, S. Deiparine, R. P. Dellavalle, K. Deribe, D. C. Des Jarlais, S. Dey, S. D. Dharmaratne, P. K. Dhillon, D. Dicker, E. L. Ding, S. Djalalinia, H. P. Do, E. R. Dorsey, K. P. B. Dos Santos, D. Douwes-Schultz, K. E. Doyle, T. R. Driscoll, M. Dubey, B. B. Duncan, Z. Z. El-Khatib, J. Ellerstrand, A. Enayati, A. Y. 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Hassanvand, R. Havmoeller, C. Hawley, R. J. Hay, S. I. Hay, N. J. Henry, I. B. Heredia-Pi, P. Heydarpour, H. W. Hoek, H. J. Hoffman, N. Horita, H. D. Hosgood, S. Hostiuc, P. J. Hotez, D. G. Hoy, A. S. Htet, G. Hu, H. Huang, C. Huynh, K. M. Iburg, E. U. Igumbor, C. Ikeda, C. M. S. Irvine, K. H. Jacobsen, N. Jahanmehr, M. B. Jakovljevic, S. K. Jassal, M. Javanbakht, S. P. Jayaraman, P. Jeemon, P. N. Jensen, V. Jha, G. Jiang, D. John, C. O. Johnson, S. C. Johnson, J. B. Jonas, M. Jürisson, Z. Kabir, R. Kadel, A. Kahsay, R. Kamal, H. Kan, N. E. Karam, A. Karch, C. K. Karema, A. Kasaeian, G. M. Kassa, N. A. Kassaw, N. J. Kassebaum, A. Kastor, S. V. Katikireddi, A. Kaul, N. Kawakami, P. N. Keiyoro, A. P. Kengne, A. Keren, Y. S. Khader, I. A. Khalil, E. A. Khan, Y.-H. H. Khang, A. Khosravi, J. Khubchandani, C. Kieling, D. Kim, P. Kim, Y. J. Kim, R. W. Kimokoti, Y. Kinfu, A. Kisa, K. A. Kissimova-Skarbek, M. Kivimaki, A. K. Knudsen, Y. Kokubo, D. Kolte, J. A. Kopec, S. Kosen, P. A. Koul, A. Koyanagi, M. Kravchenko, S. Krishnaswami, K. J. Krohn, B. Kuate Defo, B. Kucuk Bicer, G. A. Kumar, P. Kumar, S. Kumar, H. H. Kyu, D. K. Lal, R. Lalloo, N. Lambert, Q. Lan, A. Larsson, P. M. Lavados, J. L. Leasher, J.-T. T. Lee, P. H. Lee, J. Leigh, C. T. Leshargie, J. Leung, R. Leung, M. Levi, Y. Y. Li, Y. Y. Li, D. Li Kappe, X. Liang, M. L. Liben, S. S. Lim, S. Linn, A. Liu, P. Y. Liu, S. Liu, Y. Liu, R. Lodha, G. Logroscino, S. J. London, K. J. Looker, A. D. Lopez, S. Lorkowski, P. A. Lotufo, N. Low, R. Lozano, T. C. D. D. Lucas, E. R. K. Macarayan, H. Magdy Abd El Razek, M. Magdy Abd El Razek, M. Mahdavi, M. Majdan, R. Majdzadeh, A. Majeed, R. Malekzadeh, R. Malhotra, D. C. Malta, A. A. Mamun, H. Manguerra, T. Manhertz, A. Mantilla, L. G. Mantovani, C. C. Mapoma, L. B. Marczak, J. Martinez-Raga, F. R. Martins-Melo, I. Martopullo, W. März, M. R. Mathur, M. Mazidi, C. McAlinden, M. McGaughey, J. J. McGrath, M. McKee, C. McNellan, S. Mehata, M. M. Mehndiratta, T. C. Mekonnen, P. Memiah, Z. A. Memish, W. Mendoza, M. A. Mengistie, D. T. Mengistu, G. A. Mensah, A. Meretoja, T. J. Meretoja, H. B. Mezgebe, R. Micha, A. Millear, T. R. Miller, E. J. Mills, M. Mirarefin, E. M. Mirrakhimov, A. Misganaw, S. R. Mishra, P. B. Mitchell, K. A. Mohammad, A. Mohammadi, K. E. Mohammed, S. Mohammed, S. K. Mohanty, A. H. Mokdad, S. K. Mollenkopf, L. Monasta, J. M. Hernandez, M. Montico, M. Moradi-Lakeh, P. Moraga, R. Mori, C. Morozoff, S. D. Morrison, M. Moses, C. Mountjoy-Venning, K. B. Mruts, U. O. Mueller, K. Muller, M. E. Murdoch, G. V. S. Murthy, K. I. Musa, J. B. Nachega, G. Nagel, M. Naghavi, A. Naheed, K. S. Naidoo, L. Naldi, V. Nangia, G. Natarajan, D. E. Negasa, I. Negoi, R. I. Negoi, C. R. Newton, J. W. Ngunjiri, C. T. Nguyen, G. Nguyen, M. Nguyen, Q. Le Nguyen, T. H. Nguyen, E. Nichols, D. N. A. Ningrum, S. Nolte, V. M. Nong, B. Norrving, J. J. N. Noubiap, M. J. O'Donnell, F. A. Ogbo, I.-H. H. Oh, A. Okoro, O. Oladimeji, A. T. Olagunju, T. O. Olagunju, H. E. Olsen, B. O. Olusanya, J. O. Olusanya, K. Ong, J. N. Opio, E. Oren, A. Ortiz, A. Osgood-Zimmerman, M. Osman, M. O. Owolabi,

M. Pa, R. E. Pacella, A. Pana, B. K. Panda, C. Papachristou, E.-K. K. Park, C. D. Parry, M. Parsaeian, S. B. Patten, G. C. Patton, K. Paulson, N. Pearce, D. M. Pereira, N. Perico, K. Pesudovs, C. B. Peterson, M. Petzold, M. R. Phillips, D. M. Pigott, J. D. Pillay, C. Pinho, D. Plass, M. A. Pletcher, S. Popova, R. G. Poulton, F. Pourmalek, D. Prabhakaran, N. M. N. Prasad, N. M. N. Prasad, C. Purcell, M. Qorbani, R. Quansah, R. H. S. S. Rabiee, A. Radfar, A. Rafay, K. Rahimi, A. Rahimi-Movaghar, V. Rahimi-Movaghar, M. M. H. U. M. Rahman, M. M. H. U. M. Rahman, R. K. Rai, S. Rajsic, U. Ram, C. L. Ranabhat, Z. Rankin, P. C. P. V. P. C. Rao, P. C. P. V. P. C. Rao, S. Rawaf, S. E. Ray, R. C. Reiner, N. Reinig, M. B. Reitsma, G. Remuzzi, A. M. N. N. Renzaho, S. Resnikoff, S. Rezaei, A. L. Ribeiro, L. Ronfani, G. Roshandel, G. A. Roth, A. Roy, E. Rubagotti, G. M. Ruhago, S. Saadat, N. Sadat, M. Safdarian, S. Safi, S. Safiri, R. Sagar, R. Sahathevan, J. Salama, J. A. Salomon, S. S. Salvi, A. M. Samy, J. R. Sanabria, D. Santomauro, I. S. Santos, J. V. Santos, M. M. Santric Milicevic, B. Sartorius, M. Satpathy, M. Sawhney, S. Saxena, M. I. Schmidt, I. J. C. C. Schneider, B. Schöttker, D. C. Schwebel, F. Schwendicke, S. Seedat, S. G. Sepanlou, E. E. Servan-Mori, T. Setegn, K. A. Shackelford, A. Shaheen, M. A. Shaikh, M. Shamsipour, S. M. Shariful Islam, J. Sharma, R. Sharma, J. She, P. Shi, C. Shields, M. Shigematsu, Y. Shinohara, R. Shiri, R. Shirkoohi, S. Shirude, K. Shishani, M. G. Shrime, A. M. Sibai, I. D. Sigfusdottir, D. A. S. Silva, J. P. Silva, D. G. A. Silveira, J. A. Singh, N. P. Singh, D. N. Sinha, E. Skiadaresi, V. Skirbekk, E. L. Slepak, A. Sligar, D. L. Smith, M. Smith, B. H. A. A. Sobaih, E. Sobngwi, R. J. D. D. Sorensen, T. C. M. Sousa, L. A. Sposato, C. T. Sreeramareddy, V. Srinivasan, J. D. Stanaway, V. Stathopoulou, N. Steel, D. J. Stein, M. B. Stein, C. Steiner, T. J. Steiner, S. Steinke, M. A. Stokes, L. J. Stovner, B. Strub, M. Subart, M. B. Sufiyan, R. Suliankatchi Abdulkader, B. F. Sunguya, P. J. Sur, S. Swaminathan, B. L. Sykes, D. O. Sylte, R. Tabarés-Seisdedos, G. R. Taffere, J. S. Takala, N. Tandon, M. Tavakkoli, N. Taveira, H. R. Taylor, A. Tehrani-Banihashemi, T. Tekelab, G. Temam Shifa, A. S. Terkawi, D. J. Tesfaye, B. Tesssema, O. Thamsuwan, K. E. Thomas, A. G. Thrift, T. Y. Tiruye, R. Tobe-Gai, M. C. Tollanes, M. Tonelli, R. Topor-Madry, M. Tortajada, M. Touvier, B. X. Tran, S. Tripathi, C. Troeger, T. Truelsen, D. Tsoi, K. B. Tuem, E. M. Tuzcu, S. Tyrovolas, K. N. Ukwaja, E. A. Undurraga, C. J. Uneke, R. Updike, O. A. Uthman, B. S. C. C. Uzochukwu, J. F. M. Van Boven, S. Varughese, T. Vasankari, S. Venkatesh, N. Venketasubramanian, R. Vidavalur, F. S. Violante, S. K. Vladimirov, V. V. Vlassov, S. E. Vollset, F. Wadilo, T. Wakayo, Y.-P. P. Wang, M. Weaver, S. Weichenthal, E. Weiderpass, R. G. Weintraub, A. Werdecker, R. Westerman, H. A. Whiteford, T. Wijeratne, C. S. Wiysonge, C. D. A. A. Wolfe, R. Woodbrook, A. D. Woolf, A. Workicho, S. Wulf Hanson, D. Xavier, G. Xu, S. Yadgir, M. Yaghoubi, B. Yakob, L. L. Yan, Y. Yano, P. Ye, H. H. Yimam, P. Yip, N. Yonemoto, S.-J. J. Yoon, M. Yotebieng, M. Z. Younis, Z. Zaidi, M. E. S. Zaki, E. A. Zegeye, Z. M. Zenebe, X. Zhang, M. Zhou, B. Zipkin, S. Zodpey, L. J. Zuhlke, C. J. L. L. Murray, T. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, A. A. Abajobir, K. H. Abate, C. Abbafati, K. M. Abbas, F. Abd-Allah, R. S. Abdulkader, A. M. Abdulle, T. A. Abebo, S. F. Abera, V. Aboyans, L. J. Abu-Raddad, I. N. Ackerman, A. A. Adamu, O. Adetokunboh, M. Afarideh, A. Afshin, S. K. Agarwal, R. Aggarwal, A. Agrawal, S. Agrawal, H. Ahmadieh, M. B. Ahmed, M. T. E. Aichour, A. N. Aichour, I. Aichour, S. Aiyar, R. O. Akinyemi, N. Akseer, F. H. Al Lami, F. Alahdab, Z. Al-Aly, K. Alam, N. Alam, T. Alam, D. Alasfoor, K. A. Alene, R. Ali, R. Alizadeh-Navaei, A. Alkerwi, F. Alla, P. Allebeck, C. Allen, F. Al-Maskari, R. Al-Raddadi, U. Alsharif, S. Alsowaidi, K. A. Altirkawi, A. T. Amare, E. Amini, W. Ammar, Y. A. Amoako, H. H. Andersen, C. A. T. Antonio, P. Anwari, J. Ärnlöv, A. Artaman, K. K. Aryal, H. Asayesh, S. W. Asgedom, R. Assadi, T. M. Atey, N. T. Atnafu, S. R. Atre, L. Avila-Burgos, E. F. G. A. Avokphako, A. Awasthi, U. Bacha, A. Badawi, K. Balakrishnan, A. Banerjee, M. S. Bannick, A. Barac, R. M. Barber, S. L. Barker-Collo, T. Bärnighausen, S. Barquera, L. Barregard, L. H. Barrero, S. Basu, B. Battista, K. E. Battle, B. T. Baune, S. Bazargan-Hejazi, J. Beardsley, N. Bedi, E. Beghi, Y. Béjot, B. B. Bekele, M. L. Bell, D. A. Bennett, I. M. Bensenor, J. Benson, A. Berhane, D. F. Berhe, E. Bernabé, B. D. Betsu, M. Beuran, A. S. Beyene, N. Bhala, A. Bhansali, S. Bhatt, Z. A. Bhutta, S. Biadgilign, B. K. Bicer, K. Bienhoff, B. Bikbov, C. Birungi, S. Biryukov, D. Bisanzio, H. M. Bizuayehu, D. J. Boneya, S. Boufous, R. R. A. A. Bourne, A. Brazinova, T. S. Brugha, R. Buchbinder, L. N. B. Bulto, B. R. Bumgarner, Z. A. Butt, L. Cahuana-Hurtado, E. Cameron, M. Car, H. Carabin, J. R. Carapetis, R. Cárdenas, D. O. Carpenter, J. J. Carrero, A. Carter, F. Carvalho, D. C.

Casey, V. Caso, C. A. Castañeda-Orjuela, C. D. Castle, F. Catalá-López, H.-Y. Y. Chang, J.-C. C. Chang, F. J. Charlson, H. Chen, M. Chibalabala, C. E. Chibueze, V. H. Chisumpa, A. A. Chitheer, D. J. Christopher, L. G. Ciobanu, M. Cirillo, D. Colombara, C. Cooper, P. A. Cortesi, M. H. Criqui, J. A. Crump, A. F. Dadi, K. Dalal, L. Dandona, R. Dandona, J. das Neves, D. V. Davitoiu, B. de Courten, D. D. De Leo, B. K. Defo, L. Degenhardt, S. Deiparine, R. P. Dellavalle, K. Deribe, D. C. Des Jarlais, S. Dey, S. D. Dharmaratne, P. K. Dhillon, D. Dicker, E. L. Ding, S. Djalalinia, H. P. Do, E. R. Dorsey, K. P. B. dos Santos, D. Douwes-Schultz, K. E. Doyle, T. R. Driscoll, M. Dubey, B. B. Duncan, Z. Z. El-Khatib, J. Ellerstrand, A. Enayati, A. Y. Endries, S. P. Ermakov, H. E. Erskine, B. Eshrati, S. Eskandarieh, A. Esteghamati, K. Estep, F. B. B. Fanuel, C. S. E. S. S. Farinha, A. Faro, F. Farzadfar, M. S. Fazeli, V. L. Feigin, S.-M. M. Fereshtehnejad, J. C. Fernandes, A. J. Ferrari, T. R. Feyissa, I. Filip, F. Fischer, C. Fitzmaurice, A. D. Flaxman, L. S. Flor, N. Foigt, K. J. Foreman, R. C. Franklin, N. Fullman, T. Fürst, J. M. Furtado, N. D. Futran, E. Gakidou, M. Ganji, A. L. Garcia-Basteiro, T. Gebre, T. T. Gebrehiwot, A. Geleto, B. L. Gemechu, H. A. Gesesew, P. W. Gething, A. Ghajar, K. B. Gibney, P. S. Gill, R. F. Gillum, I. A. M. Ginawi, A. Z. Z. Giref, M. D. Gishu, G. Giussani, W. W. Godwin, A. L. Gold, E. M. Goldberg, P. N. Gona, A. Goodridge, S. V. Gopalani, A. Goto, A. C. Goulart, M. Griswold, H. C. Gugnani, R. R. Gupta, R. R. Gupta, T. Gupta, V. Gupta, N. Hafezi-Nejad, G. B. Hailu, A. D. Hailu, R. R. Hamadeh, S. Hamidi, A. J. Handal, G. J. Hankey, S. W. Hanson, Y. Hao, H. L. Harb, H. A. Hareri, J. M. Haro, J. Harvey, M. S. Hassanvand, R. Havmoeller, C. Hawley, S. I. Hay, R. J. Hay, N. J. Henry, I. B. Heredia-Pi, J. M. Hernandez, P. Heydarpour, H. W. Hoek, H. J. Hoffman, N. Horita, H. D. Hosgood, S. Hostiuc, P. J. Hotez, D. G. Hoy, A. S. Htet, G. Hu, H. Huang, C. Huynh, K. M. Iburg, E. U. Igumbor, C. Ikeda, C. M. S. Irvine, K. H. Jacobsen, N. Jahanmehr, M. B. Jakovljevic, S. K. Jassal, M. Javanbakht, S. P. Jayaraman, P. Jeemon, P. N. Jensen, V. Jha, G. Jiang, D. John, S. C. Johnson, C. O. Johnson, J. B. Jonas, M. Jürisson, Z. Kabir, R. Kadel, A. Kahsay, R. Kamal, H. Kan, N. E. Karam, A. Karch, C. K. Karema, A. Kasaeian, G. M. Kassa, N. A. Kassaw, N. J. Kassebaum, A. Kastor, S. V. Katikireddi, A. Kaul, N. Kawakami, P. N. Keiyoro, A. P. Kengne, A. Keren, Y. S. Khader, I. A. Khalil, E. A. Khan, Y.-H. H. Khang, A. Khosravi, J. Khubchandani, A. A. Kiadaliri, C. Kieling, Y. J. Kim, D. Kim, P. Kim, R. W. Kimokoti, Y. Kinfu, A. Kisa, K. A. Kissimova-Skarbek, M. Kivimaki, A. K. Knudsen, Y. Kokubo, D. Kolte, J. A. Kopec, S. Kosen, P. A. Koul, A. Koyanagi, M. Kravchenko, S. Krishnaswami, K. J. Krohn, G. A. Kumar, P. Kumar, S. Kumar, H. H. Kyu, D. K. Lal, R. Lalloo, N. Lambert, Q. Lan, A. Larsson, P. M. Lavados, J. L. Leasher, P. H. Lee, J.-T. T. Lee, J. Leigh, C. T. Leshargie, J. Leung, R. Leung, M. Levi, Y. Y. Li, Y. Y. Li, D. L. Kappe, X. Liang, M. L. Liben, S. S. Lim, S. Linn, P. Y. Liu, A. Liu, S. Liu, Y. Liu, R. Lodha, G. Logroscino, S. J. London, K. J. Looker, A. D. Lopez, S. Lorkowski, P. A. Lotufo, N. Low, R. Lozano, T. C. D. D. Lucas, E. R. K. Macarayan, H. M. A. El Razek, M. M. A. El Razek, M. Mahdavi, M. Majdan, R. Majdzadeh, A. Majeed, R. Malekzadeh, R. Malhotra, D. C. Malta, A. A. Mamun, H. Manguerra, T. Manhertz, A. Mantilla, L. G. Mantovani, C. C. Mapoma, L. B. Marczak, J. Martinez-Raga, F. R. Martins-Melo, I. Martopullo, W. März, M. R. Mathur, M. Mazidi, C. McAlinden, M. McGaughey, J. J. McGrath, M. McKee, C. McNellan, S. Mehata, M. M. Mehndiratta, T. C. Mekonnen, P. Memiah, Z. A. Memish, W. Mendoza, M. A. Mengistie, D. T. Mengistu, G. A. Mensah, T. J. Meretoja, A. Meretoja, H. B. Mezgebe, R. Micha, A. Millear, T. R. Miller, E. J. Mills, M. Mirarefin, E. M. Mirrakhimov, A. Misganaw, S. R. Mishra, P. B. Mitchell, K. A. Mohammad, A. Mohammadi, K. E. Mohammed, S. Mohammed, S. K. Mohanty, A. H. Mokdad, S. K. Mollenkopf, L. Monasta, M. Montico, M. Moradi-Lakeh, P. Moraga, R. Mori, C. Morozoff, S. D. Morrison, M. Moses, C. Mountjoy-Venning, K. B. Mruts, U. O. Mueller, K. Muller, M. E. Murdoch, G. V. S. Murthy, K. I. Musa, J. B. Nachega, G. Nagel, M. Naghavi, A. Naheed, K. S. Naidoo, L. Naldi, V. Nangia, G. Natarajan, D. E. Negasa, R. I. Negoi, I. Negoi, C. R. Newton, J. W. Ngunjiri, T. H. Nguyen, Q. Le Nguyen, C. T. Nguyen, G. Nguyen, M. Nguyen, E. Nichols, D. N. A. Ningrum, S. Nolte, V. M. Nong, B. Norrving, J. J. N. Noubiap, M. J. O'Donnell, F. A. Ogbo, I.-H. H. Oh, A. Okoro, O. Oladimeji, T. O. Olagunju, A. T. Olagunju, H. E. Olsen, B. O. Olusanya, J. O. Olusanya, K. Ong, J. N. Opio, E. Oren, A. Ortiz, A. Osgood-Zimmerman, M. Osman, M. O. Owolabi, M. Pa, R. E. Pacella, A. Pana, B. K. Panda, C. Papachristou, E.-K. K. Park, C. D. Parry, M. Parsaeian, S. B. Patten, G. C. Patton, K. Paulson, N. Pearce, D. M. Pereira, N. Perico, K. Pesudovs, C. B. Peterson, M. Petzold, M. R. Phillips, D. M. Pigott, J. D. Pillay, C. Pinho,

D. Plass, M. A. Pletcher, S. Popova, R. G. Poulton, F. Pourmalek, D. Prabhakaran, N. M. N. Prasad, N. M. N. Prasad, C. Purcell, M. Qorbani, R. Quansah, B. P. A. Quintanilla, R. H. S. S. Rabiee, A. Radfar, A. Rafay, K. Rahimi, A. Rahimi-Movaghar, V. Rahimi-Movaghar, M. M. H. U. M. Rahman, M. M. H. U. M. Rahman, R. K. Rai, S. Rajsic, U. Ram, C. L. Ranabhat, Z. Rankin, P. C. P. V. P. C. Rao, P. C. P. V. P. C. Rao, S. Rawaf, S. E. Ray, R. C. Reiner, N. Reinig, M. B. Reitsma, G. Remuzzi, A. M. N. N. Renzaho, S. Resnikoff, S. Rezaei, A. L. Ribeiro, L. Ronfani, G. Roshandel, G. A. Roth, A. Roy, E. Rubagotti, G. M. Ruhago, S. Saadat, N. Sadat, M. Safdarian, S. Safi, S. Safiri, R. Sagar, R. Sahathevan, J. Salama, H. O. B. Saleem, J. A. Salomon, S. S. Salvi, A. M. Samy, J. R. Sanabria, D. Santomauro, I. S. Santos, J. V. Santos, M. M. S. Milicevic, B. Sartorius, M. Satpathy, M. Sawhney, S. Saxena, M. I. Schmidt, I. J. C. C. Schneider, B. Schöttker, D. C. Schwebel, F. Schwendicke, S. Seedat, S. G. Sepanlou, E. E. Servan-Mori, T. Setegn, K. A. Shackelford, A. Shaheen, M. A. Shaikh, M. Shamsipour, S. M. S. Islam, J. Sharma, R. Sharma, J. She, P. Shi, C. Shields, G. T. Shifa, M. Shigematsu, Y. Shinohara, R. Shiri, R. Shirkoohi, S. Shirude, K. Shishani, M. G. Shrime, A. M. Sibai, I. D. Sigfusdottir, D. A. S. Silva, J. P. Silva, D. G. A. Silveira, J. A. Singh, N. P. Singh, D. N. Sinha, E. Skiadaresi, V. Skirbekk, E. L. Slepak, A. Sligar, D. L. Smith, M. Smith, B. H. A. A. Sobaih, E. Sobngwi, R. J. D. D. Sorensen, T. C. M. Sousa, L. A. Sposato, C. T. Sreeramareddy, V. Srinivasan, J. D. Stanaway, V. Stathopoulou, N. Steel, M. B. Stein, D. J. Stein, T. J. Steiner, C. Steiner, S. Steinke, M. A. Stokes, L. J. Stovner, B. Strub, M. Subart, M. B. Sufiyan, B. F. Sunguya, P. J. Sur, S. Swaminathan, B. L. Sykes, D. O. Sylte, R. Tabarés-Seisdedos, G. R. Taffere, J. S. Takala, N. Tandon, M. Tavakkoli, N. Taveira, H. R. Taylor, A. Tehrani-Banihashemi, T. Tekelab, A. S. Terkawi, D. J. Tesfaye, B. Tesssema, O. Thamsuwan, K. E. Thomas, A. G. Thrift, T. Y. Tiruye, R. Tobe-Gai, M. C. Tollanes, M. Tonelli, R. Topor-Madry, M. Tortajada, M. Touvier, B. X. Tran, S. Tripathi, C. Troeger, T. Truelsen, D. Tsoi, K. B. Tuem, E. M. Tuzcu, S. Tyrovolas, K. N. Ukwaja, E. A. Undurraga, C. J. Uneke, R. Updike, O. A. Uthman, B. S. C. C. Uzochukwu, J. F. M. van Boven, S. Varughese, T. Vasankari, S. Venkatesh, N. Venketasubramanian, R. Vidavalur, F. S. Violante, S. K. Vladimirov, V. V. Vlassov, S. E. Vollset, F. Wadilo, T. Wakayo, Y.-P. P. Wang, M. Weaver, S. Weichenthal, E. Weiderpass, R. G. Weintraub, A. Werdecker, R. Westerman, H. A. Whiteford, T. Wijeratne, C. S. Wiysonge, C. D. A. A. Wolfe, R. Woodbrook, A. D. Woolf, A. Workicho, D. Xavier, G. Xu, S. Yadgir, M. Yaghoubi, B. Yakob, L. L. Yan, Y. Yano, P. Ye, H. H. Yimam, P. Yip, N. Yonemoto, S.-J. J. Yoon, M. Yotebieng, M. Z. Younis, Z. Zaidi, M. E. S. Zaki, E. A. Zegeye, Z. M. Zenebe, X. Zhang, M. Zhou, B. Zipkin, S. Zodpey, L. J. Zuhlke, and C. J. L. L. Murray, "Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016," *Lancet*, vol. 390, no. 10100, pp. 1211–1259, Sep. 2017, doi: 10.1016/S0140-6736(17)32154-2.

[23] J. M. Simmons, L. Winsky, J. L. Zehr, and J. A. Gordon, "Priorities in stress research: a view from the U.S. National Institute of Mental Health," *Stress*, vol. 24, no. 2, pp. 123–129, Mar. 2021, doi: 10.1080/10253890.2020.1781084.

[24] E. Assary, J. P. Vincent, R. Keers, and M. Pluess, "Gene-environment interaction and psychiatric disorders: Review and future directions," *Semin. Cell Dev. Biol.*, vol. 77, pp. 133–143, 2018, doi: 10.1016/j.semcdb.2017.10.016.

[25] D. Joel and M. M. McCarthy, "Incorporating Sex As a Biological Variable in Neuropsychiatric Research: Where Are We Now and Where Should We Be?," *Neuropsychopharmacology*, vol. 42, no. 2, pp. 379–385, Jan. 2017, doi: 10.1038/npp.2016.79.

[26] M. M. McCarthy, "Multifaceted origins of sex differences in the brain," *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 371, no. 1688, 2016, doi: 10.1098/rstb.2015.0106.

[27] L. A. Martin, H. W. Neighbors, and D. M. Griffith, "The Experience of Symptoms of Depression in Men vs Women," *JAMA Psychiatry*, vol. 70, no. 10, p. 1100, Oct. 2013, doi: 10.1001/jamapsychiatry.2013.1985.

[28] M. Altemus, N. Sarvaiya, and C. Neill Epperson, "Sex differences in anxiety and depression clinical perspectives," *Front. Neuroendocrinol.*, vol. 35, no. 3, pp. 320–330,

2014, doi: 10.1016/j.yfrne.2014.05.004.

[29] L. Y. Maeng and M. R. Milad, “Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones,” *Horm. Behav.*, vol. 76, pp. 106–117, Nov. 2015, doi: 10.1016/j.yhbeh.2015.04.002.

[30] M. H. J. Bekker and J. van Mens-Verhulst, “Anxiety Disorders: Sex Differences in Prevalence, Degree, and Background, But Gender-Neutral Treatment,” *Gend. Med.*, vol. 4, no. Suppl. B, pp. S178–S193, Jan. 2007, doi: 10.1016/S1550-8579(07)80057-X.

[31] T. Vos, S. S. Lim, C. Abbafati, K. M. Abbas, M. Abbasi, M. Abbasifard, M. Abbasi-Kangevari, H. Abbastabar, F. Abd-Allah, A. Abdelalim, M. Abdollahi, I. Abdollahpour, H. Abolhassani, V. Aboyans, E. M. Abrams, L. G. Abreu, M. R. M. Abrigo, L. J. Abu-Raddad, A. I. Abushouk, A. Acebedo, I. N. Ackerman, M. Adabi, A. A. Adamu, O. M. Adebayo, V. Adekanmbi, J. D. Adelson, O. O. Adetokunboh, D. Adham, M. Afshari, A. Afshin, E. E. Agardh, G. Agarwal, K. M. Agesa, M. Aghaali, S. M. K. Aghamir, A. Agrawal, T. Ahmad, A. Ahmadi, M. Ahmadi, H. Ahmadieh, E. Ahmadpour, T. Y. Akalu, R. O. Akinyemi, T. Akinyemiju, B. Akombi, Z. Al-Aly, K. Alam, N. Alam, S. Alam, T. Alam, T. M. Alanzi, S. B. Albertson, J. E. Alcalde-Rabanal, N. M. Alema, M. Ali, S. Ali, G. Alicandro, M. Alijanzadeh, C. Alinia, V. Alipour, S. M. Aljunid, F. Alla, P. Allebeck, A. Almasi-Hashiani, J. Alonso, R. M. Al-Raddadi, K. A. Altirkawi, N. Alvis-Guzman, N. J. Alvis-Zakzuk, S. Amini, M. Amini-Rarani, A. Aminorroaya, F. Amiri, A. M. L. Amit, D. A. Amugsi, G. G. H. Amul, D. Anderlini, C. L. Andrei, T. Andrei, M. Anjomshoa, F. Ansari, I. Ansari, A. Ansari-Moghaddam, C. A. T. Antonio, C. M. Antony, E. Antriyandarti, D. Anvari, R. Anwer, J. Arabloo, M. Arab-Zozani, A. Y. Aravkin, F. Ariani, J. Ärnlöv, K. K. Aryal, A. Arzani, M. Asadi-Aliabadi, A. A. Asadi-Pooya, B. Asghari, C. Ashbaugh, D. D. Atnafu, S. R. Atre, F. Ausloos, M. Ausloos, B. P. A. Quintanilla, G. Ayano, M. A. Ayanore, Y. A. Aynalem, S. Azari, G. Azarian, Z. N. Azene, E. Babaee, A. Badawi, M. Bagherzadeh, M. H. Bakhshaei, A. Bakhtiari, S. Balakrishnan, S. Balalla, S. Balassyano, M. Banach, P. C. Banik, M. S. Bannick, A. B. Bante, A. G. Baraki, M. A. Barboza, S. L. Barker-Collo, C. M. Barthelemy, L. Barua, A. Barzegar, S. Basu, B. T. Baune, M. Bayati, G. Bazmandegan, N. Bedi, E. Beghi, Y. Béjot, A. K. Bello, R. G. Bender, D. A. Bennett, F. B. Bennitt, I. M. Bensenor, C. P. Benziger, K. Berhe, E. Bernabe, G. J. Bertolacci, R. Bhageerathy, N. Bhala, D. Bhandari, P. Bhardwaj, K. Bhattacharyya, Z. A. Bhutta, S. Bibi, M. H. Biehl, B. Bikbov, M. S. Bin Sayeed, A. Biondi, B. M. Birihane, D. Bisanzio, C. Bisignano, R. K. Biswas, S. Bohlouli, M. Bohluli, S. R. R. Bolla, A. Boloor, A. S. Boon-Dooley, G. Borges, A. M. Borzì, R. Bourne, O. J. Brady, M. Brauer, C. Brayne, N. J. K. Breitborde, H. Brenner, P. S. Briant, A. M. Briggs, N. I. Briko, G. B. Britton, D. Bryazka, R. Buchbinder, B. R. Bumgarner, R. Busse, Z. A. Butt, F. L. C. dos Santos, L. L. A. Cámera, I. R. Campos-Nonato, J. Car, R. Cárdenas, G. Carreras, J. J. Carrero, F. Carvalho, J. M. Castaldelli-Maia, C. A. Castañeda-Orjuela, G. Castelpietra, C. D. Castle, F. Castro, F. Catalá-López, K. Causey, C. R. Cederroth, K. M. Cercy, E. Cerin, J. S. Chandan, A. R. Chang, F. J. Charlson, V. K. Chattu, S. Chaturvedi, O. Chimed-Ochir, K. L. Chin, D. Y. Cho, H. Christensen, D.-T. Chu, M. T. Chung, F. M. Cicuttini, L. G. Ciobanu, M. Cirillo, E. L. Collins, K. Compton, S. Conti, P. A. Cortesi, V. M. Costa, E. Cousin, R. G. Cowden, B. C. Cowie, E. A. Cromwell, D. H. Cross, C. S. Crowe, J. A. Cruz, M. Cunningham, S. M. A. Dahlawi, G. Damiani, L. Dandona, R. Dandona, A. M. Darwesh, A. Daryani, J. K. Das, R. Das Gupta, J. das Neves, C. A. Dávila-Cervantes, K. Davletov, D. De Leo, F. E. Dean, N. K. DeCleene, A. Deen, L. Degenhardt, R. P. Dellavalle, F. M. Demeke, D. G. Demsie, E. Denova-Gutiérrez, N. D. Dereje, N. Dervenis, R. Desai, A. Desalew, G. A. Dessie, S. D. Dharmaratne, G. P. Dhungana, M. Dianatinasab, D. Diaz, Z. S. D. Forooshani, Z. V Dingels, M. A. Dirac, S. Djalalinia, H. T. Do, K. Dokova, F. Dorostkar, C. P. Doshi, L. Doshmangir, A. Douiri, M. C. Doxey, T. R. Driscoll, S. J. Dunachie, B. B. Duncan, A. R. Duraes, A. W. Eagan, M. E. Kalan, D. Edvardsson, J. R. Ehrlich, N. El Nahas, I. El Sayed, M. El Tantawi, I. Elbarazi, I. Y. Elgendy, H. R. Elhabashy, S. I. El-Jaafary, I. R. Elyazar, M. H. Emamian, S. Emmons-Bell, H. E. Erskine, B. Eshrati, S. Eskandarieh, S. Esmaeilnejad, F. Esmaeilzadeh, A. Esteghamati, K. Estep, A. Etemadi, A. E. Etisso, M. Farahmand, A. Faraj, M. Fareed, R. Faridnia, C. S. e S. Farinha, A. Farioli, A. Faro, M. Faruque, F. Farzadfar, N. Fattahi, M. Fazlzadeh, V. L. Feigin, R. Feldman, S.-M. Fereshtehnejad, E. Fernandes, A. J. Ferrari, M. L. Ferreira, I. Filip, F. Fischer, J. L.

Fisher, R. Fitzgerald, C. Flohr, L. S. Flor, N. A. Foigt, M. O. Folayan, L. M. Force, C. Fornari, M. Foroutan, J. T. Fox, M. Freitas, W. Fu, T. Fukumoto, J. M. Furtado, M. M. Gad, E. Gakidou, N. C. Galles, S. Gallus, A. Gamkrelidze, A. L. Garcia-Basteiro, W. M. Gardner, B. S. Geberemariyam, A. M. Gebrehiwot, K. B. Gebremedhin, A. A. A. A. Gebreslassie, A. G. Hayoon, P. W. Gething, M. Ghadimi, K. Ghadiri, M. Ghafourifard, A. Ghajar, F. Ghamari, A. Ghashghaee, H. Ghiasvand, N. Ghith, A. Gholamian, S. A. Gilani, P. S. Gill, M. Gitimoghaddam, G. Giussani, S. Goli, R. S. Gomez, S. V. Gopalani, G. Gorini, T. M. Gorman, H. C. Gottlich, H. Goudarzi, A. C. Goulart, B. N. G. Goulart, A. Grada, M. Grivna, G. Grosso, M. I. M. Gubari, H. C. Gugnani, A. L. S. Guimaraes, R. A. Guimarães, R. A. Guled, G. Guo, Y. Guo, R. Gupta, J. A. Haagsma, B. Haddock, N. Hafezi-Nejad, A. Hafiz, H. Hagins, L. M. Haile, B. J. Hall, I. Halvaei, R. R. Hamadeh, K. H. Abdullah, E. B. Hamilton, C. Han, H. Han, G. J. Hankey, J. M. Haro, J. D. Harvey, A. I. Hasaballah, A. Hasanzadeh, M. Hashemian, S. Hassanipour, H. Hassankhani, R. J. Havmoeller, R. J. Hay, S. I. Hay, K. Hayat, B. Heidari, G. Heidari, R. Heidari-Soureshjani, D. Hendrie, H. J. Henrikson, N. J. Henry, C. Herteliu, F. Heydarpour, T. R. Hird, H. W. Hoek, M. K. Hole, R. Holla, P. Hoogar, H. D. Hosgood, M. Hosseinzadeh, M. Hostiuc, S. Hostiuc, M. Househ, D. G. Hoy, M. Hsairi, V. C. Hsieh, G. Hu, T. M. Huda, F. N. Hugo, C. K. Huynh, B.-F. Hwang, V. C. Iannucci, S. E. Ibitoye, K. S. Ikuta, O. S. Ilesanmi, I. M. Ilic, M. D. Ilic, L. R. Inbaraj, H. Ippolito, S. S. N. Irvani, M. M. Islam, M. Islam, S. M. S. Islam, F. Islami, H. Iso, R. Q. Ivers, C. C. D. Iwu, I. O. Iyamu, J. Jaafari, K. H. Jacobsen, F. Jadidi-Niaragh, H. Jafari, M. Jafarinia, D. Jahagirdar, M. A. Jahani, N. Jahanmehr, M. Jakovljevic, A. Jalali, F. Jalilian, S. L. James, H. Janjani, M. D. Janodia, A. U. Jayatilleke, P. Jeemon, E. Jenabi, R. P. Jha, V. Jha, J. S. Ji, P. Jia, O. John, Y. O. John-Akinola, C. O. Johnson, S. C. Johnson, J. B. Jonas, T. Joo, A. Joshi, J. J. Jozwiak, M. Jürisson, A. Kabir, Z. Kabir, H. Kalani, R. Kalani, L. R. Kalankesh, R. Kalhor, Z. Kamiab, T. Kanchan, B. K. Matin, A. Karch, M. A. Karim, S. E. Karimi, G. M. Kassa, N. J. Kassebaum, S. V. Katikireddi, N. Kawakami, G. A. Kayode, S. H. Keddie, C. Keller, M. Kereselidze, M. A. Khafaie, N. Khalid, M. Khan, K. Khatab, M. M. Khater, M. N. Khatib, M. Khayamzadeh, M. T. Khodayari, R. Khundkar, N. Kianipour, C. Kieling, D. Kim, Y.-E. Kim, Y. J. Kim, R. W. Kimokoti, A. Kisa, S. Kisa, K. Kissimova-Skarbek, M. Kivimäki, C. J. Kneib, A. K. S. Knudsen, J. M. Kocarnik, T. Kolola, J. A. Kopec, S. Kosen, P. A. Koul, A. Koyanagi, M. A. Kravchenko, K. Krishan, K. J. Krohn, B. K. Defo, B. K. Bicer, G. A. Kumar, M. Kumar, P. Kumar, V. Kumar, G. Kumaresh, O. P. Kurmi, D. Kusuma, H. H. Kyu, C. La Vecchia, B. Lacey, D. K. Lal, R. Lalloo, J. O. Lam, F. H. Lami, I. Landires, J. J. Lang, V. C. Lansingh, S. L. Larson, A. O. Larsson, S. Lasrado, Z. S. Lassi, K. M.-M. Lau, P. M. Lavados, J. V Lazarus, J. R. Ledesma, P. H. Lee, S. W. H. Lee, K. E. LeGrand, J. Leigh, M. Leonardi, H. Lescinsky, J. Leung, M. Levi, S. Lewington, S. Li, L.-L. Lim, C. Lin, R.-T. Lin, C. Linehan, S. Linn, H.-C. Liu, S. Liu, Z. Liu, K. J. Looker, A. D. Lopez, P. D. Lopukhov, S. Lorkowski, P. A. Lotufo, T. C. D. Lucas, A. Lugo, R. Lunevicius, R. A. Lyons, J. Ma, J. H. MacLachlan, E. R. Maddison, R. Maddison, F. Madotto, P. W. Mahasha, H. T. Mai, A. Majeed, V. Maled, S. Maleki, R. Malekzadeh, D. C. Malta, A. A. Mamun, A. Manafi, N. Manafi, H. Manguerra, B. Mansouri, M. A. Mansournia, A. M. M. Herrera, J. C. Maravilla, A. Marks, F. R. Martins-Melo, I. Martopullo, S. Z. Masoumi, J. Massano, B. B. Massenburg, M. R. Mathur, P. K. Maulik, C. McAlinden, J. J. McGrath, M. McKee, M. M. Mehndiratta, F. Mehri, K. M. Mehta, W. B. Meitei, P. T. N. Memiah, W. Mendoza, R. G. Menezes, E. W. Mengesha, M. B. Mengesha, A. Mereke, A. Meretoja, T. J. Meretoja, T. Mestrovic, B. Miazgowski, T. Miazgowski, I. M. Michalek, K. M. Mihretie, T. R. Miller, E. J. Mills, A. Mirica, E. M. Mirrakhimov, H. Mirzaei, M. Mirzaei, M. Mirzaei-Alavijeh, A. T. Misganaw, P. Mithra, B. Moazen, M. Moghadaszadeh, E. Mohamadi, D. K. Mohammad, Y. Mohammad, N. M. G. Mezerji, A. Mohammadian-Hafshejani, N. Mohammadifard, R. Mohammadpourhodki, S. Mohammed, A. H. Mokdad, M. Molokhia, N. C. Momen, L. Monasta, S. Mondello, M. D. Mooney, M. Moosazadeh, G. Moradi, M. Moradi, M. Moradi-Lakeh, R. Moradzadeh, P. Moraga, L. Morales, L. Morawska, I. M. Velásquez, J. Morgado-da-Costa, S. D. Morrison, J. F. Mosser, S. Mouodi, S. M. Mousavi, A. M. Khaneghah, U. O. Mueller, S. B. Munro, M. K. Muriithi, K. I. Musa, S. Muthupandian, M. Naderi, A. J. Nagarajan, G. Nagel, B. Naghshtabrizi, S. Nair, A. K. Nandi, V. Nangia, J. R. Nansseu, V. C. Nayak, J. Nazari, I. Negoi, R. I. Negoi, H. B. N. Netsere, J. W. Ngunjiri, C. T. Nguyen, J. Nguyen, M. Nguyen, M. Nguyen, E. Nichols, D. Nigatu, Y. T. Nigatu, R. Nikbakhsh, M. R. Nixon, C. A. Nnaji, S.

Nomura, B. Norrving, J. J. Noubiap, C. Nowak, V. Nunez-Samudio, A. Oţoiu, B. Oancea, C. M. Odell, F. A. Ogbo, I.-H. Oh, E. W. Okunga, M. Oladnabi, A. T. Olagunju, B. O. Olusanya, J. O. Olusanya, M. M. Oluwasanu, A. O. Bali, M. O. Omer, K. L. Ong, O. E. Onwujekwe, A. U. Orji, H. M. Orpana, A. Ortiz, S. M. Ostroff, N. Otstavnov, S. S. Otstavnov, S. Øverland, M. O. Owolabi, M. P. A, J. R. Padubidri, A. P. Pakhare, R. Palladino, A. Pana, S. Panda-Jonas, A. Pandey, E.-K. Park, P. G. K. Parmar, D. K. Pasupula, S. K. Patel, A. J. Paternina-Caicedo, A. Pathak, M. Pathak, S. B. Patten, G. C. Patton, D. Paudel, H. P. Toroudi, A. E. Peden, A. Pennini, V. C. F. Pepito, E. K. Peprah, A. Pereira, D. M. Pereira, N. Perico, H. Q. Pham, M. R. Phillips, D. M. Pigott, T. Pilgrim, T. M. Pilz, M. Pirsaheb, O. Plana-Ripoll, D. Plass, K. N. Pokhrel, R. V Polibin, S. Polinder, K. R. Polkinghorne, M. J. Postma, H. Pourjafar, F. Pourmalek, R. P. Kalhori, A. Pourshams, A. Poznańska, S. I. Prada, V. Prakash, D. R. A. Pribadi, E. Pupillo, Z. Q. Syed, M. Rabiee, N. Rabiee, A. Radfar, A. Rafiee, A. Rafiei, A. Raggi, A. Rahimi-Movaghar, M. A. Rahman, A. Rajabpour-Sanati, F. Rajati, K. Ramezanzadeh, C. L. Ranabhat, P. C. Rao, S. J. Rao, D. Rasella, P. Rastogi, P. Rathi, D. L. Rawaf, S. Rawaf, L. Rawal, C. Razo, S. B. Redford, R. C. Reiner, N. Reinig, M. B. Reitsma, G. Remuzzi, V. Renjith, A. M. N. Renzaho, S. Resnikoff, N. Rezaei, M. sadegh Rezai, A. Rezapour, P.-A. Rhinehart, S. M. Riahi, A. L. P. Ribeiro, D. C. Ribeiro, D. Ribeiro, J. Rickard, N. L. S. Roberts, S. Roberts, S. R. Robinson, L. Roever, S. Rolfe, L. Ronfani, G. Roshandel, G. A. Roth, E. Rubagotti, S. F. Rumisha, S. Sabour, P. S. Sachdev, B. Saddik, E. Sadeghi, M. Sadeghi, S. Saeidi, S. Safi, S. Safiri, R. Sagar, A. Sahebkar, M. A. Sahraian, S. M. Sajadi, M. R. Salahshoor, P. Salamati, S. S. Zahabi, H. Salem, M. R. R. Salem, H. Salimzadeh, J. A. Salomon, I. Salz, Z. Samad, A. M. Samy, J. Sanabria, D. F. Santomauro, I. S. Santos, J. V. Santos, M. M. Santric-Milicevic, S. Y. I. Saraswathy, R. Sarmiento-Suárez, N. Sarrafzadegan, B. Sartorius, A. Sarveazad, B. Sathian, T. Sathish, D. Sattin, A. N. Sbarra, L. E. Schaeffer, S. Schiavolin, M. I. Schmidt, A. E. Schutte, D. C. Schwebel, F. Schwendicke, A. M. Senbeta, S. Senthilkumaran, S. G. Sepanlou, K. A. Shackelford, J. Shadid, S. Shahabi, A. A. Shaheen, M. A. Shaikh, A. S. Shalash, M. Shams-Beyranvand, M. Shamsizadeh, M. Shannawaz, K. Sharafi, F. Sharara, B. S. Sheena, A. Sheikhtaheri, R. S. Shetty, K. Shibuya, W. S. Shiferaw, M. Shigematsu, J. Il Shin, R. Shiri, R. Shirkoohi, M. G. Shrime, K. Shuval, S. Siabani, I. D. Sigfusdottir, R. Sigurvinsdottir, J. P. Silva, K. E. Simpson, A. Singh, J. A. Singh, E. Skiadaresi, S. T. S. Skou, V. Y. Skryabin, E. Sobngwi, A. Sokhan, S. Soltani, R. J. D. Sorensen, J. B. Soriano, M. B. Sorrie, I. N. Soyiri, C. T. Sreeramareddy, J. D. Stanaway, B. A. Stark, S. C. Ştefan, C. Stein, C. Steiner, T. J. Steiner, M. A. Stokes, L. J. Stovner, J. L. Stubbs, A. Sudaryanto, M. B. Sufiyan, G. Sulo, I. Sultan, B. L. Sykes, D. O. Sylte, M. Szócska, R. Tabarés-Seisdedos, K. M. Tabb, S. K. Tadakamadla, A. Taherkhani, M. Tajdini, K. Takahashi, N. Taveira, W. L. Teagle, H. Teame, A. Tehrani-Banihashemi, B. F. Teklehaimanot, S. Terrason, Z. T. Tessema, K. R. Thankappan, A. M. Thomson, H. R. Tohidinik, M. Tonelli, R. Topor-Madry, A. E. Torre, M. Touvier, M. R. R. Tovani-Palone, B. X. Tran, R. Travillian, C. E. Troeger, T. C. Truelsen, A. C. Tsai, A. Tsatsakis, L. T. Car, S. Tyrovolas, R. Uddin, S. Ullah, E. A. Undurraga, B. Unnikrishnan, M. Vacante, A. Vakilian, P. R. Valdez, S. Varughese, T. J. Vasankari, Y. Vasseghian, N. Venketasubramanian, F. S. Violante, V. Vlassov, S. E. Vollset, A. Vongpradith, A. Vukovic, R. Vukovic, Y. Waheed, M. K. Walters, J. Wang, Y. Wang, Y.-P. Wang, J. L. Ward, A. Watson, J. Wei, R. G. Weintraub, D. J. Weiss, J. Weiss, R. Westerman, J. L. Whisnant, H. A. Whiteford, T. Wiangkham, K. E. Wiens, T. Wijeratne, L. B. Wilner, S. Wilson, B. Wojtyniak, C. D. A. Wolfe, E. E. Wool, A.-M. Wu, S. W. Hanson, H. Y. Wunrow, G. Xu, R. Xu, S. Yadgir, S. H. Y. Jabbari, K. Yamagishi, M. Yaminfirooz, Y. Yano, S. Yaya, V. Yazdi-Feyzabadi, J. A. Yearwood, T. Y. Yeheyis, Y. G. Yeshitila, P. Yip, N. Yonemoto, S.-J. Yoon, J. Y. Lebni, M. Z. Younis, T. P. Younker, Z. Yousefi, M. Yousefifard, T. Yousefinezhadi, A. Y. Yousuf, C. Yu, H. Yusefzadeh, T. Z. Moghadam, L. Zaki, S. Bin Zaman, M. Zamani, M. Zamanian, H. Zandian, A. Zangeneh, M. S. Zastrozhin, K. A. Zewdie, Y. Zhang, Z.-J. Zhang, J. T. Zhao, Y. Zhao, P. Zheng, M. Zhou, A. Ziapour, S. R. M. Zimsen, M. Naghavi, and C. J. L. Murray, “Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019,” *Lancet*, vol. 396, no. 10258, pp. 1204–1222, Oct. 2020, doi: 10.1016/S0140-6736(20)30925-9.

[32] A. R. Gobinath, E. Choleris, and L. A. M. Galea, “Sex, hormones, and genotype interact to influence psychiatric disease, treatment, and behavioral research,” *J. Neurosci. Res.*, vol. 95, no. 1–2, pp. 50–64, 2017, doi: 10.1002/jnr.23872.

[33] T. A. LeGates, M. D. Kvarta, and S. M. Thompson, “Sex differences in antidepressant efficacy,” *Neuropsychopharmacology*, vol. 44, no. 1. Nature Publishing Group, pp. 140–154, Jan. 01, 2019. doi: 10.1038/s41386-018-0156-z.

[34] N. Kokras and C. Dalla, “Preclinical sex differences in depression and antidepressant response: Implications for clinical research,” *J. Neurosci. Res.*, vol. 95, no. 1–2, pp. 731–736, Jan. 2017, doi: 10.1002/jnr.23861.

[35] J. J. Sramek, M. F. Murphy, and N. R. Cutler, “Sex differences in the psychopharmacological treatment of depression,” *Dialogues Clin. Neurosci.*, vol. 18, no. 4, pp. 447–457, Dec. 2016, Accessed: Nov. 08, 2017. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/28179816

[36] S. G. Kornstein, A. F. Schatzberg, M. E. Thase, K. A. Yonkers, J. P. McCullough, G. I. Keitner, A. J. Gelenberg, S. M. Davis, W. M. Harrison, and M. B. Keller, “Gender Differences in Treatment Response to Sertraline Versus Imipramine in Chronic Depression,” *Am. J. Psychiatry*, vol. 157, no. 9, pp. 1445–1452, Sep. 2000, doi: 10.1176/appi.ajp.157.9.1445.

[37] B. K. Chen, V. M. Luna, C. T. LaGamma, X. Xu, S.-X. Deng, R. F. Suckow, T. B. Cooper, A. Shah, R. A. Brachman, I. Mendez-David, D. J. David, A. M. Gardier, D. W. Landry, and C. A. Denny, “Sex-specific neurobiological actions of prophylactic (R,S)-ketamine, (2R,6R)-hydroxynorketamine, and (2S,6S)-hydroxynorketamine,” *Neuropsychopharmacology*, vol. 45, no. 9, pp. 1545–1556, Aug. 2020, doi: 10.1038/s41386-020-0714-z.

[38] C. L. Hyde, M. W. Nagle, C. Tian, X. Chen, S. A. Paciga, J. R. Wendland, J. Y. Tung, D. A. Hinds, R. H. Perlis, and A. R. Winslow, “Identification of 15 genetic loci associated with risk of major depression in individuals of European descent,” *Nat. Genet.*, vol. 48, no. 9, pp. 1031–1036, Aug. 2016, doi: 10.1038/ng.3623.

[39] A. K. Merikangas and L. Almasy, “Using the tools of genetic epidemiology to understand sex differences in neuropsychiatric disorders,” *Genes, Brain Behav.*, vol. 19, no. 6, Jul. 2020, doi: 10.1111/gbb.12660.

[40] J. Martin, K. Tammimies, R. Karlsson, Y. Lu, H. Larsson, P. Lichtenstein, and P. K. E. Magnusson, “Copy number variation and neuropsychiatric problems in females and males in the general population,” *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.*, vol. 180, no. 6, pp. 341–350, Sep. 2019, doi: 10.1002/AJMG.B.32685.

[41] F. R. Wendt, G. A. Pathak, D. S. Tylee, A. Goswami, and R. Polimanti, “Heterogeneity and Polygenicity in Psychiatric Disorders: A Genome-Wide Perspective,” *Chronic Stress*, vol. 4, 2020, doi: 10.1177/2470547020924844.

[42] J. P. Herman, “Regulation of the HPA Axis by Acute and Chronic Stress,” in *Encyclopedia of Behavioral Neuroscience*, Elsevier, 2010, pp. 149–153. doi: 10.1016/B978-0-08-045396-5.00230-X.

[43] S. M. Smith and W. W. Vale, “The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress,” *Dialogues Clin. Neurosci.*, vol. 8, no. 4, pp. 383–395, 2006, doi: 10.31887/dcns.2006.8.4/ssmith.

[44] M. G. Oyola and R. J. Handa, “Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: sex differences in regulation of stress responsivity,” *Stress*, vol. 20, no. 5. pp. 476–494, Sep. 03, 2017. doi: 10.1080/10253890.2017.1369523.

[45] C. Rivier and W. Vale, “Effects of corticotropin-releasing factor, neurohypophyseal peptides, and catecholamines on pituitary function.,” *Fed. Proc.*, vol. 44, no. 1 Pt 2, pp. 189–95, Jan. 1985, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/2981740

[46] W. Vale, J. Spiess, C. Rivier, and J. Rivier, “Characterization of a 41-residue ovine

hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin," *Science (80-.).*, vol. 213, no. 4514, pp. 1394–1397, Sep. 1981, doi: 10.1126/science.6267699.

[47] J. M. Deussing and A. Chen, "The Corticotropin-Releasing Factor Family: Physiology of the Stress Response," *Physiol. Rev.*, vol. 98, no. 4, pp. 2225–2286, Oct. 2018, doi: 10.1152/physrev.00042.2017.

[48] M. E. Keller-Wood and M. F. Dallman, "Corticosteroid inhibition of ACTH secretion," *Endocr. Rev.*, vol. 5, no. 1, pp. 1–24, 1984, doi: 10.1210/edrv-5-1-1.

[49] B. S. McEwen and P. J. Gianaros, "Stress- and Allostasis-Induced Brain Plasticity," *Annu. Rev. Med.*, vol. 62, no. 1, pp. 431–445, Feb. 2011, doi: 10.1146/annurev-med-052209-100430.

[50] E. R. de Kloet, M. Joëls, and F. Holsboer, "Stress and the brain: from adaptation to disease," *Nat. Rev. Neurosci.*, vol. 6, no. 6, pp. 463–475, Jun. 2005, doi: 10.1038/nrn1683.

[51] B. S. McEwen, "Protective and Damaging Effects of Stress Mediators," *N. Engl. J. Med.*, vol. 338, no. 3, pp. 171–179, Jan. 1998, doi: 10.1056/NEJM199801153380307.

[52] A. O'Donovan, G. M. Slavich, E. S. Epel, and T. C. Neylan, "Exaggerated neurobiological sensitivity to threat as a mechanism linking anxiety with increased risk for diseases of aging," *Neurosci. Biobehav. Rev.*, vol. 37, no. 1, pp. 96–108, Jan. 2013, doi: 10.1016/j.neubiorev.2012.10.013.

[53] L. D. Godoy, M. T. Rossignoli, P. Delfino-Pereira, N. Garcia-Cairasco, and E. H. de L. Umeoka, "A comprehensive overview on stress neurobiology: Basic concepts and clinical implications," *Frontiers in Behavioral Neuroscience*, vol. 12. Frontiers Media S.A., p. 127, Jul. 03, 2018. doi: 10.3389/fnbeh.2018.00127.

[54] J. P. Herman, M. M. Ostrander, N. K. Mueller, and H. Figueiredo, "Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis," *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, vol. 29, no. 8, pp. 1201–1213, Dec. 2005, doi: 10.1016/j.pnpbp.2005.08.006.

[55] D. A. Bangasser and R. J. Valentino, "Sex differences in stress-related psychiatric disorders: Neurobiological perspectives," *Front. Neuroendocrinol.*, vol. 35, no. 3, pp. 303–319, Aug. 2014, doi: 10.1016/j.yfrne.2014.03.008.

[56] J. P. Herman, H. Figueiredo, N. K. Mueller, Y. Ulrich-Lai, M. M. Ostrander, D. C. Choi, and W. E. Cullinan, "Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness.," *Front. Neuroendocrinol.*, vol. 24, no. 3, pp. 151–80, Jul. 2003, Accessed: Jul. 30, 2019. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/14596810

[57] L. Musazzi, P. Tornese, N. Sala, and M. Popoli, "Acute or Chronic? A Stressful Question," *Trends Neurosci.*, vol. 40, no. 9, pp. 525–535, Sep. 2017, doi: 10.1016/j.tins.2017.07.002.

[58] P. Sheldon Cohen, P. Denise Janicki-Deverts, and P. Gregory E. Miller, "Psychological Stress and Disease," *J. Am. Med. Assoc.*, vol. 298, no. 14, pp. 1685–1687, 2007, [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/17925521/

[59] K. S. Kendler, L. M. Thornton, and C. A. Prescott, "Gender Differences in the Rates of Exposure to Stressful Life Events and Sensitivity to Their Depressogenic Effects," *Am. J. Psychiatry*, vol. 158, no. 4, pp. 587–593, Apr. 2001, doi: 10.1176/appi.ajp.158.4.587.

[60] J. M. Breton, M. Barraza, K. Y. Hu, S. Joy Frias, K. L. Long, and D. Kaufer, "Juvenile exposure to acute traumatic stress leads to long-lasting alterations in grey matter myelination in adult female but not male rats," *bioRxiv*, p. 2020.12.14.422686, Dec. 2020, doi: 10.1101/2020.12.14.422686.

[61] C. J. Peña, M. Smith, A. Ramakrishnan, H. M. Cates, R. C. Bagot, H. G. Kronman, B. Patel, A. B. Chang, I. Purushothaman, J. Dudley, H. Morishita, L. Shen, and E. J. Nestler, "Early life stress alters transcriptomic patterning across reward circuitry in male and female mice," *Nat. Commun.*, vol. 10, no. 1, p. 5098, Dec. 2019, doi: 10.1038/s41467-019-13085-

6.

[62] M. Di Segni, D. Andolina, and R. Ventura, “Long-term effects of early environment on the brain: Lesson from rodent models,” *Semin. Cell Dev. Biol.*, vol. 77, pp. 81–92, May 2018, doi: 10.1016/j.semcdb.2017.09.039.

[63] J. I. Herzog and C. Schmahl, “Adverse Childhood Experiences and the Consequences on Neurobiological, Psychosocial, and Somatic Conditions Across the Lifespan,” *Front. Psychiatry*, vol. 9, p. 420, Sep. 2018, doi: 10.3389/fpsyt.2018.00420.

[64] C. Heim, M. Shugart, W. E. Craighead, and C. B. Nemeroff, “Neurobiological and psychiatric consequences of child abuse and neglect,” *Dev. Psychobiol.*, vol. 52, no. 7, pp. 671–690, Nov. 2010, doi: 10.1002/dev.20494.

[65] S. Maccari, D. Polese, M.-L. Reynaert, T. Amici, S. Morley-Fletcher, and F. Fagioli, “Early-life experiences and the development of adult diseases with a focus on mental illness: The Human Birth Theory,” *Neuroscience*, vol. 342, pp. 232–251, Feb. 2017, doi: 10.1016/j.neuroscience.2016.05.042.

[66] C. Heim and E. B. Binder, “Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics,” *Exp. Neurol.*, vol. 233, no. 1, pp. 102–111, Jan. 2012, doi: 10.1016/J.EXPNEUROL.2011.10.032.

[67] C. Heim, T. Mletzko, D. Purselle, D. L. Musselman, and C. B. Nemeroff, “The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma,” *Biol. Psychiatry*, vol. 63, no. 4, pp. 398–405, Feb. 2008, doi: 10.1016/J.BIOPSYCH.2007.07.002.

[68] M. Fatima, S. Srivastav, and A. C. Mondal, “Prenatal stress and depression associated neuronal development in neonates,” *Int. J. Dev. Neurosci.*, vol. 60, pp. 1–7, Aug. 2017, doi: 10.1016/j.ijdevneu.2017.04.001.

[69] B. R. H. Van den Bergh, M. I. van den Heuvel, M. Lahti, M. Braeken, S. R. de Rooij, S. Entringer, D. Hoyer, T. Roseboom, K. Räikkönen, S. King, and M. Schwab, “Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy,” *Neurosci. Biobehav. Rev.*, Jul. 2017, doi: 10.1016/j.neubiorev.2017.07.003.

[70] M. R. Infurna, C. Reichl, P. Parzer, A. Schimmenti, A. Bifulco, and M. Kaess, “Associations between depression and specific childhood experiences of abuse and neglect: A meta-analysis,” *J. Affect. Disord.*, vol. 190, pp. 47–55, Jan. 2016, doi: 10.1016/J.JAD.2015.09.006.

[71] M. W. Enns, B. J. Cox, and I. Clara, “Parental bonding and adult psychopathology: results from the US National Comorbidity Survey,” *Psychol. Med.*, vol. 32, no. 6, pp. 997–1008, Aug. 2002, doi: 10.1017/S0033291702005937.

[72] A. Bifulco, P. M. Moran, R. Baines, A. Bunn, and K. Stanford, “Exploring psychological abuse in childhood: II. Association with other abuse and adult clinical depression,” *Bull. Menninger Clin.*, vol. 66, no. 3, pp. 241–258, Jun. 2002, doi: 10.1521/BUMC.66.3.241.23366.

[73] C. B. Dyer, V. N. Pavlik, K. P. Murphy, and D. J. Hyman, “The High Prevalence of Depression and Dementia in Elder Abuse or Neglect,” *J. Am. Geriatr. Soc.*, vol. 48, no. 2, pp. 205–208, Feb. 2000, doi: 10.1111/J.1532-5415.2000.TB03913.X.

[74] M. J. Farah, “The Neuroscience of Socioeconomic Status: Correlates, Causes, and Consequences,” *Neuron*, vol. 96, no. 1, pp. 56–71, Sep. 2017, doi: 10.1016/j.neuron.2017.08.034.

[75] A. Freeman, S. Tyrovolas, A. Koyanagi, S. Chatterji, M. Leonardi, J. L. Ayuso-Mateos, B. Tobiasz-Adamczyk, S. Koskinen, C. Rummel-Kluge, and J. M. Haro, “The role of socio-economic status in depression: Results from the COURAGE (aging survey in Europe),” *BMC Public Health*, vol. 16, no. 1, pp. 1–8, 2016, doi: 10.1186/s12889-016-3638-0.

[76] J. Hoebel, U. E. Maske, H. Zeeb, and T. Lampert, “Social inequalities and depressive symptoms in adults: The role of objective and subjective socioeconomic status,” *PLoS One*, vol. 12, no. 1, pp. 1–18, 2017, doi: 10.1371/journal.pone.0169764.

[77] B. S. McEwen and P. J. Gianaros, “Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease,” *Ann. N. Y. Acad. Sci.*, vol. 1186, no. 1, pp. 190–222, Feb. 2010, doi: 10.1111/j.1749-6632.2009.05331.x.

[78] D. Shaked, M. Williams, M. K. Evans, and A. B. Zonderman, “Indicators of subjective social status: Differential associations across race and sex,” *SSM - Popul. Heal.*, vol. 2, no. May, pp. 700–707, Dec. 2016, doi: 10.1016/j.ssmph.2016.09.009.

[79] K. Wetherall, K. A. Robb, and R. C. O’Connor, “Social rank theory of depression: A systematic review of self-perceptions of social rank and their relationship with depressive symptoms and suicide risk,” *J. Affect. Disord.*, vol. 246, pp. 300–319, Mar. 2019, doi: 10.1016/j.jad.2018.12.045.

[80] S. Checkley, “Neuroendocrine Mechanisms and the Precipitation of Depression by Life Events,” *Br. J. Psychiatry*, vol. 160, no. S15, pp. 7–17, Feb. 1992, doi: 10.1192/S0007125000296633.

[81] H. M. Van Praag, “Can stress cause depression?,” *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, vol. 28, no. 5, pp. 891–907, Aug. 2004, doi: 10.1016/j.pnpbp.2004.05.031.

[82] R. C. Kessler, “The effects of stressful life events on depression,” *Annu. Rev. Psychol.*, vol. 48, no. 1, pp. 191–214, Feb. 1997, doi: 10.1146/annurev.psych.48.1.191.

[83] C. Kubo, “Stress and depression,” *Nippon rinsho. Japanese J. Clin. Med.*, vol. 65, no. 9, pp. 1706–1709, 2007, doi: 10.1146/annurev.clinpsy.1.102803.143938.

[84] C. Hammen, “Generation of Stress in the Course of Unipolar Depression,” *J. Abnorm. Psychol.*, vol. 100, no. 4, pp. 555–561, 1991, doi: 10.1037/0021-843X.100.4.555.

[85] M. Weger and C. Sandi, “High anxiety trait: A vulnerable phenotype for stress-induced depression,” *Neurosci. Biobehav. Rev.*, vol. 87, pp. 27–37, Apr. 2018, doi: 10.1016/j.neubiorev.2018.01.012.

[86] A. B. Levine, L. M. Levine, and T. B. Levine, “Posttraumatic Stress Disorder and Cardiometabolic Disease,” *Cardiology*, vol. 127, no. 1, pp. 1–19, Dec. 2014, doi: 10.1159/000354910.

[87] N. Goel, J. L. Workman, T. T. Lee, L. Innala, and V. Viau, “Sex differences in the HPA axis,” *Compr. Physiol.*, vol. 4, no. 3, pp. 1121–1155, Jun. 2014, doi: 10.1002/cphy.c130054.

[88] E. McCrory, S. A. De Brito, and E. Viding, “The link between child abuse and psychopathology: A review of neurobiological and genetic research,” *J. R. Soc. Med.*, vol. 105, no. 4, pp. 151–156, Apr. 2012, doi: 10.1258/jrsm.2011.110222.

[89] L. Musazzi, P. Tornese, N. Sala, and M. Popoli, “What Acute Stress Protocols Can Tell Us About PTSD and Stress-Related Neuropsychiatric Disorders,” *Front. Pharmacol.*, vol. 9, p. 758, Jul. 2018, doi: 10.3389/fphar.2018.00758.

[90] B. M. Kudielka and C. Kirschbaum, “Sex differences in HPA axis responses to stress: A review,” *Biol. Psychol.*, vol. 69, no. 1 SPEC. ISS., pp. 113–132, Apr. 2005, doi: 10.1016/j.biopsycho.2004.11.009.

[91] T. L. Bale and C. N. Epperson, “Sex differences and stress across the lifespan,” *Nat Neurosci.*, vol. 18, no. 10, pp. 1413–1420, Oct. 2015, doi: 10.1038/nn.4112.Sex.

[92] L. Arborelius, M. J. Owens, P. M. Plotsky, and C. B. Nemeroff, “The role of corticotropin-releasing factor in depression and anxiety disorders,” *J. Endocrinol.*, vol. 160, no. 1, pp. 1–12, 1999, doi: 10.1677/JOE.0.1600001.

[93] N. Goel and T. L. Bale, “Examining the intersection of sex and stress in modelling

neuropsychiatric disorders," *J. Neuroendocrinol.*, vol. 21, no. 4, pp. 415–420, Mar. 2009, doi: 10.1111/j.1365-2826.2009.01843.x.

[94] S. G. Kornstein, "Gender differences in depression: implications for treatment.," *J. Clin. Psychiatry*, vol. 58 Suppl 1, pp. 12–8, 1997, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/9427872

[95] D. Kaul, S. G. Schwab, N. Mechawar, and N. Matosin, "How stress physically re-shapes the brain: Impact on brain cell shapes, numbers and connections in psychiatric disorders," *Neurosci. Biobehav. Rev.*, vol. 124, pp. 193–215, May 2021, doi: 10.1016/j.neubiorev.2021.01.025.

[96] R. S. Eid, A. R. Gobinath, and L. A. M. Galea, "Sex differences in depression: Insights from clinical and preclinical studies," *Prog. Neurobiol.*, vol. 176, no. December 2018, pp. 86–102, 2019, doi: 10.1016/j.pneurobio.2019.01.006.

[97] B. Planchez, A. Surget, and C. Belzung, "Animal models of major depression: drawbacks and challenges," *J. Neural Transm.*, vol. 126, no. 11, pp. 1383–1408, Nov. 2019, doi: 10.1007/s00702-019-02084-y.

[98] J. R. Scarpa, M. Fatma, Y.-H. E. Loh, S. R. Traore, T. Stefan, T. H. Chen, E. J. Nestler, and B. Labonté, "Shared Transcriptional Signatures in Major Depressive Disorder and Mouse Chronic Stress Models," *Biol. Psychiatry*, vol. 88, no. 2, pp. 159–168, Jul. 2020, doi: 10.1016/j.biopsych.2019.12.029.

[99] M. Q. Steinman and B. C. Trainor, "Sex differences in the effects of social defeat on brain and behavior in the California mouse: Insights from a monogamous rodent," *Semin. Cell Dev. Biol.*, vol. 61, pp. 92–98, Jan. 2017, doi: 10.1016/j.semcdb.2016.06.021.

[100] G. E. Hodes and C. N. Epperson, "Sex Differences in Vulnerability and Resilience to Stress Across the Life Span," *Biol. Psychiatry*, vol. 86, no. 6, pp. 421–432, Sep. 2019, doi: 10.1016/j.biopsych.2019.04.028.

[101] M. Grossman and W. Wood, "Sex Differences in Intensity of Emotional Experience: A Social Role Interpretation," *J. Pers. Soc. Psychol.*, vol. 65, no. 5, pp. 1010–1022, 1993, doi: 10.1037/0022-3514.65.5.1010.

[102] G. Domes, L. Schulze, M. Böttger, A. Grossmann, K. Hauenstein, P. H. Wirtz, M. Heinrichs, and S. C. Herpertz, "The neural correlates of sex differences in emotional reactivity and emotion regulation," *Hum. Brain Mapp.*, vol. 31, no. 5, pp. 758–769, Dec. 2009, doi: 10.1002/hbm.20903.

[103] J. S. Stevens and S. Hamann, "Sex differences in brain activation to emotional stimuli: A meta-analysis of neuroimaging studies," *Neuropsychologia*, vol. 50, no. 7, pp. 1578–1593, Jun. 2012, doi: 10.1016/j.neuropsychologia.2012.03.011.

[104] R. T. Rao and I. P. Androulakis, "Modeling the sex differences and interindividual variability in the activity of the hypothalamic-pituitary-adrenal axis," *Endocrinology*, vol. 158, no. 11, pp. 4017–4037, Nov. 2017, doi: 10.1210/en.2017-00544.

[105] H. M. Burke, M. C. Davis, C. Otte, and D. C. Mohr, "Depression and cortisol responses to psychological stress: a meta-analysis," *Psychoneuroendocrinology*, vol. 30, no. 9, pp. 846–856, Oct. 2005, doi: 10.1016/J.PSYNEUEN.2005.02.010.

[106] M. Uhart, R. Y. Chong, L. Oswald, P. I. Lin, and G. S. Wand, "Gender differences in hypothalamic-pituitary-adrenal (HPA) axis reactivity," *Psychoneuroendocrinology*, vol. 31, no. 5, pp. 642–652, 2006, doi: 10.1016/j.psyneuen.2006.02.003.

[107] E. A. Young, "Glucocorticoid cascade hypothesis revisited: Role of gonadal steroids," *Depression*, vol. 3, no. 1–2, pp. 20–27, Jan. 1995, doi: 10.1002/DEPR.3050030105.

[108] F. Peeters, N. A. Nicholson, and J. Berkhof, "Cortisol Responses to Daily Events in Major Depressive Disorder," *Psychosom. Med.*, vol. 65, no. 5, pp. 836–841, Sep. 2003, doi: 10.1097/01.PSY.0000088594.17747.2E.

[109] K. K. Chopra, A. Ravindran, S. H. Kennedy, B. Mackenzie, S. Matthews, H. Anisman, R.

M. Bagby, P. Farvolden, and R. D. Levitan, “Sex differences in hormonal responses to a social stressor in chronic major depression,” *Psychoneuroendocrinology*, vol. 34, no. 8, pp. 1235–1241, 2009, doi: 10.1016/j.psyneuen.2009.03.014.

[110] D. R. Rubinow and P. J. Schmidt, “Sex differences and the neurobiology of affective disorders,” *Neuropsychopharmacology*, vol. 44, no. 1, pp. 111–128, Jan. 2019, doi: 10.1038/s41386-018-0148-z.

[111] R. J. Handa, L. H. Burgess, J. E. Kerr, and J. A. O’Keefe, “Gonadal Steroid Hormone Receptors and Sex Differences in the Hypothalamo-Pituitary-Adrenal Axis,” *Horm. Behav.*, vol. 28, no. 4, pp. 464–476, Dec. 1994, doi: 10.1006/hbeh.1994.1044.

[112] A. L. Heck and R. J. Handa, “Sex differences in the hypothalamic–pituitary–adrenal axis’ response to stress: an important role for gonadal hormones,” *Neuropsychopharmacology*, vol. 44, no. 1, pp. 45–58, Jan. 2019, doi: 10.1038/s41386-018-0167-9.

[113] V. Viau and M. J. Meaney, “Testosterone-dependent variations in plasma and intrapituitary corticosteroid binding globulin and stress hypothalamic-pituitary-adrenal activity in the male rat,” *J. Endocrinol.*, vol. 181, no. 2, pp. 223–231, May 2004, doi: 10.1677/joe.0.1810223.

[114] G. E. Hodes, “A primer on sex differences in the behavioral response to stress,” *Curr. Opin. Behav. Sci.*, vol. 23, pp. 75–83, Oct. 2018, doi: 10.1016/J.COBEHA.2018.03.012.

[115] N. Goel and T. L. Bale, “Organizational and activational effects of testosterone on masculinization of female physiological and behavioral stress responses,” *Endocrinology*, vol. 149, no. 12, pp. 6399–6405, 2008, doi: 10.1210/en.2008-0433.

[116] K. Kosidou, C. Dalman, M. Lundberg, J. Hallqvist, G. Isacsson, and C. Magnusson, “Socioeconomic status and risk of psychological distress and depression in the Stockholm Public Health Cohort: A population-based study,” *J. Affect. Disord.*, vol. 134, no. 1–3, pp. 160–167, Nov. 2011, doi: 10.1016/j.jad.2011.05.024.

[117] J. Mwinyi, C. Pisanu, E. Castelao, S. Stringhini, M. Preisig, and H. B. Schiöth, “Anxiety Disorders are Associated with Low Socioeconomic Status in Women but Not in Men,” *Women’s Heal. Issues*, vol. 27, no. 3, pp. 302–307, May 2017, doi: 10.1016/j.whi.2017.01.001.

[118] B. Peplinski, R. McClelland, and M. Szklo, “Associations between socioeconomic status markers and depressive symptoms by race and gender: results from the Multi-Ethnic Study of Atherosclerosis (MESA),” *Ann. Epidemiol.*, vol. 28, no. 8, pp. 535-542.e1, Aug. 2018, doi: 10.1016/j.annepidem.2018.05.005.

[119] S. Karamihalev, E. Brivio, C. Flachskamm, R. Stoffel, M. V. Schmidt, and A. Chen, “Social dominance mediates behavioral adaptation to chronic stress in a sex-specific manner,” *Elife*, vol. 9, pp. 1–18, Oct. 2020, doi: 10.7554/eLife.58723.

[120] Y. Shemesh, Y. Sztainberg, O. Forkosh, T. Shlapobersky, A. Chen, and E. Schneidman, “High-order social interactions in groups of mice,” *Elife*, vol. 2, pp. 1–19, Sep. 2013, doi: 10.7554/eLife.00759.

[121] O. Forkosh, S. Karamihalev, S. Roeh, U. Alon, S. Anpilov, C. Touma, M. Nussbaumer, C. Flachskamm, P. M. Kaplick, Y. Shemesh, and A. Chen, “Identity domains capture individual differences from across the behavioral repertoire,” *Nat. Neurosci.*, pp. 1–6, Nov. 2019, doi: 10.1038/s41593-019-0516-y.

[122] J. S. Danska, “Sex matters for mechanism,” *Sci. Transl. Med.*, vol. 6, no. 258, p. 258fs40, Oct. 2014, doi: 10.1126/scitranslmed.3009859.

[123] J. E. Garrett and C. L. Wellman, “Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence,” *Neuroscience*, vol. 162, no. 1, pp. 195–207, Aug. 2009, doi: 10.1016/j.neuroscience.2009.04.057.

[124] N. Nava, G. Treccani, A. Alabsi, H. Kaastrup Mueller, B. Elfving, M. Popoli, G. Wegener, and J. R. Nyengaard, “Temporal Dynamics of Acute Stress-Induced Dendritic Remodeling

in Medial Prefrontal Cortex and the Protective Effect of Desipramine.," *Cereb. Cortex*, vol. 27, no. 1, pp. 694–705, Nov. 2017, doi: 10.1093/cercor/bhv254.

[125] A. Izquierdo, C. L. Wellman, and A. Holmes, "Brief Uncontrollable Stress Causes Dendritic Retraction in Infralimbic Cortex and Resistance to Fear Extinction in Mice," *J. Neurosci.*, vol. 26, no. 21, pp. 5733–5738, May 2006, doi: 10.1523/JNEUROSCI.0474-06.2006.

[126] M. R. Breach, K. M. Moench, and C. L. Wellman, "Social instability in adolescence differentially alters dendritic morphology in the medial prefrontal cortex and its response to stress in adult male and female rats," *Dev. Neurobiol.*, vol. 79, no. 9–10, pp. 839–856, Sep. 2019, doi: 10.1002/DNEU.22723.

[127] R. M. Shansky, C. Hamo, P. R. Hof, W. Lou, B. S. McEwen, and J. H. Morrison, "Estrogen promotes stress sensitivity in a prefrontal cortex-amygdala pathway," *Cereb. Cortex*, vol. 20, no. 11, pp. 2560–2567, 2010, doi: 10.1093/cercor/bhq003.

[128] R. M. Shansky, C. Hamo, P. R. Hof, B. S. McEwen, and J. H. Morrison, "Stress-Induced Dendritic Remodeling in the Prefrontal Cortex is Circuit Specific," *Cereb. Cortex*, vol. 19, no. 10, pp. 2479–2484, Oct. 2009, doi: 10.1093/CERCOR/BHP003.

[129] T. Hajszan, A. Dow, J. L. Warner-Schmidt, K. Szigeti-Buck, N. L. Sallam, A. Parducz, C. Leranth, and R. S. Duman, "Remodeling of hippocampal spine synapses in the rat learned helplessness model of depression," *Biol. Psychiatry*, vol. 65, no. 5, pp. 392–400, Mar. 2009, doi: 10.1016/J.BIOPSYCH.2008.09.031.

[130] Y. Chen, C. S. Rex, C. J. Rice, C. M. Dubé, C. M. Gall, G. Lynch, and T. Z. Barama, "Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling," *Proc. Natl. Acad. Sci.*, vol. 107, no. 29, pp. 13123–13128, Jul. 2010, doi: 10.1073/PNAS.1003825107.

[131] L. A. . Galea, B. . McEwen, P. Tanapat, T. Deak, R. . Spencer, and F. . Dhabhar, "Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress," *Neuroscience*, vol. 81, no. 3, pp. 689–697, Sep. 1997, doi: 10.1016/S0306-4522(97)00233-9.

[132] T. J. Shors, C. Chua, and J. Falduto, "Sex Differences and Opposite Effects of Stress on Dendritic Spine Density in the Male Versus Female Hippocampus," *J. Neurosci.*, vol. 21, no. 16, pp. 6292–6297, Aug. 2001, doi: 10.1523/JNEUROSCI.21-16-06292.2001.

[133] E. W. Salter, J. K. Sunstrum, S. Matovic, and W. Inoue, "Chronic stress dampens excitatory synaptic gain in the paraventricular nucleus of the hypothalamus," *J. Physiol.*, vol. 596, no. 17, pp. 4157–4172, Sep. 2018, doi: 10.1113/JP275669.

[134] J. S. Bains, J. I. W. Cusulin, and W. Inoue, "Stress-related synaptic plasticity in the hypothalamus," *Nature Reviews Neuroscience*, vol. 16, no. 7. Nat Rev Neurosci, pp. 377–388, Jul. 22, 2015. doi: 10.1038/nrn3881.

[135] M. C. Palumbo, S. Dominguez, and H. Dong, "Sex differences in hypothalamic–pituitary–adrenal axis regulation after chronic unpredictable stress," *Brain Behav.*, vol. 10, no. 4, p. e01586, Apr. 2020, doi: 10.1002/brb3.1586.

[136] D. Karandrea, C. Kittas, and E. Kitraki, "Forced Swimming Differentially Affects Male and Female Brain Corticosteroid Receptors," *Neuroendocrinology*, vol. 75, no. 4, pp. 217–226, Apr. 2002, doi: 10.1159/000054713.

[137] D. Karandrea, C. Kittas, and E. Kitraki, "Contribution of Sex and Cellular Context in the Regulation of Brain Corticosteroid Receptors following Restraint Stress," *Neuroendocrinology*, vol. 71, no. 6, pp. 343–353, Jun. 2000, doi: 10.1159/000054555.

[138] E. Brivio, J. P. Lopez, and A. Chen, "Sex differences: Transcriptional signatures of stress exposure in male and female brains," *Genes, Brain Behav.*, vol. 19, no. 3, pp. 1–22, Mar. 2020, doi: 10.1111/gbb.12643.

[139] O. Issler and E. J. Nestler, "The molecular basis for sex differences in depression susceptibility," *Curr. Opin. Behav. Sci.*, vol. 23, pp. 1–6, Oct. 2018, doi:

10.1016/j.cobeha.2017.12.019.

[140] G. E. Hodes, M. L. Pfau, I. Purushothaman, H. F. Ahn, S. A. Golden, D. J. Christoffel, J. Magida, A. Brancato, A. Takahashi, M. E. Flanigan, C. Menard, H. Aleyasin, J. W. J. W. Koo, Z. S. Lorsch, J. Feng, M. Heshmati, M. Wang, G. Turecki, R. Neve, B. Zhang, L. L. Shen, E. J. Nestler, and S. J. Russo, "Sex Differences in Nucleus Accumbens Transcriptome Profiles Associated with Susceptibility versus Resilience to Subchronic Variable Stress," *J. Neurosci.*, vol. 35, no. 50, pp. 16362–16376, Dec. 2015, doi: 10.1523/JNEUROSCI.1392-15.2015.

[141] Q. LaPlant, V. Vialou, H. E. Covington, D. Dumitriu, J. Feng, B. L. Warren, I. Maze, D. M. Dietz, E. L. Watts, S. D. Iñiguez, J. W. Koo, E. Mouzon, W. Renthal, F. Hollis, H. Wang, M. a Noonan, Y. Ren, A. J. Eisch, C. a Bolaños, M. Kabbaj, G. Xiao, R. L. Neve, Y. L. Hurd, R. S. Oosting, G. Fan, J. H. Morrison, and E. J. Nestler, "Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens.," *Nat. Neurosci.*, vol. 13, no. 9, pp. 1137–43, Sep. 2010, doi: 10.1038/nn.2619.

[142] B. B. Nair, Z. Khant Aung, R. Porteous, M. Prescott, K. A. Glendining, D. E. Jenkins, R. A. Augustine, M. S. B. Silva, S. H. Yip, G. T. Bouwer, C. H. Brown, C. L. Jasoni, R. E. Campbell, S. J. Bunn, G. M. Anderson, D. R. Grattan, A. E. Herbison, and K. J. Iremonger, "Impact of chronic variable stress on neuroendocrine hypothalamus and pituitary in male and female C57BL/6J mice," *J. Neuroendocrinol.*, vol. 33, no. 5, 2021, doi: 10.1111/jne.12972.

[143] F. Mena and L. Benoit, "Molecular programs underlying differences in the expression of mood disorders in males and females," *Brain Res.*, vol. 1719, no. April, pp. 89–103, Sep. 2019, doi: 10.1016/j.brainres.2019.05.016.

[144] A. P. Borrow, N. J. Bales, S. A. Stover, and R. J. Handa, "Chronic variable stress induces sex-specific alterations in social behavior and neuropeptide expression in the mouse," *Endocrinology*, vol. 159, no. 7, pp. 2803–2814, Jul. 2018, doi: 10.1210/en.2018-00217.

[145] K. M. Moench, M. R. Breach, and C. L. Wellman, "Chronic stress produces enduring sex- and region-specific alterations in novel stress-induced c-Fos expression," *Neurobiol. Stress*, vol. 10, p. 100147, Feb. 2019, doi: 10.1016/j.ynstr.2019.100147.

[146] B. Labonté, O. Engmann, I. Purushothaman, C. Menard, J. Wang, C. Tan, J. R. Scarpa, G. Moy, Y.-H. H. E. Loh, M. Cahill, Z. S. Lorsch, P. J. Hamilton, E. S. Calipari, G. E. Hodes, O. Issler, H. Kronman, M. Pfau, A. L. J. J. Obradovic, Y. Dong, R. L. Neve, S. J. Russo, A. Kazarskis, C. Tamminga, N. Mechawar, G. Turecki, B. Zhang, L. Shen, and E. J. Nestler, "Sex-specific transcriptional signatures in human depression," *Nat. Med.*, vol. 23, no. 9, pp. 1102–1111, Aug. 2017, doi: 10.1038/nm.4386.

[147] D. M. Gerhard and R. S. Duman, "Sex-Specific Molecular Changes in Depression," *Biol. Psychiatry*, vol. 84, no. 1, pp. 2–4, Jul. 2018, doi: 10.1016/j.biopsych.2018.05.005.

[148] M. L. Seney, Z. Huo, K. Cahill, L. French, R. Puralewski, J. Zhang, R. W. Logan, G. Tseng, D. A. Lewis, and E. Sibille, "Opposite Molecular Signatures of Depression in Men and Women," *Biol. Psychiatry*, vol. 84, no. 1, pp. 18–27, Jul. 2018, doi: 10.1016/j.biopsych.2018.01.017.

[149] J. Marrocco, G. H. Petty, M. B. Ríos, J. D. Gray, J. F. Kogan, E. M. Waters, E. F. Schmidt, F. S. Lee, and B. S. McEwen, "A sexually dimorphic pre-stressed translational signature in CA3 pyramidal neurons of BDNF Val66Met mice," *Nat. Commun.*, vol. 8, no. 1, p. 808, Dec. 2017, doi: 10.1038/s41467-017-01014-4.

[150] R. C. Bagot, H. M. Cates, I. Purushothaman, Z. S. Lorsch, D. M. Walker, J. Wang, X. Huang, O. M. Schlüter, I. Maze, C. J. Peña, E. A. Heller, O. Issler, M. Wang, W.-M. Song, J. L. Stein, X. Liu, M. A. Doyle, K. N. Scobie, H. S. Sun, R. L. Neve, D. Geschwind, Y. Dong, L. Shen, B. Zhang, and E. J. Nestler, "Circuit-wide Transcriptional Profiling Reveals Brain Region-Specific Gene Networks Regulating Depression Susceptibility," *Neuron*, vol. 90, no. 5, pp. 969–983, Jun. 2016, doi: 10.1016/j.neuron.2016.04.015.

[151] J. R. Rainville, T. Lipuma, and G. E. Hodes, "Translating the Transcriptome: Sex

Differences in the Mechanisms of Depression and Stress, Revisited," *Biol. Psychiatry*, no. 40, pp. 1–11, 2021, doi: 10.1016/j.biopsych.2021.02.003.

[152] B. C. Karisetty, N. Khandelwal, A. Kumar, S. Chakravarty, P. C. Joshi, A. Kumar, and S. Chakravarty, "Sex difference in mouse hypothalamic transcriptome profile in stress-induced depression model," *Biochem. Biophys. Res. Commun.*, vol. 486, no. 4, pp. 1122–1128, Jul. 2017, doi: 10.1016/j.bbrc.2017.04.005.

[153] E. Mereu, A. Lafzi, C. Moutinho, C. Ziegenhain, D. J. McCarthy, A. Álvarez-Varela, E. Batlle, Sagar, D. Grün, J. K. Lau, S. C. Boutet, C. Sanada, A. Ooi, R. C. Jones, K. Kaihara, C. Brampton, Y. Talaga, Y. Sasagawa, K. Tanaka, T. Hayashi, C. Braeuning, C. Fischer, S. Sauer, T. Trefzer, C. Conrad, X. Adiconis, L. T. Nguyen, A. Regev, J. Z. Levin, S. Parekh, A. Janjic, L. E. Wange, J. W. Bagnoli, W. Enard, M. Gut, R. Sandberg, I. Nikaido, I. Gut, O. Stegle, and H. Heyn, "Benchmarking single-cell RNA-sequencing protocols for cell atlas projects," *Nat. Biotechnol.*, pp. 1–9, Apr. 2020, doi: 10.1038/s41587-020-0469-4.

[154] D. Ofengeim, N. Giagtzoglou, D. Huh, C. Zou, and J. Yuan, "Single-Cell RNA Sequencing: Unraveling the Brain One Cell at a Time," *Trends Mol. Med.*, vol. 23, no. 6, pp. 563–576, Jun. 2017, doi: 10.1016/j.molmed.2017.04.006.

[155] Takara, "Single-cell transcriptome studies: a powerful way to highlight subtle differences between cells that may be hidden in a population." https://www.takarabio.com/learning-centers/next-generation-sequencing/technical-notes/single-cell-mrna-seq (accessed Jul. 03, 2018).

[156] J. Poulin, B. Tasic, J. Hjerling-Leffler, J. M. Trimarchi, and R. Awatramani, "Disentangling neural cell diversity using single-cell transcriptomics," *Nat. Neurosci.*, vol. 19, no. 9, pp. 1131–1141, Aug. 2016, doi: 10.1038/nn.4366.

[157] Q. H. Nguyen, N. Pervolarakis, K. Nee, and K. Kessenbrock, "Experimental Considerations for Single-Cell RNA Sequencing Approaches," *Front. Cell Dev. Biol.*, vol. 6, p. 108, Sep. 2018, doi: 10.3389/fcell.2018.00108.

[158] A. A. Kolodziejczyk and T. Lönnberg, "Global and targeted approaches to single-cell transcriptome characterization," *Brief. Funct. Genomics*, Sep. 2017, doi: 10.1093/bfgp/elx025.

[159] B. D. Aevermann, M. Novotny, T. Bakken, J. A. Miller, A. D. Diehl, D. Osumi-Sutherland, R. S. Lasken, E. S. Lein, and R. H. Scheuermann, "Cell type discovery using single-cell transcriptomics: implications for ontological representation.," *Hum. Mol. Genet.*, vol. 27, no. R1, pp. R40–R47, 2018, doi: 10.1093/hmg/ddy100.

[160] C. M. Stein, R. Weiskirchen, F. Damm, and P. M. Strzelecka, "Single-cell omics: Overview, analysis, and application in biomedical science," *J. Cell. Biochem.*, no. July, 2021, doi: 10.1002/jcb.30134.

[161] K. D. Birnbaum, "Power in Numbers: Single-Cell RNA-Seq Strategies to Dissect Complex Tissues," *Annu. Rev. Genet.*, vol. 52, no. 1, pp. 203–221, Nov. 2018, doi: 10.1146/annurev-genet-120417-031247.

[162] A. Haque, J. Engel, S. A. Teichmann, and T. Lönnberg, "A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications.," *Genome Med.*, vol. 9, no. 1, p. 75, 2017, doi: 10.1186/s13073-017-0467-4.

[163] M. S. Cembrowski, "Single-cell transcriptomics as a framework and roadmap for understanding the brain," *J. Neurosci. Methods*, vol. 326, p. 108353, Oct. 2019, doi: 10.1016/j.jneumeth.2019.108353.

[164] B. Tasic, V. Menon, T. N. Nguyen, T. K. Kim, T. Jarsky, Z. Yao, B. Levi, L. T. Gray, S. A. Sorensen, T. Dolbeare, D. Bertagnolli, J. Goldy, N. Shapovalova, S. Parry, C. Lee, K. Smith, A. Bernard, L. Madisen, S. M. Sunkin, M. Hawrylycz, C. Koch, and H. Zeng, "Adult mouse cortical cell taxonomy revealed by single cell transcriptomics," *Nat. Neurosci.*, vol. 19, no. 2, pp. 335–346, 2016, doi: 10.1038/nn.4216.

[165] A. Zeisel, A. B. Muñoz-Manchado, S. Codeluppi, P. Lönnerberg, G. La Manno, A. Juréus, S. Marques, H. Munguba, L. He, C. Betsholtz, C. Rolny, G. Castelo-Branco, J. Hjerling-Leffler, and S. Linnarsson, "Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq," *Science (80-.).*, vol. 347, no. 6226, pp. 1138–1142, Mar. 2015, doi: 10.1126/science.aaa1934.

[166] J. R. Moffitt, D. Bambah-Mukku, S. W. Eichhorn, E. Vaughn, K. Shekhar, J. D. Perez, N. D. Rubinstein, J. Hao, A. Regev, C. Dulac, and X. Zhuang, "Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region," *Science (80-.).*, vol. 362, no. 6416, p. eaau5324, Nov. 2018, doi: 10.1126/science.aau5324.

[167] A. Zeisel, H. Hochgerner, P. Lönnerberg, A. Johnsson, F. Memic, J. van der Zwan, M. Häring, E. Braun, L. E. Borm, G. La Manno, S. Codeluppi, A. Furlan, K. Lee, N. Skene, K. D. Harris, J. Hjerling-Leffler, E. Arenas, P. Ernfors, U. Marklund, and S. Linnarsson, "Molecular Architecture of the Mouse Nervous System," *Cell*, vol. 174, no. 4, pp. 999-1014.e22, Aug. 2018, doi: 10.1016/j.cell.2018.06.021.

[168] J. Sun, Y. Song, · Zhiheng Chen, J. Qiu, S. Zhu, · Liucheng Wu, and L. Xing, "Heterogeneity and Molecular Markers for CNS Glial Cells Revealed by Single-Cell Transcriptomics," *Cell. Mol. Neurobiol. 2021*, pp. 1–14, Oct. 2021, doi: 10.1007/S10571-021-01159-3.

[169] J. N. Campbell, E. Z. Macosko, H. Fenselau, T. H. Pers, A. Lyubetskaya, D. Tenen, M. Goldman, A. M. J. Verstegen, J. M. Resch, S. A. McCarroll, E. D. Rosen, B. B. Lowell, and L. T. Tsai, "A molecular census of arcuate hypothalamus and median eminence cell types," *Nat. Neurosci.*, vol. 20, no. 3, pp. 484–496, Feb. 2017, doi: 10.1038/nn.4495.

[170] R. Chen, X. Wu, L. Jiang, and Y. Zhang, "Single-Cell RNA-Seq Reveals Hypothalamic Cell Diversity," *Cell Rep.*, vol. 18, no. 13, pp. 3227–3241, Mar. 2017, doi: 10.1016/j.celrep.2017.03.004.

[171] P. Hasel, I. V. L. Rose, J. S. Sadick, R. D. Kim, and S. A. Liddelow, "Neuroinflammatory astrocyte subtypes in the mouse brain," *Nat. Neurosci.*, vol. 24, no. 10, pp. 1475–1487, Oct. 2021, doi: 10.1038/s41593-021-00905-6.

[172] S. Marques, A. Zeisel, S. Codeluppi, D. Van Bruggen, A. M. Falcão, L. Xiao, H. Li, M. Häring, H. Hochgerner, R. A. Romanov, D. Gyllborg, A. B. Muñoz-Manchado, G. La Manno, P. Lönnerberg, E. M. Floriddia, F. Rezayee, P. Ernfors, E. Arenas, J. Hjerling-Leffler, T. Harkany, W. D. Richardson, S. Linnarsson, G. Castelo-Branco, A. Mendanha Falcao, L. Xiao, H. Li, M. Haring, H. Hochgerner, R. A. Romanov, D. Gyllborg, A. B. Munoz-Manchado, G. La Manno, P. Lonnerberg, E. M. Floriddia, F. Rezayee, P. Ernfors, E. Arenas, J. Hjerling-Leffler, T. Harkany, W. D. Richardson, S. Linnarsson, and G. Castelo-Branco, "Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system," *Science (80-.).*, vol. 352, no. 6291, pp. 1326–1329, Jun. 2016, doi: 10.1126/science.aaf6463.

[173] H. Van Hove, L. Martens, I. Scheyltjens, K. De Vlaminck, A. R. Pombo Antunes, S. De Prijck, N. Vandamme, S. De Schepper, G. Van Isterdael, C. L. Scott, J. Aerts, G. Berx, G. E. Boeckxstaens, R. E. Vandenbroucke, L. Vereecke, D. Moechars, M. Guilliams, J. A. Van Ginderachter, Y. Saeys, and K. Movahedi, "A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment," *Nat. Neurosci.*, vol. 22, no. 6, pp. 1021–1035, Jun. 2019, doi: 10.1038/s41593-019-0393-4.

[174] E. Klingler, U. Tomasello, J. Prados, J. M. Kebschull, A. Contestabile, G. L. Galiñanes, S. Fièvre, A. Santinha, R. Platt, D. Huber, A. Dayer, C. Bellone, and D. Jabaudon, "Temporal controls over inter-areal cortical projection neuron fate diversity," *Nat. 2021*, pp. 1–5, Nov. 2021, doi: 10.1038/s41586-021-04048-3.

[175] H. Mathys, J. Davila-Velderrain, Z. Peng, F. Gao, S. Mohammadi, J. Z. Young, M. Menon, L. He, F. Abdurrob, X. Jiang, A. J. Martorell, R. M. Ransohoff, B. P. Hafler, D. A. Bennett, M. Kellis, and L. H. Tsai, "Single-cell transcriptomic analysis of Alzheimer's disease," *Nature*, vol. 570, no. 7761, pp. 332–337, Jun. 2019, doi: 10.1038/S41586-019-1195-2.

[176] D. Velmeshev, L. Schirmer, D. Jung, M. Haeussler, Y. Perez, S. Mayer, A. Bhaduri, N. Goyal, D. H. Rowitch, and A. R. Kriegstein, "Single-cell genomics identifies cell type–specific molecular changes in autism," *Science (80-.).*, vol. 364, no. 6441, pp. 685–689, May 2019, doi: 10.1126/science.aav8130.

[177] N. Cai, S. Chang, Y. Li, Q. Li, J. Hu, J. Liang, L. Song, W. Kretzschmar, X. Gan, J. Nicod, M. Rivera, H. Deng, B. Du, K. Li, W. Sang, J. Gao, S. Gao, B. Ha, H.-Y. Ho, C. Hu, J. Hu, Z. Hu, G. Huang, G. Jiang, T. Jiang, W. Jin, G. Li, K. Li, Y. Li, Y. Li, Y. Li, Y.-T. Lin, L. Liu, T. Liu, Y. Liu, Y. Liu, Y. Lu, L. Lv, H. Meng, P. Qian, H. Sang, J. Shen, J. Shi, J. Sun, M. Tao, G. Wang, G. Wang, J. Wang, L. Wang, X. Wang, X. Wang, H. Yang, L. Yang, Y. Yin, J. Zhang, K. Zhang, N. Sun, W. Zhang, X. Zhang, Z. Zhang, H. Zhong, G. Breen, J. Wang, J. Marchini, Y. Chen, Q. Xu, X. Xu, R. Mott, G.-J. Huang, K. Kendler, and J. Flint, "Molecular Signatures of Major Depression," *Curr. Biol.*, vol. 25, no. 9, pp. 1146–1156, May 2015, doi: 10.1016/j.cub.2015.03.008.

[178] S. L. Byers, M. V. Wiles, S. L. Dunn, and R. A. Taft, "Mouse Estrous Cycle Identification Tool and Images," *PLoS One*, vol. 7, no. 4, p. e35538, Apr. 2012, doi: 10.1371/journal.pone.0035538.

[179] A. Johnson, J. R. Rainville, G. N. Rivero-Ballon, K. Dhimitri, and G. E. Hodes, "Testing the Limits of Sex Differences Using Variable Stress," *Neuroscience*, vol. 454, pp. 72–84, Feb. 2021, doi: 10.1016/j.neuroscience.2019.12.034.

[180] András Péter, "Solomon Coder." Budapest, 2019. [Online]. Available: https://solomon.andraspeter.com/

[181] B. Schwanhäusser, D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, and M. Selbach, "Global quantification of mammalian gene expression control," *Nature*, vol. 473, no. 7347, pp. 337–342, May 2011, doi: 10.1038/nature10098.

[182] R Core Development Team, "A language and environment for statistical computing." R Foundation for Statistical Computing, Vienna, Austria, 2013. [Online]. Available: http://www.r-project.org/

[183] T. Stuart, A. Butler, P. Hoffman, C. Hafemeister, E. Papalexi, W. M. Mauck, Y. Hao, M. Stoeckius, P. Smibert, and R. Satija, "Comprehensive Integration of Single-Cell Data," *Cell*, vol. 177, no. 7, pp. 1888-1902.e21, Jun. 2019, doi: 10.1016/j.cell.2019.05.031.

[184] M. D. Luecken and F. J. Theis, "Current best practices in single-cell RNA-seq analysis: a tutorial," *Mol. Syst. Biol.*, vol. 15, no. 6, Jun. 2019, doi: 10.15252/msb.20188746.

[185] A. T. L. Lun, D. J. McCarthy, and J. C. Marioni, "A step-by-step workflow for low-level analysis of single-cell RNA-seq data with Bioconductor," *F1000Research*, vol. 5, p. 2122, Oct. 2016, doi: 10.12688/f1000research.9501.2.

[186] J. P. Lopez, E. Brivio, A. Santambrogio, C. De Donno, A. Kos, M. Peters, N. Rost, D. Czamara, T. M. Brückl, S. Roeh, M. L. Pöhlmann, C. Engelhardt, A. Ressle, R. Stoffel, A. Tontsch, J. M. Villamizar, M. Reincke, A. Riester, S. Sbiera, M. Fassnacht, H. S. Mayberg, W. E. Craighead, B. W. Dunlop, C. B. Nemeroff, M. V Schmidt, E. B. Binder, F. J. Theis, F. Beuschlein, C. L. Andoniadou, and A. Chen, "Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation," *Sci. Adv.*, vol. 7, no. 5, p. eabe4497, Jan. 2021, doi: 10.1126/sciadv.abe4497.

[187] C. Soneson and M. D. Robinson, "Bias, robustness and scalability in single-cell differential expression analysis," *Nat. Methods*, vol. 15, no. 4, pp. 255–261, Apr. 2018, doi: 10.1038/nmeth.4612.

[188] G. Finak, A. McDavid, M. Yajima, J. Deng, V. Gersuk, A. K. Shalek, C. K. Slichter, H. W. Miller, M. J. McElrath, M. Prlic, P. S. Linsley, and R. Gottardo, "MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data," *Genome Biol.*, vol. 16, no. 1, p. 278, Dec. 2015, doi: 10.1186/s13059-015-0844-5.

[189] M. Krassowski and CyrilLagger, "krassowski/complex-upset: v1.2.1." Apr. 25, 2021. doi:

10.5281/ZENODO.4718811.

[190] A. Lex, N. Gehlenborg, H. Strobelt, R. Vuillemot, and H. Pfister, “UpSet: Visualization of intersecting sets,” *IEEE Trans. Vis. Comput. Graph.*, vol. 20, no. 12, pp. 1983–1992, Dec. 2014, doi: 10.1109/TVCG.2014.2346248.

[191] L. Micallef and P. Rodgers, “eulerAPE: Drawing Area-Proportional 3-Venn Diagrams Using Ellipses,” *PLoS One*, vol. 9, no. 7, p. e101717, Jul. 2014, doi: 10.1371/journal.pone.0101717.

[192] L. Wilkinson, “Exact and approximate area-proportional circular venn and euler diagrams,” *IEEE Trans. Vis. Comput. Graph.*, vol. 18, no. 2, pp. 321–331, 2012, doi: 10.1109/TVCG.2011.56.

[193] M. V. Kuleshov, M. R. Jones, A. D. Rouillard, N. F. Fernandez, Q. Duan, Z. Wang, S. Koplev, S. L. Jenkins, K. M. Jagodnik, A. Lachmann, M. G. McDermott, C. D. Monteiro, G. W. Gundersen, and A. Ma’ayan, “Enrichr: a comprehensive gene set enrichment analysis web server 2016 update,” *Nucleic Acids Res.*, vol. 44, no. W1, pp. W90–W97, Jul. 2016, doi: 10.1093/nar/gkw377.

[194] K. M. Cahill, Z. Huo, G. C. Tseng, R. W. Logan, and M. L. Seney, “Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach,” *Sci. Rep.*, vol. 8, no. 1, pp. 1–11, Dec. 2018, doi: 10.1038/s41598-018-27903-2.

[195] S. B. Plaisier, R. Taschereau, J. A. Wong, and T. G. Graeber, “Rank-rank hypergeometric overlap: Identification of statistically significant overlap between gene-expression signatures,” *Nucleic Acids Res.*, vol. 38, no. 17, Jul. 2010, doi: 10.1093/nar/gkq636.

[196] “GitHub - BaderLab/CCInx: Build predicted cell-cell interaction networks from single-cell data.,” 2020. https://github.com/BaderLab/CCInx (accessed Mar. 08, 2021).

[197] “CellCellInteractions - Bader Lab @ The University of Toronto,” 2020. http://baderlab.org/CellCellInteractions (accessed Mar. 08, 2021).

[198] C. Nagy, M. Maitra, A. Tanti, M. Suderman, J. F. Théroux, M. A. Davoli, K. Perlman, V. Yerko, Y. C. Wang, S. J. Tripathy, P. Pavlidis, N. Mechawar, J. Ragoussis, and G. Turecki, “Single-nucleus transcriptomics of the prefrontal cortex in major depressive disorder implicates oligodendrocyte precursor cells and excitatory neurons,” *Nat. Neurosci.*, vol. 23, no. 6, pp. 771–781, Apr. 2020, doi: 10.1038/s41593-020-0621-y.

[199] Z. Gu, L. Gu, R. Eils, M. Schlesner, and B. Brors, “Circlize implements and enhances circular visualization in R,” *Bioinformatics*, vol. 30, no. 19, pp. 2811–2812, Apr. 2014, doi: 10.1093/bioinformatics/btu393.

[200] J. Cao, M. Spielmann, X. Qiu, X. Huang, D. M. Ibrahim, A. J. Hill, F. Zhang, S. Mundlos, L. Christiansen, F. J. Steemers, C. Trapnell, and J. Shendure, “The single-cell transcriptional landscape of mammalian organogenesis,” *Nature*, vol. 566, no. 7745, pp. 496–502, Feb. 2019, doi: 10.1038/s41586-019-0969-x.

[201] C. Trapnell, D. Cacchiarelli, J. Grimsby, P. Pokharel, S. Li, M. Morse, N. J. Lennon, K. J. Livak, T. S. Mikkelsen, and J. L. Rinn, “The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells,” *Nat. Biotechnol.*, vol. 32, no. 4, pp. 381–386, 2014, doi: 10.1038/nbt.2859.

[202] X. Qiu, Q. Mao, Y. Tang, L. Wang, R. Chawla, H. A. Pliner, and C. Trapnell, “Reversed graph embedding resolves complex single-cell trajectories,” *Nat. Methods*, vol. 14, no. 10, pp. 979–982, Oct. 2017, doi: 10.1038/nmeth.4402.

[203] R Core Team, “R: A language and environment for statistical computing.” R Foundation for Statistical Computing, Vienna, Austria, 2020. [Online]. Available: https://www.r-project.org/

[204] G. Korotkevich, V. Sukhov, N. Budin, B. Shpak, M. Artyomov, and A. Sergushichev, “Fast gene set enrichment analysis,” *bioRxiv*, p. 060012, Feb. 2016, doi: 10.1101/060012.

[205] I. Dolgalev, “msigdbr: MSigDB Gene Sets for Multiple Organisms in a Tidy Data Format.” CRAN, 2019. [Online]. Available: https://cran.r-project.org/package=msigdbr

[206] Y. Zhou, B. Zhou, L. Pache, M. Chang, A. H. Khodabakhshi, O. Tanaseichuk, C. Benner, and S. K. Chanda, “Metascape provides a biologist-oriented resource for the analysis of systems-level datasets,” *Nat. Commun.*, vol. 10, no. 1, p. 1523, Dec. 2019, doi: 10.1038/s41467-019-09234-6.

[207] M. H. Longair, D. A. Baker, and J. D. Armstrong, “Simple neurite tracer: Open source software for reconstruction, visualization and analysis of neuronal processes,” *Bioinformatics*, vol. 27, no. 17, pp. 2453–2454, Sep. 2011, doi: 10.1093/bioinformatics/btr390.

[208] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, and A. Cardona, “Fiji: an open-source platform for biological-image analysis,” *Nat. Methods 2012 97*, vol. 9, no. 7, pp. 676–682, Jun. 2012, doi: 10.1038/nmeth.2019.

[209] T. A. Ferreira, A. V. Blackman, J. Oyrer, S. Jayabal, A. J. Chung, A. J. Watt, P. J. Sjöström, and D. J. Van Meyel, “Neuronal morphometry directly from bitmap images,” *Nature Methods*, vol. 11, no. 10. Nature Publishing Group, pp. 982–984, Jan. 01, 2014. doi: 10.1038/nmeth.3125.

[210] D. Bates, M. Mächler, B. Bolker, and S. Walker, “Fitting Linear Mixed-Effects Models Using lme4,” *J. Stat. Softw.*, vol. 67, no. 1, 2015, doi: 10.18637/jss.v067.i01.

[211] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, and R Core Team, “nlme: Linear and Nonlinear Mixed Effects Models.” 2020.

[212] A. Kuznetsova, P. B. Brockhoff, and R. H. B. Christensen, “lmerTest Package: Tests in Linear Mixed Effects Models,” *J. Stat. Softw.*, vol. 82, no. 13, 2017, doi: 10.18637/jss.v082.i13.

[213] A. Zucco, “adrigabzu/sholl_analysis_in_R: AUC calculation and stats,” Jan. 2018, doi: 10.5281/ZENODO.1158612.

[214] M. D. Wilson, S. Sethi, P. J. Lein, and K. P. Keil, “Valid statistical approaches for analyzing sholl data: Mixed effects versus simple linear models,” *J. Neurosci. Methods*, vol. 279, pp. 33–43, Mar. 2017, doi: 10.1016/j.jneumeth.2017.01.003.

[215] J. F. Ouyang, U. S. Kamaraj, E. Y. Cao, and O. J. L. Rackham, “Gene expression ShinyCell : simple and sharable visualization of single-cell gene expression data,” no. March, pp. 1–3, 2021, doi: 10.1093/bioinformatics/btab209.

[216] W. Chang, J. Cheng, J. Allaire, C. Sievert, B. Schloerke, Y. Xie, J. Allen, J. McPherson, A. Dipert, B. Borges, and RStudio, “Package shiny.” Comprehensive R Archive Network (CRAN), 2020. Accessed: Nov. 23, 2021. [Online]. Available: https://rdocumentation.org/packages/shiny/versions/1.4.0.2

[217] C. Mason-Thom, “Package shinyhelper.” Comprehensive R Archive Network (CRAN), 2019. Accessed: Nov. 23, 2021. [Online]. Available: https://github.com/cwthom/shinyhelper/issues

[218] RStudio Team, “RStudio: Integrated Development for R.” RStudio, Inc., Boston, MA, 2019. [Online]. Available: http://www.rstudio.com

[219] H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani, “Welcome to the Tidyverse,” *J. Open Source Softw.*, vol. 4, no. 43, p. 1686, Nov. 2019, doi: 10.21105/joss.01686.

[220] H. Wickham, *ggplot2: Elegant Graphics for Data Analysis*. New York, 2016. [Online]. Available: https://ggplot2.tidyverse.org

[221] S. Garnier, “viridis: Default Color Maps from ‘matplotlib’. R package.” 2018. [Online]. Available: https://cran.r-project.org/package=viridis

[222] R. V. Lenth, “emmeans: Estimated Marginal Means, aka Least-Squares Means.” 2021. [Online]. Available: https://cran.r-project.org/package=emmeans

[223] K. J. Brown and N. E. Grunberg, “Effects of housing on male and female rats: Crowding stresses males but calms females,” *Physiol. Behav.*, vol. 58, no. 6, pp. 1085–1089, 1995, doi: 10.1016/0031-9384(95)02043-8.

[224] P. Palanza, L. Gioiosa, and S. Parmigiani, “Social stress in mice: Gender differences and effects of estrous cycle and social dominance,” *Physiol. Behav.*, vol. 73, no. 3, pp. 411–420, Jun. 2001, doi: 10.1016/S0031-9384(01)00494-2.

[225] N. Liu, Y. Wang, A. Y. An, C. Banker, Y. H. Qian, and J. M. O’Donnell, “Single housing-induced effects on cognitive impairment and depression-like behavior in male and female mice involve neuroplasticity-related signaling,” *Eur. J. Neurosci.*, vol. 52, no. 1, pp. 2694–2704, Jul. 2020, doi: 10.1111/ejn.14565.

[226] K. Kondrakiewicz, M. Kostecki, W. Szadzińska, and E. Knapska, “Ecological validity of social interaction tests in rats and mice,” *Genes, Brain Behav.*, vol. 18, no. 1, pp. 1–14, 2019, doi: 10.1111/gbb.12525.

[227] Y. Horii, T. Nagasawa, H. Sakakibara, A. Takahashi, A. Tanave, Y. Matsumoto, H. Nagayama, K. Yoshimi, M. T. Yasuda, K. Shimoi, and T. Koide, “Hierarchy in the home cage affects behaviour and gene expression in group-housed C57BL/6 male mice,” *Sci. Rep.*, vol. 7, no. 1, p. 6991, Dec. 2017, doi: 10.1038/s41598-017-07233-5.

[228] J. A. Varholick, A. Pontiggia, E. Murphy, V. Daniele, R. Palme, B. Voelkl, H. Würbel, and J. D. Bailoo, “Social dominance hierarchy type and rank contribute to phenotypic variation within cages of laboratory mice,” *Sci. Rep.*, vol. 9, no. 1, p. 13650, Dec. 2019, doi: 10.1038/s41598-019-49612-0.

[229] F. Wang, H. W. Kessels, and H. Hu, “The mouse that roared: Neural mechanisms of social hierarchy,” *Trends Neurosci.*, vol. 37, no. 11, pp. 674–682, 2014, doi: 10.1016/j.tins.2014.07.005.

[230] B. Schuhr, “Social structure and plasma corticosterone level in female albino mice,” *Physiol. Behav.*, vol. 40, no. 6, pp. 689–693, 1987, doi: 10.1016/0031-9384(87)90269-1.

[231] W. E. Van Den Berg, S. Lamballais, and S. A. Kushner, “Sex-Specific Mechanism of Social Hierarchy in Mice,” *Neuropsychopharmacology*, vol. 40, no. 6, pp. 1364–1372, May 2015, doi: 10.1038/npp.2014.319.

[232] J. A. Varholick, J. D. Bailoo, R. Palme, and H. Würbel, “Phenotypic variability between Social Dominance Ranks in laboratory mice,” *Sci. Rep.*, vol. 8, no. 1, p. 6593, Dec. 2018, doi: 10.1038/s41598-018-24624-4.

[233] C. M. Williamson, W. Lee, A. R. DeCasien, A. Lanham, R. D. Romeo, and J. P. Curley, “Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression,” *bioRxiv*, vol. 9, no. 1, p. 529131, Jan. 2019, doi: 10.1101/529131.

[234] T. Buynitsky and D. I. Mostofsky, “Restraint stress in biobehavioral research: Recent developments,” *Neuroscience and Biobehavioral Reviews*, vol. 33, no. 7. Neurosci Biobehav Rev, pp. 1089–1098, Jul. 2009. doi: 10.1016/j.neubiorev.2009.05.004.

[235] A. Franceschelli, S. Herchick, C. Thelen, Z. Papadopoulou-Daifoti, and P. M. Pitychoutis, “Sex differences in the chronic mild stress model of depression,” *Behav. Pharmacol.*, vol. 25, no. 5–6, p. 1, Jul. 2014, doi: 10.1097/FBP.0000000000000062.

[236] P. Willner, “Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation.,” *Psychopharmacology (Berl).*, vol. 134, no. 4, pp. 319–29, Dec. 1997, Accessed: Oct. 18, 2017. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/9452163

[237] P. Willner, “Chronic Mild Stress (CMS) Revisited: Consistency and Behavioural-Neurobiological Concordance in the Effects of CMS,” *Neuropsychobiology*, vol. 52, no. 2, pp. 90–110, Aug. 2005, doi: 10.1159/000087097.

[238] P. Willner, A. Towell, D. Sampson, S. Sophokleous, and R. Muscat, “Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant.,” *Psychopharmacology (Berl).*, vol. 93, no. 3, pp. 358–64, 1987, Accessed: Oct. 18, 2017. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/3124165

[239] S. Antoniuk, M. Bijata, E. Ponimaskin, and J. Wlodarczyk, “Chronic unpredictable mild stress for modeling depression in rodents: Meta-analysis of model reliability,” *Neurosci. Biobehav. Rev.*, vol. 99, pp. 101–116, Apr. 2019, doi: 10.1016/j.neubiorev.2018.12.002.

[240] S. Monteiro, S. Roque, D. de Sá-Calçada, N. Sousa, M. Correia-Neves, and J. J. Cerqueira, “An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice.,” *Front. psychiatry*, vol. 6, p. 6, 2015, doi: 10.3389/fpsyt.2015.00006.

[241] A. Lajtha and J. D. Blaustein, *Handbook of neurochemistry and molecular neurobiology: Behavioral neurochemistry, neuroendocrinology and molecular neurobiology*, 3rd ed. Springer Science and Business Media LLC, 2007.

[242] N. A. Karp, J. Mason, A. L. Beaudet, Y. Benjamini, L. Bower, R. E. Braun, S. D. M. Brown, E. J. Chesler, M. E. Dickinson, A. M. Flenniken, H. Fuchs, M. H. de Angelis, X. Gao, S. Guo, S. Greenaway, R. Heller, Y. Herault, M. J. Justice, N. Kurbatova, C. J. Lelliott, K. C. K. Lloyd, A.-M. Mallon, J. E. Mank, H. Masuya, C. McKerlie, T. F. Meehan, R. F. Mott, S. A. Murray, H. Parkinson, R. Ramirez-Solis, L. Santos, J. R. Seavitt, D. Smedley, T. Sorg, A. O. Speak, K. P. Steel, K. L. Svenson, Y. Obata, T. Suzuki, M. Tamura, H. Kaneda, T. Furuse, K. Kobayashi, I. Miura, I. Yamada, N. Tanaka, A. Yoshiki, S. Ayabe, D. A. Clary, H. A. Tolentino, M. A. Schuchbauer, T. Tolentino, J. A. Aprile, S. M. Pedroia, L. Kelsey, I. Vukobradovic, Z. Berberovic, C. Owen, D. Qu, R. Guo, S. Newbigging, L. Morikawa, N. Law, X. Shang, P. Feugas, Y. Wang, M. Eskandarian, Y. Zhu, L. M. J. Nutter, P. Penton, V. Laurin, S. Clarke, Q. Lan, K. Sohel, D. Miller, G. Clark, J. Hunter, J. Cabezas, M. Bubshait, T. Carroll, S. Tondat, S. MacMaster, M. Pereira, M. Gertsenstein, O. Danisment, E. Jacob, A. Creighton, G. Sleep, J. Clark, L. Teboul, M. Fray, A. Caulder, J. Loeffler, G. Codner, J. Cleak, S. Johnson, Z. Szoke-Kovacs, A. Radage, M. Maritati, J. Mianne, W. Gardiner, S. Allen, H. Cater, M. Stewart, P. Keskivali-Bond, C. Sinclair, E. Brown, B. Doe, H. Wardle-Jones, E. Grau, N. Griggs, M. Woods, H. Kundi, M. N. D. Griffiths, C. Kipp, D. G. Melvin, N. P. S. Raj, S. A. Holroyd, D. J. Gannon, R. Alcantara, A. Galli, Y. E. Hooks, C. L. Tudor, A. L. Green, F. L. Kussy, E. J. Tuck, E. J. Siragher, S. A. Maguire, D. T. Lafont, V. E. Vancollie, S. A. Pearson, A. S. Gates, M. Sanderson, C. Shannon, L. F. E. Anthony, M. T. Sumowski, R. S. B. McLaren, A. Swiatkowska, C. M. Isherwood, E. L. Cambridge, H. M. Wilson, S. S. Caetano, C. I. Mazzeo, M. H. Dabrowska, C. Lillistone, J. Estabel, A. K. B. Maguire, L.-A. Roberson, G. Pavlovic, M.-C. Birling, W.-D. Marie, S. Jacquot, A. Ayadi, D. Ali-Hadji, P. Charles, P. André, E. Le Marchand, A. El Amri, L. Vasseur, A. Aguilar-Pimentel, L. Becker, I. Treise, K. Moreth, T. Stoeger, O. V. Amarie, F. Neff, W. Wurst, R. Bekeredjian, M. Ollert, T. Klopstock, J. Calzada-Wack, S. Marschall, R. Brommage, R. Steinkamp, C. Lengger, M. A. Östereicher, H. Maier, C. Stoeger, S. Leuchtenberger, A. Yildrim, L. Garrett, S. M. Hölter, A. Zimprich, C. Seisenberger, A. Bürger, J. Graw, O. Eickelberg, A. Zimmer, E. Wolf, D. H. Busch, M. Klingenspor, C. Schmidt-Weber, V. Gailus-Durner, J. Beckers, B. Rathkolb, J. Rozman, S. Wakana, D. West, S. Wells, H. Westerberg, S. Yaacoby, and J. K. White, “Prevalence of sexual dimorphism in mammalian phenotypic traits,” *Nat. Commun.*, vol. 8, no. 721, p. 15475, Jun. 2017, doi: 10.1038/ncomms15475.

[243] J.-P. Guilloux, M. Seney, N. Edgar, and E. Sibille, “Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: Relevance to emotionality and sex,” *J. Neurosci. Methods*, vol. 197, no. 1, pp. 21–31, Apr. 2011, doi: 10.1016/j.jneumeth.2011.01.019.

[244] C. Dournes, J. Dine, J. P. Lopez, E. Brivio, E. Anderzhanova, S. Roeh, C. Kuehne, M. Holzapfel, R. E. Huettl, R. Stoffel, L. Tietze, C. Eggert, M. Schieven, M. Jakovcevski, J. M. Deussing, and A. Chen, "Hypothalamic glucocorticoid receptor in CRF neurons is essential for HPA axis habituation to repeated stressor," *bioRxiv*. bioRxiv, p. 2020.11.30.402024, Nov. 30, 2020. doi: 10.1101/2020.11.30.402024.

[245] J. P. Herman, "Neural control of chronic stress adaptation," *Front. Behav. Neurosci.*, vol. 7, no. MAY, p. 61, May 2013, doi: 10.3389/fnbeh.2013.00061.

[246] N. Grissom and S. Bhatnagar, "Habituation to repeated stress: Get used to it," *Neurobiol. Learn. Mem.*, vol. 92, no. 2, pp. 215–224, Sep. 2009, doi: 10.1016/j.nlm.2008.07.001.

[247] B. Valério-Gomes, D. M. Guimarães, D. Szczupak, and R. Lent, "The Absolute Number of Oligodendrocytes in the Adult Mouse Brain," *Front. Neuroanat.*, vol. 12, p. 90, Oct. 2018, doi: 10.3389/fnana.2018.00090.

[248] B. D. Rood and G. J. De Vries, "Vasopressin innervation of the mouse (Mus musculus) brain and spinal cord," *J. Comp. Neurol.*, vol. 519, no. 12, pp. 2434–2474, Aug. 2011, doi: 10.1002/cne.22635.

[249] C. Decavel and A. N. Van Den Pol, "GABA: A dominant neurotransmitter in the hypothalamus," *J. Comp. Neurol.*, vol. 302, no. 4, pp. 1019–1037, 1990, doi: 10.1002/cne.903020423.

[250] Y. Kim, G. R. Yang, K. Pradhan, K. U. Venkataraju, M. Bota, L. C. García del Molino, G. Fitzgerald, K. Ram, M. He, J. M. Levine, P. Mitra, Z. J. Huang, X.-J. Wang, and P. Osten, "Brain-wide Maps Reveal Stereotyped Cell-Type-Based Cortical Architecture and Subcortical Sexual Dimorphism," *Cell*, vol. 171, no. 2, pp. 456-469.e22, Oct. 2017, doi: 10.1016/j.cell.2017.09.020.

[251] T. Goodman and M. K. Hajihosseini, "Hypothalamic tanycytes—masters and servants of metabolic, neuroendocrine, and neurogenic functions," *Front. Neurosci.*, vol. 9, no. OCT, p. 387, Oct. 2015, doi: 10.3389/fnins.2015.00387.

[252] A. B. Bialkowska, V. W. Yang, and S. K. Mallipattu, "Krüppel-like factors in mammalian stem cells and development," *Dev.*, vol. 144, no. 5, pp. 737–754, Mar. 2017, doi: 10.1242/dev.145441.

[253] B. B. McConnell and V. W. Yang, "Mammalian Krüppel-Like Factors in Health and Diseases," *Physiol. Rev.*, vol. 90, no. 4, p. 1337, Oct. 2010, doi: 10.1152/PHYSREV.00058.2009.

[254] C. E. Manning, A. L. Eagle, C. C. Kwiatkowski, R. Achargui, H. Woodworth, E. Potter, Y. Ohnishi, G. M. Leinninger, and A. J. Robison, "Hippocampal Subgranular Zone FosB Expression Is Critical for Neurogenesis and Learning," *Neuroscience*, vol. 406, pp. 225–233, May 2019, doi: 10.1016/J.NEUROSCIENCE.2019.03.022.

[255] A. R. Malik, E. Liszewska, and J. Jaworski, "Matricellular proteins of the Cyr61/CTGF/NOV (CCN) family and the nervous system," *Front. Cell. Neurosci.*, vol. 9, no. JUNE, pp. 1–13, 2015, doi: 10.3389/fncel.2015.00237.

[256] L. Yuniati, B. Scheijen, L. T. van der Meer, and F. N. van Leeuwen, "Tumor suppressors BTG1 and BTG2: Beyond growth control," *J. Cell. Physiol.*, vol. 234, no. 5, p. 5379, May 2019, doi: 10.1002/JCP.27407.

[257] A. Rodríguez-Rodríguez, I. Lazcano, E. Sánchez-Jaramillo, R. M. Uribe, L. Jaimes-Hoy, P. Joseph-Bravo, and J. L. Charli, "Tanycytes and the control of thyrotropin-releasing hormone flux into portal capillaries," *Frontiers in Endocrinology*, vol. 10, no. JUN. Frontiers Media S.A., p. 401, Jun. 25, 2019. doi: 10.3389/fendo.2019.00401.

[258] J. Chow and E. Heard, "X inactivation and the complexities of silencing a sex chromosome.," *Curr. Opin. Cell Biol.*, vol. 21, no. 3, pp. 359–66, Jun. 2009, doi: 10.1016/j.ceb.2009.04.012.

[259] R. H. Oakley and J. A. Cidlowski, "The biology of the glucocorticoid receptor: New

signaling mechanisms in health and disease," *J. Allergy Clin. Immunol.*, vol. 132, no. 5, pp. 1033–1044, Nov. 2013, doi: 10.1016/j.jaci.2013.09.007.

[260] W. C. Langston and B. L. Robinson, "Castration atrophy a chronological study of uterine changes following bilateral ovariectomy in the albino rat," *Endocrinology*, vol. 19, no. 1, pp. 51–62, Jan. 1935, doi: 10.1210/endo-19-1-51.

[261] E. Boda, "Myelin and oligodendrocyte lineage cell dysfunctions: New players in the etiology and treatment of depression and stress-related disorders," *Eur. J. Neurosci.*, vol. 53, no. 1, pp. 281–297, Jan. 2021, doi: 10.1111/ejn.14621.

[262] R. E. Pepper, K. A. Pitman, C. L. Cullen, and K. M. Young, "How do cells of the oligodendrocyte lineage affect neuronal circuits to influence motor function, memory and mood?," *Front. Cell. Neurosci.*, vol. 12, no. November, pp. 1–14, Nov. 2018, doi: 10.3389/fncel.2018.00399.

[263] C. Habermacher, M. C. Angulo, and N. Benamer, "Glutamate versus GABA in neuron–oligodendroglia communication," *GLIA*, vol. 67, no. 11. John Wiley and Sons Inc., pp. 2092–2106, Nov. 01, 2019. doi: 10.1002/glia.23618.

[264] L. M. De Biase, A. Nishiyama, and D. E. Bergles, "Excitability and synaptic communication within the oligodendrocyte lineage," *J. Neurosci.*, vol. 30, no. 10, pp. 3600–3611, Mar. 2010, doi: 10.1523/JNEUROSCI.6000-09.2010.

[265] K. M. Young, K. Psachoulia, R. B. Tripathi, S. J. Dunn, L. Cossell, D. Attwell, K. Tohyama, and W. D. Richardson, "Oligodendrocyte dynamics in the healthy adult CNS: Evidence for myelin remodeling," *Neuron*, vol. 77, no. 5, pp. 873–885, Mar. 2013, doi: 10.1016/j.neuron.2013.01.006.

[266] K. A. Nave, "Myelination and support of axonal integrity by glia," *Nature*, vol. 468, no. 7321. Nature Publishing Group, pp. 244–252, Nov. 11, 2010. doi: 10.1038/nature09614.

[267] N. Golan, K. Adamsky, E. Kartvelishvily, D. Brockschnieder, W. Möbius, I. Spiegel, A. D. Roth, C. E. Thomson, G. Rechavi, and E. Peles, "Identification of Tmem10/Opalin as an oligodendrocyte enriched gene using expression profiling combined with genetic cell ablation," *Glia*, vol. 56, no. 11, pp. 1176–1186, Aug. 2008, doi: 10.1002/glia.20688.

[268] M. Wegner, "Expression of transcription factors during oligodendroglial development," *Microsc. Res. Tech.*, vol. 52, no. 6, pp. 746–752, 2001, doi: 10.1002/jemt.1059.

[269] K. V. Wagner, D. Marinescu, J. Hartmann, X. D. Wang, C. Labermaier, S. H. Scharf, C. Liebl, M. Uhr, F. Holsboer, M. B. Müller, and M. V. Schmidt, "Differences in FKBP51 regulation following chronic social defeat stress correlate with individual stress sensitivity: Influence of paroxetine treatment," *Neuropsychopharmacology*, vol. 37, no. 13, pp. 2797–2808, Dec. 2012, doi: 10.1038/NPP.2012.150.

[270] Y. M. Ulrich-Lai, H. F. Figueiredo, M. M. Ostrander, D. C. Choi, W. C. Engeland, and J. P. Herman, "Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner," *Am. J. Physiol. Endocrinol. Metab.*, vol. 291, no. 5, 2006, doi: 10.1152/AJPENDO.00070.2006.

[271] P. Willner, "The chronic mild stress (CMS) model of depression: History, evaluation and usage," *Neurobiol. Stress*, vol. 6, pp. 78–93, Feb. 2017, doi: 10.1016/j.ynstr.2016.08.002.

[272] H. Dadomo, L. Gioiosa, J. Cigalotti, G. Ceresini, S. Parmigiani, and P. Palanza, "What is stressful for females? Differential effects of unpredictable environmental or social stress in CD1 female mice," *Horm. Behav.*, vol. 98, pp. 22–32, Feb. 2018, doi: 10.1016/j.yhbeh.2017.11.013.

[273] N. Kokras and C. Dalla, "Sex differences in animal models of psychiatric disorders," *British journal of pharmacology*, vol. 171, no. 20. pp. 4595–4619, Oct. 01, 2014. doi: 10.1111/bph.12710.

[274] C. Dalla, K. Antoniou, G. Drossopoulou, M. Xagoraris, N. Kokras, A. Sfikakis, and Z. Papadopoulou-Daifoti, "Chronic mild stress impact: are females more vulnerable?,"

Neuroscience, vol. 135, no. 3, pp. 703–14, Jan. 2005, doi: 10.1016/j.neuroscience.2005.06.068.

[275] A. P. Borrow, A. L. Heck, A. M. Miller, J. A. Sheng, S. A. Stover, R. M. Daniels, N. J. Bales, T. K. Fleury, and R. J. Handa, "Chronic variable stress alters hypothalamic-pituitary-adrenal axis function in the female mouse," *Physiol. Behav.*, vol. 209, no. 7, p. 112613, Oct. 2019, doi: 10.1016/j.physbeh.2019.112613.

[276] "Volume 134, issue 4," *Psychopharmacology (Berl).*, vol. 134, no. 4, 1997, [Online]. Available: https://link.springer.com/journal/213/volumes-and-issues/134-4

[277] C. Dalla, K. Antoniou, G. Drossopoulou, M. Xagoraris, N. Kokras, a Sfikakis, and Z. Papadopoulou-Daifoti, "Chronic mild stress impact: are females more vulnerable?," *Neuroscience*, vol. 135, no. 3, pp. 703–14, Jan. 2005, doi: 10.1016/j.neuroscience.2005.06.068.

[278] K. Malki, Y. S. Mineur, M. G. Tosto, J. Campbell, P. Karia, I. Jumabhoy, F. Sluyter, W. E. Crusio, and L. C. Schalkwyk, "Pervasive and opposing effects of Unpredictable Chronic Mild Stress (UCMS) on hippocampal gene expression in BALB/cJ and C57BL/6J mouse strains," *BMC Genomics*, vol. 16, no. 1, p. 262, Dec. 2015, doi: 10.1186/s12864-015-1431-6.

[279] M. C. Schweizer, M. S. H. H. Henniger, and I. Sillaber, "Chronic mild stress (CMS) in mice: Of anhedonia, 'anomalous anxiolysis' and activity," *PLoS One*, vol. 4, no. 1, p. e4326, Jan. 2009, doi: 10.1371/journal.pone.0004326.

[280] Z. J. Rosinger, J. S. Jacobskind, R. M. De Guzman, N. J. Justice, and D. G. Zuloaga, "A sexually dimorphic distribution of corticotropin-releasing factor receptor 1 in the paraventricular hypothalamus," *Neuroscience*, vol. 409, no. May, pp. 195–203, Jun. 2019, doi: 10.1016/j.neuroscience.2019.04.045.

[281] P. A. Fletcher, K. Smiljanic, R. Maso Prévide, J. R. Iben, T. Li, M. B. Rokic, A. Sherman, S. L. Coon, and S. S. Stojilkovic, "Cell Type- and Sex-Dependent Transcriptome Profiles of Rat Anterior Pituitary Cells," *Front. Endocrinol. (Lausanne).*, vol. 10, p. 623, Sep. 2019, doi: 10.3389/fendo.2019.00623.

[282] G. J. de Vries, "Sex Differences in Neurotransmitters Systems; Vasopressin as an Example," in *Handbook of Neurochemistry and Molecular Neurobiology*, Boston, MA: Springer US, 2007, pp. 487–512. doi: 10.1007/978-0-387-30405-2_12.

[283] R. Stoop, "Neuromodulation by Oxytocin and Vasopressin," *Neuron*, vol. 76, no. 1, pp. 142–159, Oct. 2012, doi: 10.1016/J.NEURON.2012.09.025.

[284] G. J. de Vries, "Sex differences in vasopressin and oxytocin innervation of the brain," *Prog. Brain Res.*, vol. 170, no. 08, pp. 17–27, Jan. 2008, doi: 10.1016/S0079-6123(08)00402-0.

[285] K. M. Dumais and A. H. Veenema, "Vasopressin and oxytocin receptor systems in the brain: Sex differences and sex-specific regulation of social behavior," *Front. Neuroendocrinol.*, vol. 40, pp. 1–23, Jan. 2016, doi: 10.1016/j.yfrne.2015.04.003.

[286] C. J. W. Smith, B. T. DiBenedictis, and A. H. Veenema, "Comparing vasopressin and oxytocin fiber and receptor density patterns in the social behavior neural network: Implications for cross-system signaling," *Front. Neuroendocrinol.*, vol. 53, p. 100737, Apr. 2019, doi: 10.1016/j.yfrne.2019.02.001.

[287] T. A. Ishunina and D. F. Swaab, "Vasopressin and oxytocin neurons of the human supraoptic and paraventricular nucleus; size changes in relation to age and sex," *J. Clin. Endocrinol. Metab.*, vol. 84, no. 12, pp. 4637–4644, Dec. 1999, doi: 10.1210/jcem.84.12.6187.

[288] M. Q. Steinman, S. A. Laredo, E. M. Lopez, C. E. Manning, R. C. Hao, I. E. Doig, K. L. Campi, A. E. Flowers, J. K. Knight, and B. C. Trainor, "Hypothalamic vasopressin systems are more sensitive to the long term effects of social defeat in males versus females," *Psychoneuroendocrinology*, vol. 51, pp. 122–134, Jan. 2015, doi: 10.1016/j.psyneuen.2014.09.009.

[289] S. C. C. Robins, I. Stewart, D. E. E. McNay, V. Taylor, C. Giachino, M. Goetz, J. Ninkovic, N. Briancon, E. Maratos-Flier, J. S. S. Flier, M. V. V. Kokoeva, and M. Placzek, "α-Tanycytes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors," *Nat. Commun.*, vol. 4, no. 1, p. 2049, Oct. 2013, doi: 10.1038/ncomms3049.

[290] K. Rizzoti and R. Lovell-Badge, "Pivotal role of median eminence tanycytes for hypothalamic function and neurogenesis," *Mol. Cell. Endocrinol.*, vol. 445, pp. 7–13, Apr. 2017, doi: 10.1016/j.mce.2016.08.020.

[291] D. A. Lee, S. Yoo, T. Pak, J. Salvatierra, E. Velarde, S. Aja, and S. Blackshaw, "Dietary and sex-specific factors regulate hypothalamic neurogenesis in young adult mice," *Front. Neurosci.*, vol. 8, no. 8 JUN, pp. 1–11, 2014, doi: 10.3389/fnins.2014.00157.

[292] D. Trabzuni, A. Ramasamy, S. Imran, R. Walker, C. Smith, M. E. Weale, J. Hardy, M. Ryten, and N. A. B. E. Consortium, "Widespread sex differences in gene expression and splicing in the adult human brain," *Nat. Commun.*, vol. 4, no. 1, p. 2771, Dec. 2013, doi: 10.1038/ncomms3771.

[293] B. Gegenhuber and J. Tollkuhn, "Signatures of sex: Sex differences in gene expression in the vertebrate brain," *WIREs Dev. Biol.*, vol. 9, no. 1, Jan. 2020, doi: 10.1002/wdev.348.

[294] S. Naqvi, A. K. Godfrey, J. F. Hughes, M. L. Goodheart, R. N. Mitchell, and D. C. Page, "Conservation, acquisition, and functional impact of sex-biased gene expression in mammals," *Science (80-.).*, vol. 365, no. 6450, p. eaaw7317, Jul. 2019, doi: 10.1126/science.aaw7317.

[295] M. Oliva, M. Muñoz-Aguirre, S. Kim-Hellmuth, V. Wucher, A. D. H. Gewirtz, D. J. Cotter, P. Parsana, S. Kasela, B. Balliu, A. Viñuela, S. E. Castel, P. Mohammadi, F. Aguet, Y. Zou, E. A. Khramtsova, A. D. Skol, D. Garrido-Martín, F. Reverter, A. Brown, P. Evans, E. R. Gamazon, A. Payne, R. Bonazzola, A. N. Barbeira, A. R. Hamel, A. Martinez-Perez, J. M. Soria, B. L. Pierce, M. Stephens, E. Eskin, E. T. Dermitzakis, A. V. Segrè, H. K. Im, B. E. Engelhardt, K. G. Ardlie, S. B. Montgomery, A. J. Battle, T. Lappalainen, R. Guigó, and B. E. Stranger, "The impact of sex on gene expression across human tissues," *Science (80-.).*, vol. 369, no. 6509, p. eaba3066, Sep. 2020, doi: 10.1126/science.aba3066.

[296] C. J. Walker, M. E. Schroeder, B. A. Aguado, K. S. Anseth, and L. A. Leinwand, "Matters of the heart: Cellular sex differences," *J. Mol. Cell. Cardiol.*, vol. 160, pp. 42–55, Nov. 2021, doi: 10.1016/J.YJMCC.2021.04.010.

[297] L. Huang, J. Liao, Y. Chen, C. Zou, H. Zhang, X. Yang, Q. Zhang, T. Li, L. Mo, Y. Zeng, M. Bao, F. Zhang, Y. Ye, Z. Yang, J. Cheng, and Z. Mo, "Single-cell transcriptomes reveal characteristic features of cell types within the human adrenal microenvironment," *Journal of Cellular Physiology*, vol. 236, no. 11. pp. 7308–7321, 2021. doi: 10.1002/jcp.30398.

[298] Z. Huang, B. Chen, X. Liu, H. Li, L. Xie, Y. Gao, R. Duan, Z. Li, J. Zhang, Y. Zheng, and W. Su, "Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 118, no. 33, Aug. 2021, doi: 10.1073/PNAS.2023216118/-/DCSUPPLEMENTAL.

[299] R. Puralewski, G. Vasilakis, and M. L. Seney, "Sex-related factors influence expression of mood-related genes in the basolateral amygdala differentially depending on age and stress exposure," *Biol. Sex Differ.*, vol. 7, no. 1, p. 50, 2016, doi: 10.1186/s13293-016-0106-6.

[300] K. Barko, W. Paden, K. M. Cahill, M. L. Seney, and R. W. Logan, "Sex-Specific Effects of Stress on Mood-Related Gene Expression," *Mol. Neuropsychiatry*, vol. 5, no. 3, pp. 162–176, Jun. 2019, doi: 10.1159/000499105.

[301] J. Kalucka, L. P. M. H. de Rooij, J. Goveia, K. Rohlenova, S. J. Dumas, E. Meta, N. V. Conchinha, F. Taverna, L. A. Teuwen, K. Veys, M. García-Caballero, S. Khan, V. Geldhof, L. Sokol, R. Chen, L. Treps, M. Borri, P. de Zeeuw, C. Dubois, T. K. Karakach, K. D. Falkenberg, M. Parys, X. Yin, S. Vinckier, Y. Du, R. A. Fenton, L. Schoonjans, M. Dewerchin, G. Eelen, B. Thienpont, L. Lin, L. Bolund, X. Li, Y. Luo, and P. Carmeliet,

"Single-Cell Transcriptome Atlas of Murine Endothelial Cells," *Cell*, vol. 180, no. 4, pp. 764-779.e20, 2020, doi: 10.1016/j.cell.2020.01.015.

[302] H. Wolburg, S. Noell, A. Mack, K. Wolburg-Buchholz, and P. Fallier-Becker, "Brain endothelial cells and the glio-vascular complex," *Cell Tissue Res.*, vol. 335, no. 1, pp. 75–96, 2009, doi: 10.1007/s00441-008-0658-9.

[303] S. Pong, R. Karmacharya, M. Sofman, J. R. Bishop, and P. Lizano, "The Role of Brain Microvascular Endothelial Cell and Blood-Brain Barrier Dysfunction in Schizophrenia," *Complex Psychiatry*, vol. 6, no. 1–2, pp. 30–46, 2020, doi: 10.1159/000511552.

[304] S. P. Pantazatos, Y. Y. Huang, G. B. Rosoklija, A. J. Dwork, V. Arango, and J. J. Mann, "Whole-transcriptome brain expression and exon-usage profiling in major depression and suicide: Evidence for altered glial, endothelial and ATPase activity," *Mol. Psychiatry*, vol. 22, no. 5, pp. 760–773, 2017, doi: 10.1038/mp.2016.130.

[305] C. Greene, N. Hanley, and M. Campbell, "Blood-brain barrier associated tight junction disruption is a hallmark feature of major psychiatric disorders," *Transl. Psychiatry 2020 101*, vol. 10, no. 1, pp. 1–10, Nov. 2020, doi: 10.1038/s41398-020-01054-3.

[306] K. A. Dudek, L. Dion-Albert, M. Lebel, K. LeClair, S. Labrecque, E. Tuck, C. Ferrer Perez, S. A. Golden, C. Tamminga, G. Turecki, N. Mechawar, S. J. Russo, and C. Menard, "Molecular adaptations of the blood–brain barrier promote stress resilience vs. depression," *Proc. Natl. Acad. Sci.*, vol. 117, no. 6, pp. 3326–3336, Feb. 2020, doi: 10.1073/pnas.1914655117.

[307] J. Kealy, C. Greene, and M. Campbell, "Blood-brain barrier regulation in psychiatric disorders," *Neurosci. Lett.*, vol. 726, no. June, pp. 0–1, May 2020, doi: 10.1016/j.neulet.2018.06.033.

[308] D. N. Krause, S. P. Duckles, and D. A. Pelligrino, "Influence of sex steroid hormones on cerebrovascular function," *J. Appl. Physiol.*, vol. 101, no. 4, pp. 1252–1261, 2006, doi: 10.1152/JAPPLPHYSIOL.01095.2005.

[309] M. J. Girgenti, E. S. Wohleb, S. Mehta, S. Ghosal, M. V. Fogaca, and R. S. Duman, "Prefrontal cortex interneurons display dynamic sex-specific stress-induced transcriptomes," *Transl. Psychiatry*, vol. 9, no. 1, pp. 1–13, Dec. 2019, doi: 10.1038/s41398-019-0642-z.

[310] Z. S. Lorsch, Y.-H. E. Loh, I. Purushothaman, D. M. Walker, E. M. Parise, M. Salery, M. E. Cahill, G. E. Hodes, M. L. Pfau, H. Kronman, P. J. Hamilton, O. Issler, B. Labonté, A. E. Symonds, M. Zucker, T. Y. Zhang, M. J. Meaney, S. J. Russo, L. Shen, R. C. Bagot, and E. J. Nestler, "Estrogen receptor α drives pro-resilient transcription in mouse models of depression," *Nat. Commun.*, vol. 9, no. 1, p. 1116, Dec. 2018, doi: 10.1038/s41467-018-03567-4.

[311] C. H. Holland, J. Tanevski, J. Perales-Patón, J. Gleixner, M. P. Kumar, E. Mereu, B. A. Joughin, O. Stegle, D. A. Lauffenburger, H. Heyn, B. Szalai, and J. Saez-Rodriguez, "Robustness and applicability of transcription factor and pathway analysis tools on single-cell RNA-seq data," *Genome Biol.*, vol. 21, no. 1, p. 36, Dec. 2020, doi: 10.1186/s13059-020-1949-z.

[312] C. H. Holland, B. Szalai, and J. Saez-Rodriguez, "Transfer of regulatory knowledge from human to mouse for functional genomics analysis," *Biochim. Biophys. Acta - Gene Regul. Mech.*, vol. 1863, no. 6, p. 194431, Jun. 2020, doi: 10.1016/j.bbagrm.2019.194431.

[313] L. Garcia-Alonso, C. H. Holland, M. M. Ibrahim, D. Turei, and J. Saez-Rodriguez, "Benchmark and integration of resources for the estimation of human transcription factor activities," *Genome Res.*, vol. 29, no. 8, pp. 1363–1375, Aug. 2019, doi: 10.1101/GR.240663.118.

[314] R. S. Duman, G. Sanacora, and J. H. Krystal, "Altered neurotransmitter deficits and reversal by novel treatments," *Neuron*, vol. 102, no. 62, pp. 75–90, Apr. 2020, doi: 10.1016/j.neuron.2019.03.013.Altered.

[315] K. Yasuda, T. Maki, H. Kinoshita, S. Kaji, M. Toyokawa, R. Nishigori, Y. Kinoshita, Y. Ono, A. Kinoshita, and R. Takahashi, "Sex-specific differences in transcriptomic profiles and cellular characteristics of oligodendrocyte precursor cells," *Stem Cell Res.*, vol. 46, no. May, p. 101866, Jul. 2020, doi: 10.1016/j.scr.2020.101866.

[316] M. Cerghet, R. P. Skoff, D. Bessert, Z. Zhang, C. Mullins, and M. S. Ghandour, "Proliferation and death of oligodendrocytes and myelin proteins are differentially regulated in male and female rodents," *J. Neurosci.*, vol. 26, no. 5, pp. 1439–1447, 2006, doi: 10.1523/JNEUROSCI.2219-05.2006.

[317] M. Swamydas, D. Bessert, and R. Skoff, "Sexual dimorphism of oligodendrocytes is mediated by differential regulation of signaling pathways," *J. Neurosci. Res.*, vol. 87, no. 15, pp. 3306–3319, Nov. 2009, doi: 10.1002/jnr.21943.

[318] M. Marin-Husstege, M. Muggironi, D. Raban, R. P. Skoff, and P. Casaccia-Bonnefil, "Oligodendrocyte progenitor proliferation and maturation is differentially regulated by male and female sex steroid hormones," *Dev. Neurosci.*, vol. 26, no. 2–4, pp. 245–254, 2004, doi: 10.1159/000082141.

[319] G. Alonso, "Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain," *Glia*, vol. 31, no. 3, pp. 219–231, Sep. 2000, doi: 10.1002/1098-1136(200009)31:3<219::AID-GLIA30>3.0.CO;2-R.

[320] R. R. Voskuhl and S. M. Gold, "Sex-related factors in multiple sclerosis susceptibility and progression," *Nature Reviews Neurology*, vol. 8, no. 5. Nat Rev Neurol, pp. 255–263, May 2012. doi: 10.1038/nrneurol.2012.43.

[321] A. P. Arnold, "Neuroscience and Biobehavioral Reviews Four Core Genotypes and XY * mouse models : Update on impact on SABV research," *Neurosci. Biobehav. Rev.*, vol. 119, no. September, pp. 1–8, 2020, doi: 10.1016/j.neubiorev.2020.09.021.

[322] R. A. Romanov, A. Zeisel, J. Bakker, F. Girach, A. Hellysaz, R. Tomer, A. Alpár, J. Mulder, F. Clotman, E. Keimpema, B. Hsueh, A. K. Crow, H. Martens, C. Schwindling, D. Calvigioni, J. S. Bains, Z. Máté, G. Szabó, Y. Yanagawa, M.-D. D. Zhang, A. Rendeiro, M. Farlik, M. Uhlén, P. Wulff, C. Bock, C. Broberger, K. Deisseroth, T. Hökfelt, S. Linnarsson, T. L. Horvath, and T. Harkany, "Molecular interrogation of hypothalamic organization reveals distinct dopamine neuronal subtypes," *Nat. Neurosci.*, vol. 20, no. 2, pp. 176–188, Feb. 2017, doi: 10.1038/nn.4462.

[323] L. E. Mickelsen, M. Bolisetty, B. R. Chimileski, A. Fujita, E. J. Beltrami, J. T. Costanzo, J. R. Naparstek, P. Robson, and A. C. Jackson, "Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons," *Nat. Neurosci.*, vol. 22, no. 4, pp. 642–656, Apr. 2019, doi: 10.1038/s41593-019-0349-8.

[324] M. M. McCarthy, L. A. Pickett, J. W. VanRyzin, and K. E. Kight, "Surprising origins of sex differences in the brain," *Horm. Behav.*, vol. 76, pp. 3–10, Nov. 2015, doi: 10.1016/j.yhbeh.2015.04.013.

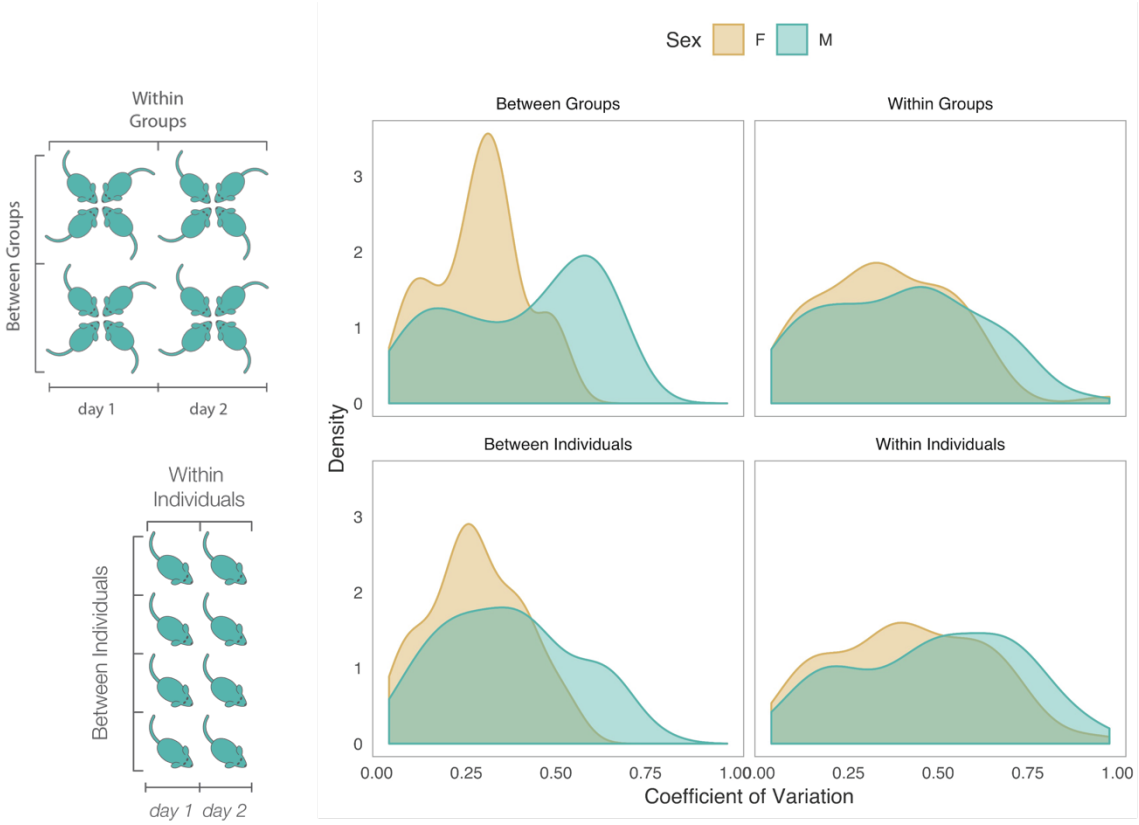
[325] "Package ComplexUpset," *CRAN*. 2021. Accessed: Apr. 28, 2021. [Online]. Available: https://cran.r-project.org/web/packages/ComplexUpset/index.html

[326] W. Chang, "Package shinythemes." Comprehensive R Archive Network (CRAN), Jan. 25, 2021. Accessed: Nov. 23, 2021. [Online]. Available: https://cran.r-project.org/package=shinythemes

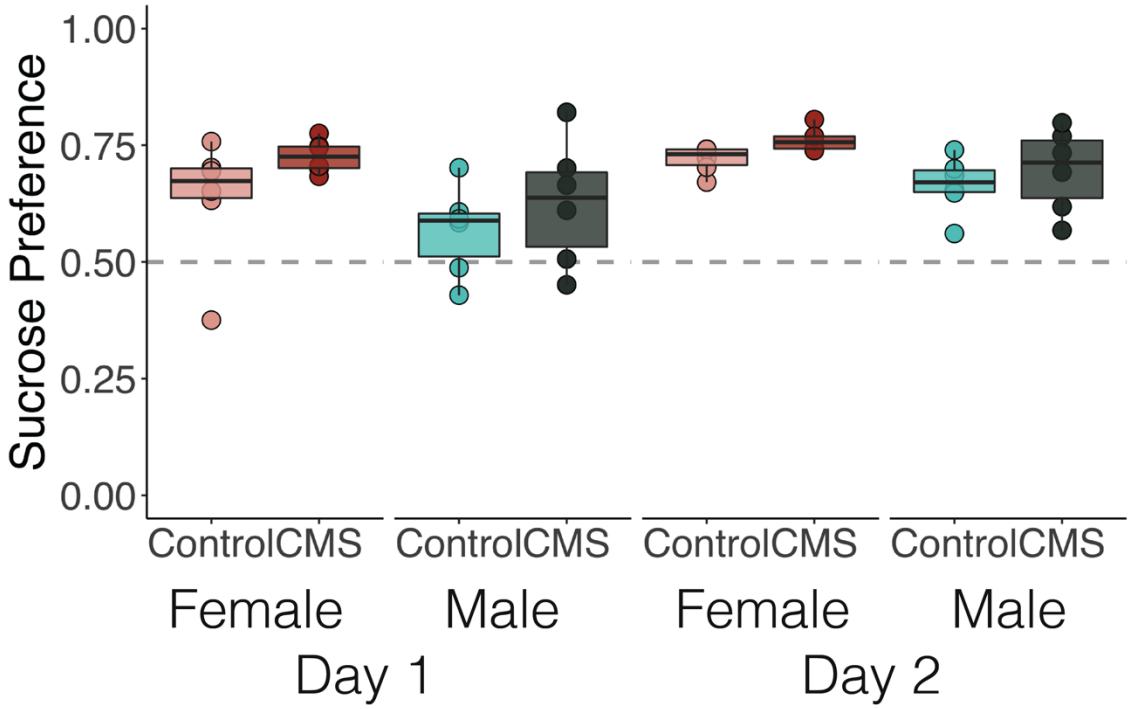
[327] "ANY-maze." https://www.any-maze.com/ (accessed Oct. 15, 2021).

7 | Appendix

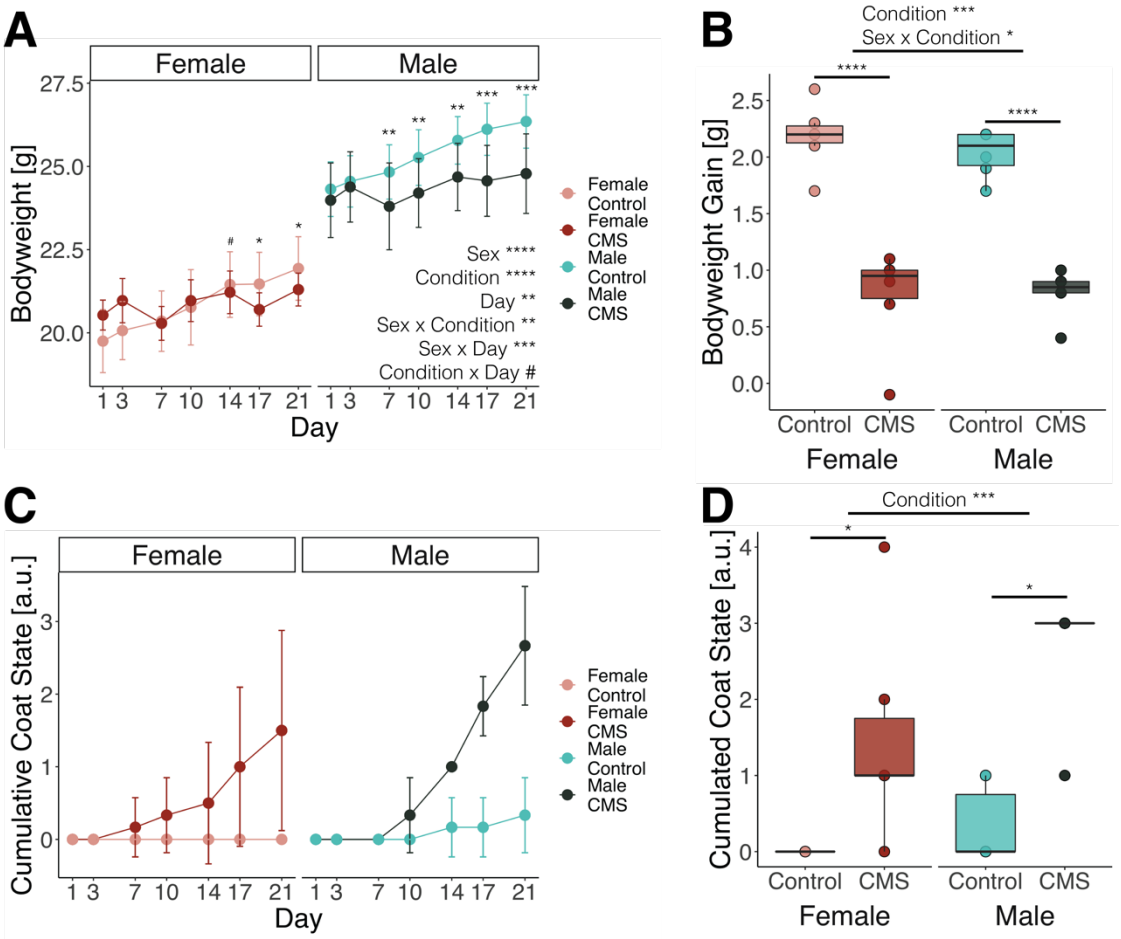
7.1 Supplementary Figures



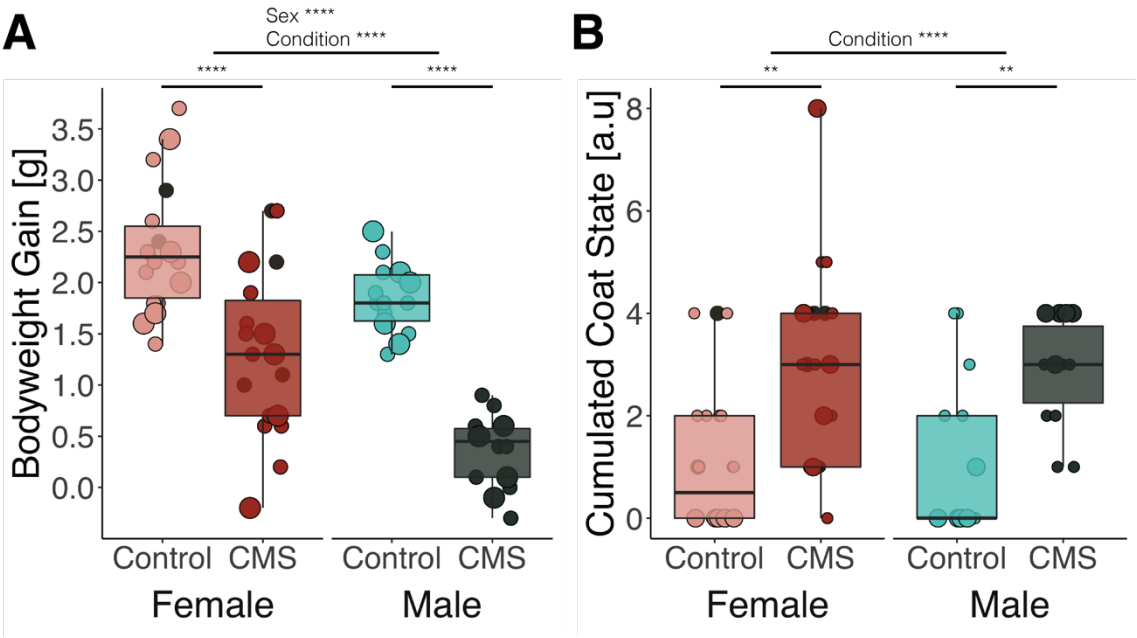
Supplementary Figure 1: Variability in Behaviour in Groups of Male or Female Mice. Groups of four male or four female CD1 mice was housed in semi-naturalistic environment and tens of behavioural features for continuously tracked for days with an automatized video tracking (information about the technology can be found in [119], [121]). Variability (measured as coefficient of variability) is consistently lower in groups of females and in female individuals.



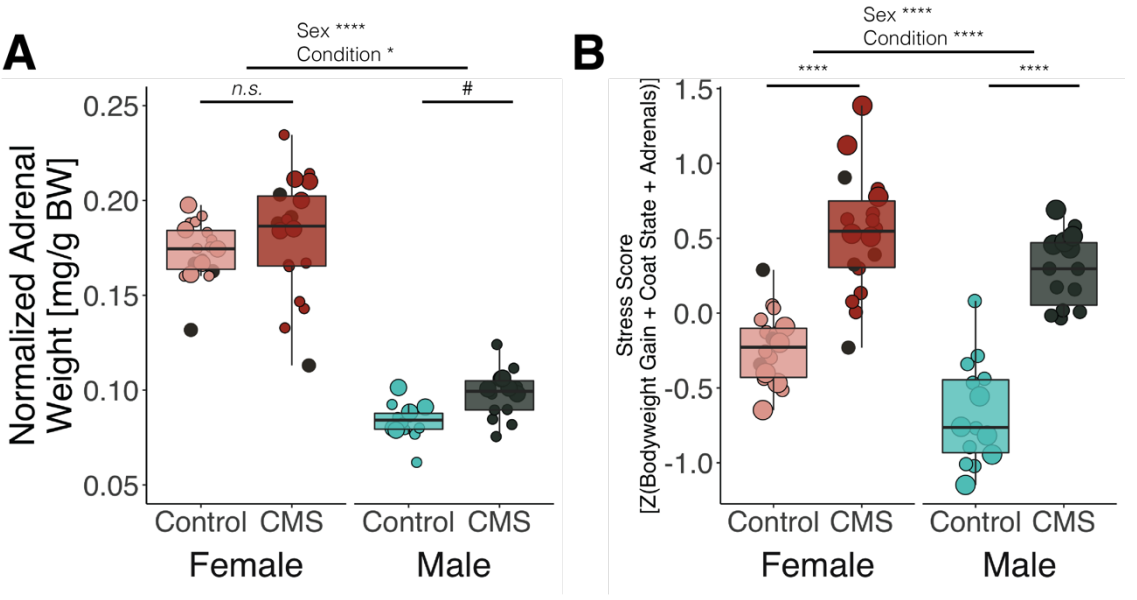
Supplementary Figure 2: Sucrose Preference Test. Sucrose preference displayed separately for the two days of testing.



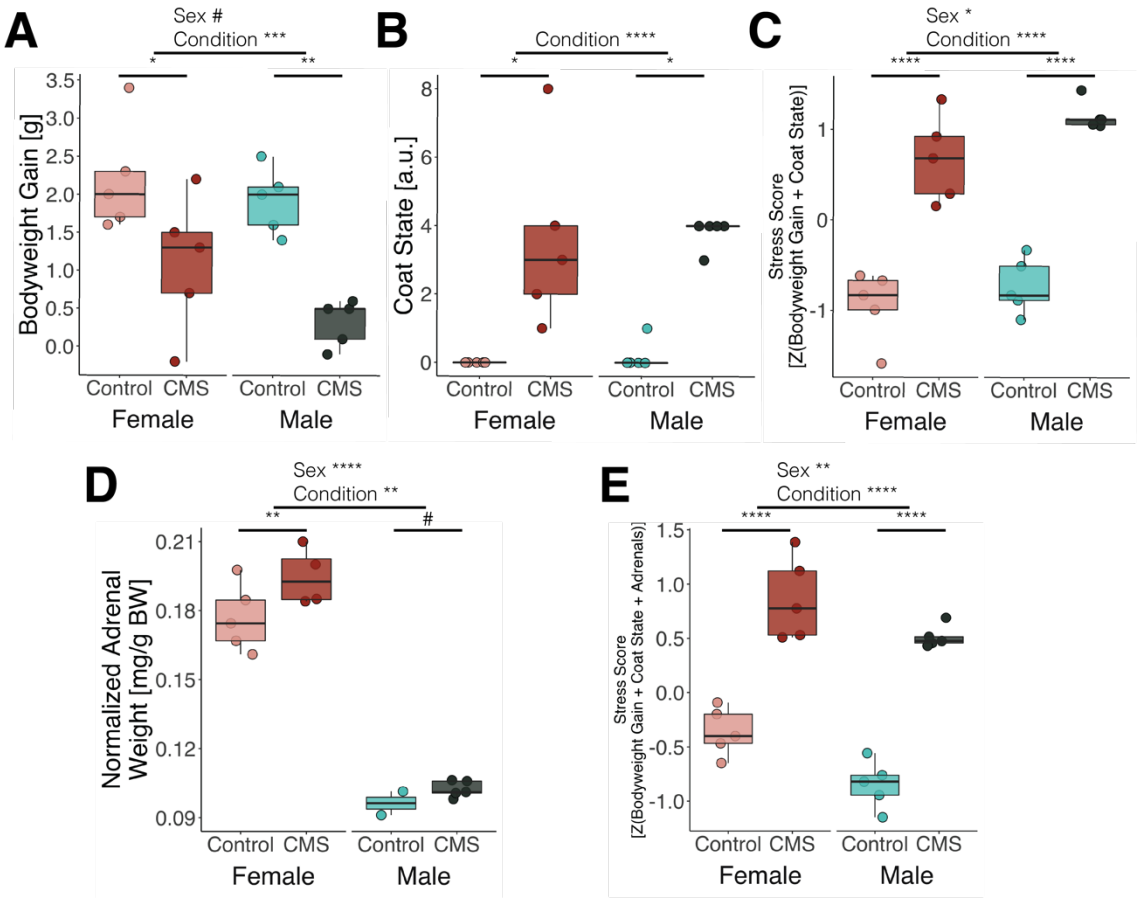
Supplementary Figure 3: Bodyweight Change and Coat State during CMS Monitoring - CORT Experiment. (**A-B**) Bodyweight and (**C-D**) coat state were regularly monitored during the CMS protocol before collecting CORT response to ARS. (**A**) Control male and female mice gained considerable weight during the three weeks of protocol, while CMS mice had a considerable smaller growth (RM two-way ANOVA, Tukey's post-hoc correction). (**B**) As a result, on Day 21 CMS mice were considerable lighter than controls (two-way ANOVA, Tukey's post-hoc correction). (**C**) The coat of CMS mice deteriorated across the three weeks of stress paradigm, (**D**) so that on day 21 they showed a statistically worse coat state (Kruskal-Wallis rank sum test, Dunn's post-hoc test). Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.



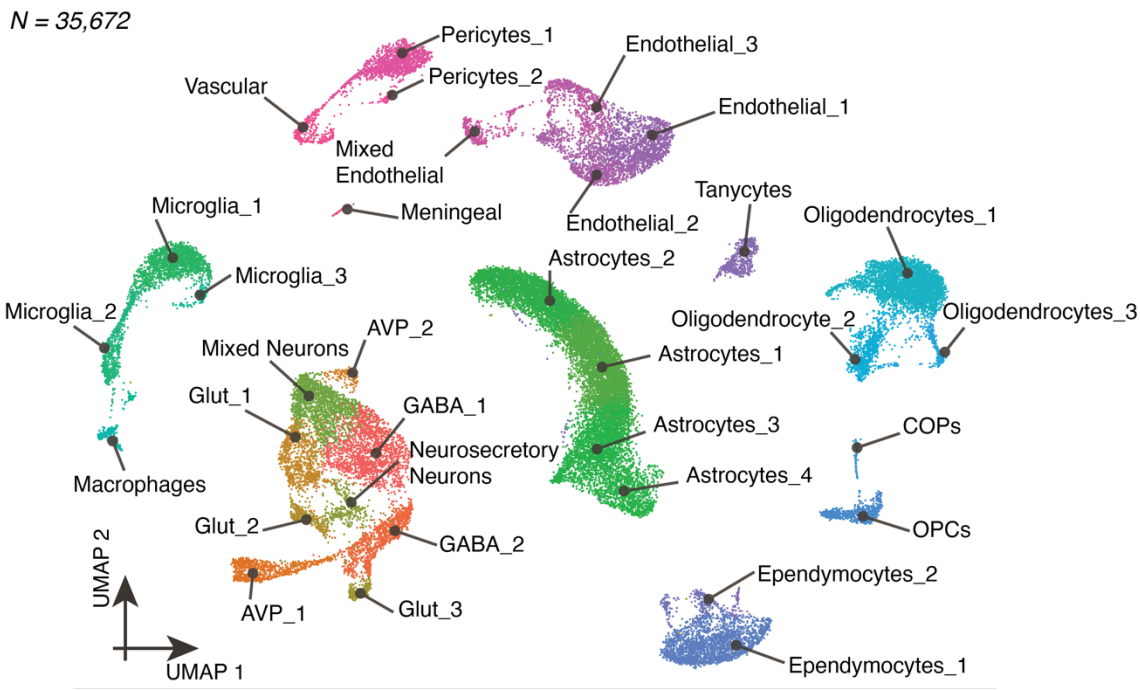
Supplementary Figure 4: Bodyweight Gain and Coat State after CMS - scRNA-seq Experiment. (**A**) Bodyweight and (**B**) coat state on Day 21 of CMS in the scRNA-seq experiment, which were used to compute the stress score. (**A**) CMS mice were considerable lighter than controls (two-way ANOVA, Tukey's post-hoc correction) and (**B**) their coat showed a statistically worse coat state (Kruskal-Wallis rank sum test, Dunn's post-hoc test). Bigger dots show the mice used for the scRNA-seq dataset. Grey dots represent female mice excluded due to poor cycling. Two-way ANOVA, Tukey's post-hoc corrected p-values.



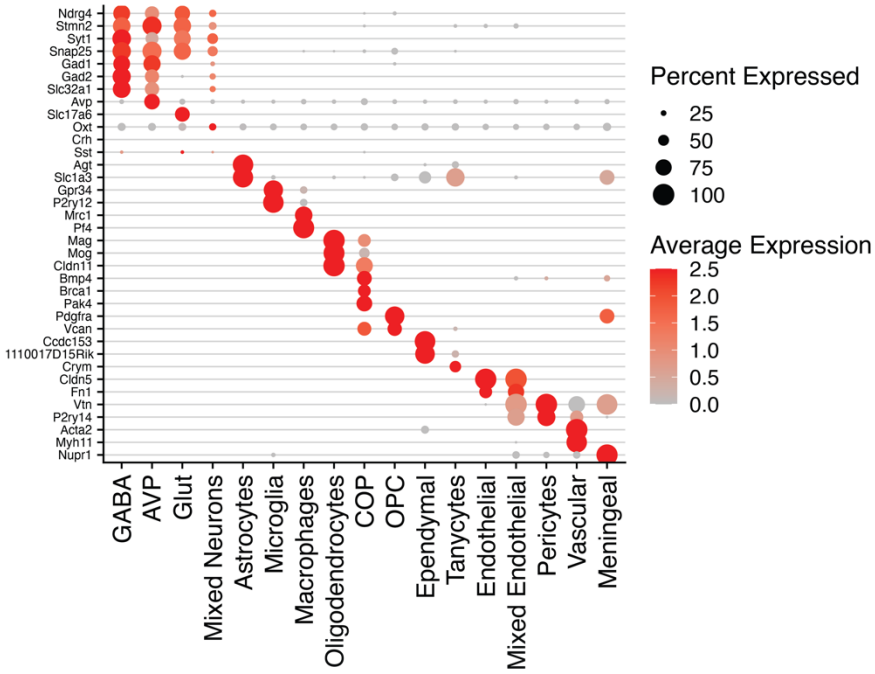
Supplementary Figure 5: Adrenal Weight and Stress Score after CMS - scRNA-seq Experiment. (**A**) Normalized adrenal weight was slightly increased after CMS. (**B**) Stress score integrating the adrenal weight showed a clear stress phenotype for mice exposed to CMS. Bigger dots show the mice used for the scRNA-seq dataset. Grey dots represent female mice excluded due to poor cycling. Two-way ANOVA, Tukey's post-hoc corrected p-values.



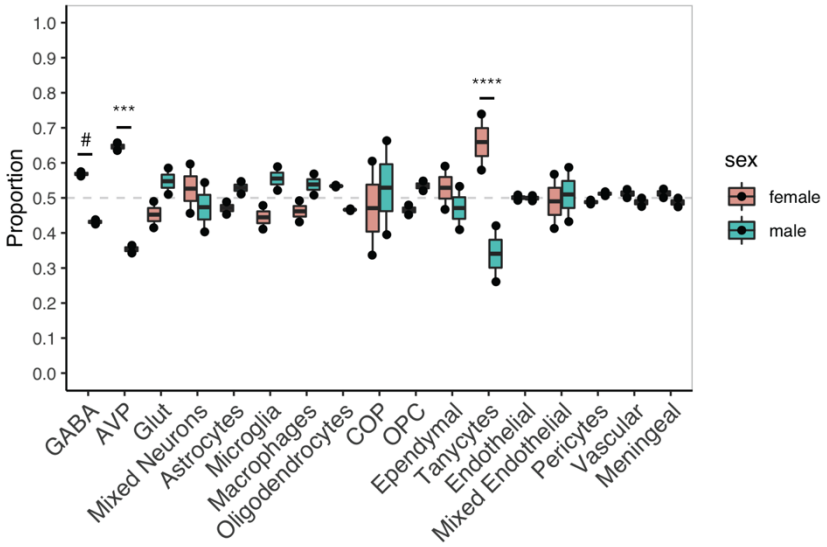
Supplementary Figure 6: Bodyweight Change, Coat State, Adrenal Weight and Stress Score for CMS Mice in the scRNA-seq. Measures from the CMS monitoring for the mice selected for the scRNA-seq experiment which displayed a significant stress phenotype. This included the (**A**) bodyweight gain, and (**B**) coat state at the end of the 21 days, which were combined in a stress score (**C**). After sacrifice, (**D**) adrenal weight was also collected and used to calculate a new stress score (**E**). Boxplots represent the interquartile range (IQR) and median, whiskers are minimum and maximum value ± 1.5 IQR. Two-way ANOVA, Tukey's post-hoc corrected p-values.



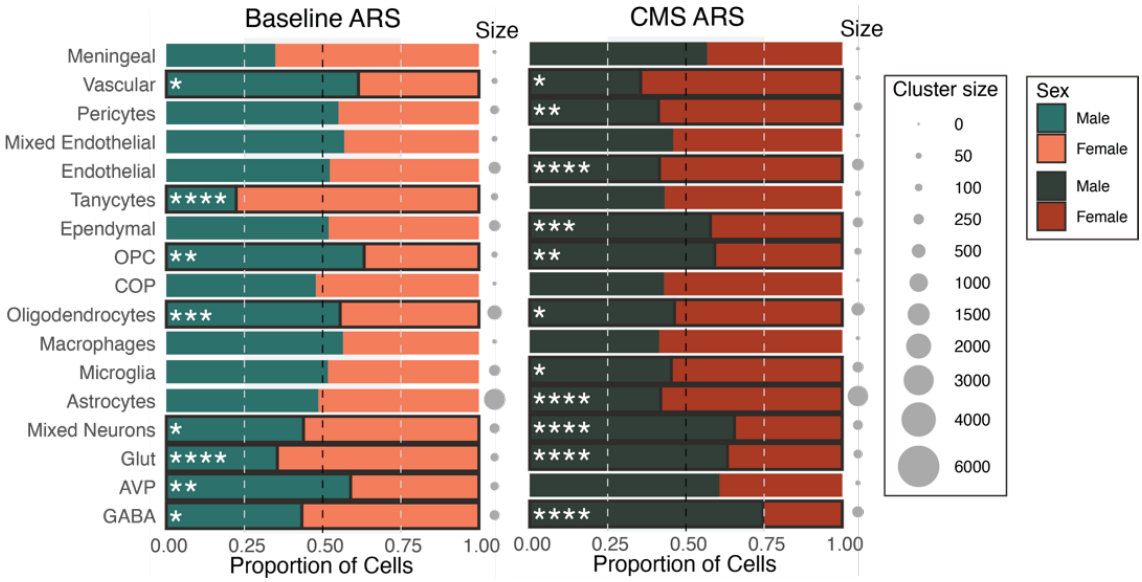
Supplementary Figure 7: High Resolution Clustering of the scRNA-seq Dataset. UMAP plot of the scRNA-seq showing the 33 clusters identified.



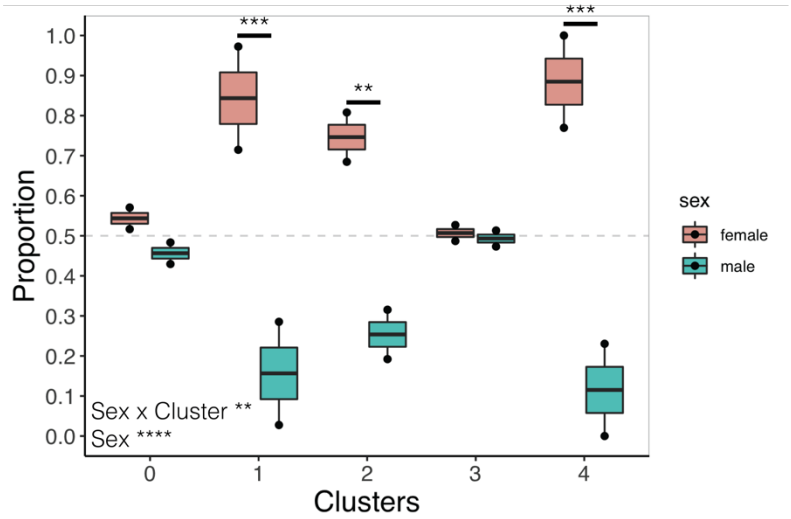
Supplementary Figure 8: Clustering Markers for the scRNA-seq Dataset. Markers used to identify the 17 cell types in the scRNA-seq dataset.



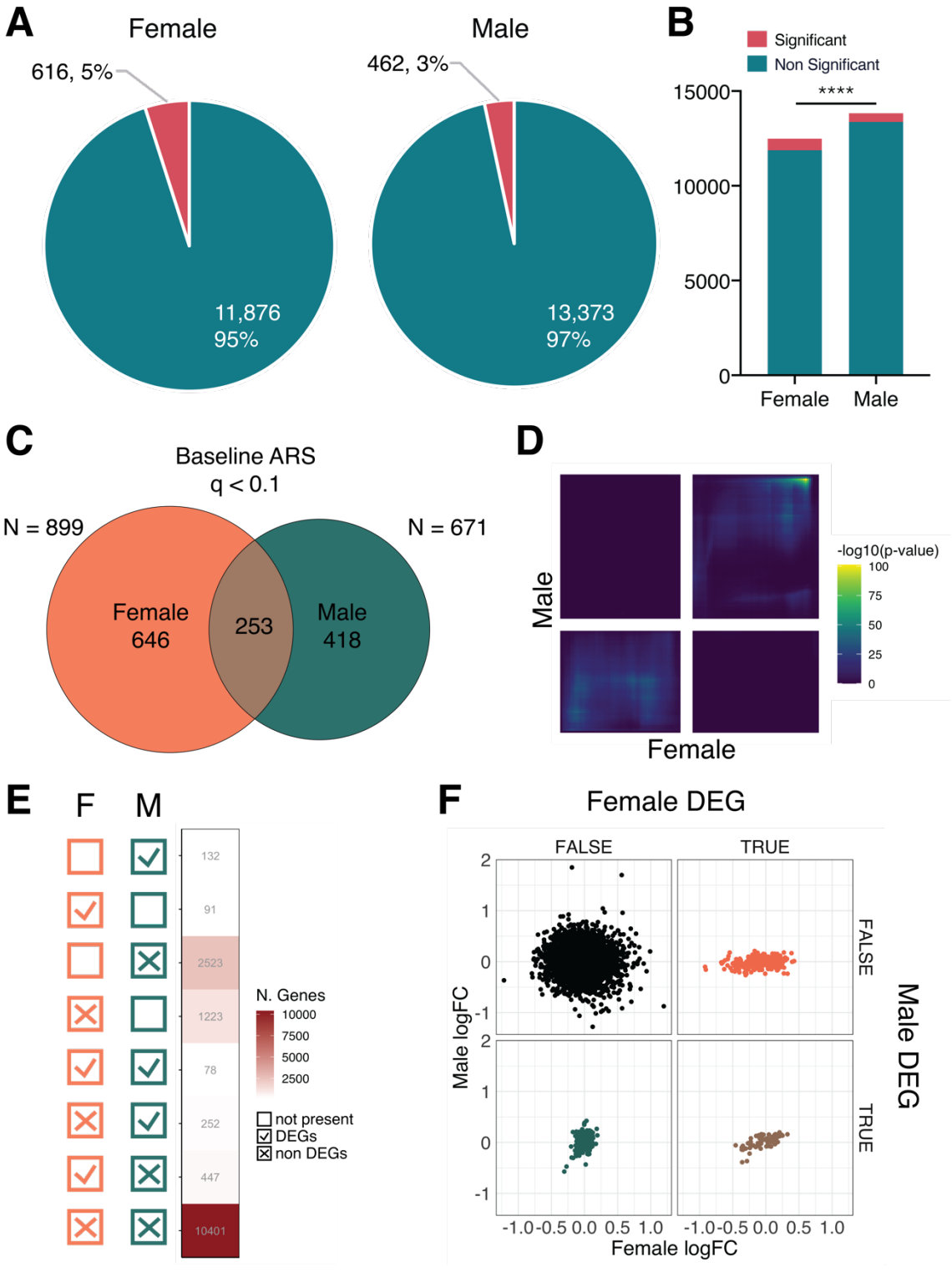
Supplementary Figure 9: Male and Female Cells Distribution in the Two Control Replicates. Proportion of male and female cells across the 17 cell types. Two-way ANOVA, Tukey's post-hoc corrected p-values.



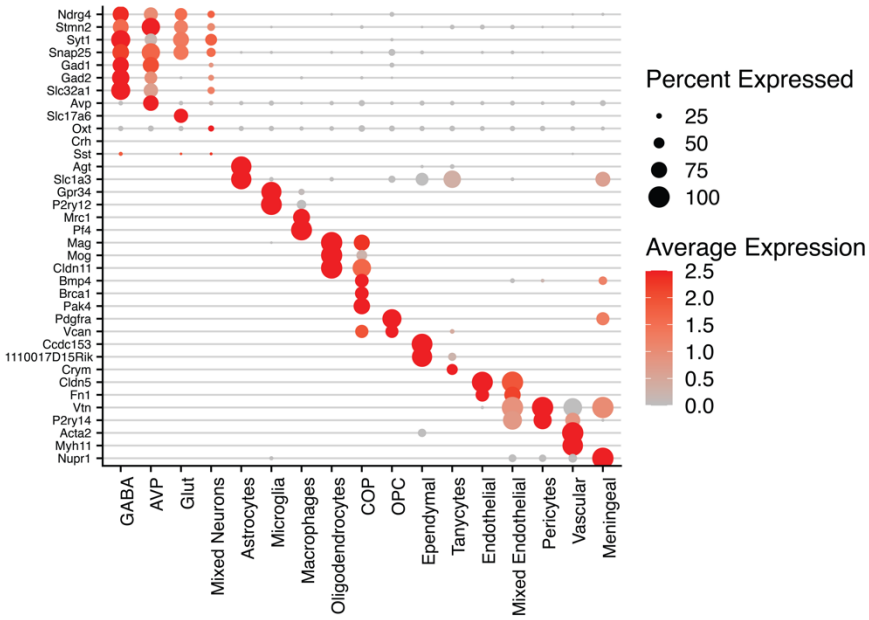
Supplementary Figure 10: Male and Female Cells Distribution in Baseline and CMS ARS Conditions. Distribution of male and female cells (bar plot) in the two stress conditions (Baseline ARS, and CMS ARS) and cluster size as total number of cells per cluster (dot plot). Cell numbers are represented as normalized proportions. Clusters significantly unbalanced (q-values < 0.05) are highlighted with a black border. Fisher's exact test, Benjamini-Hochberg post-hoc corrected p-values.



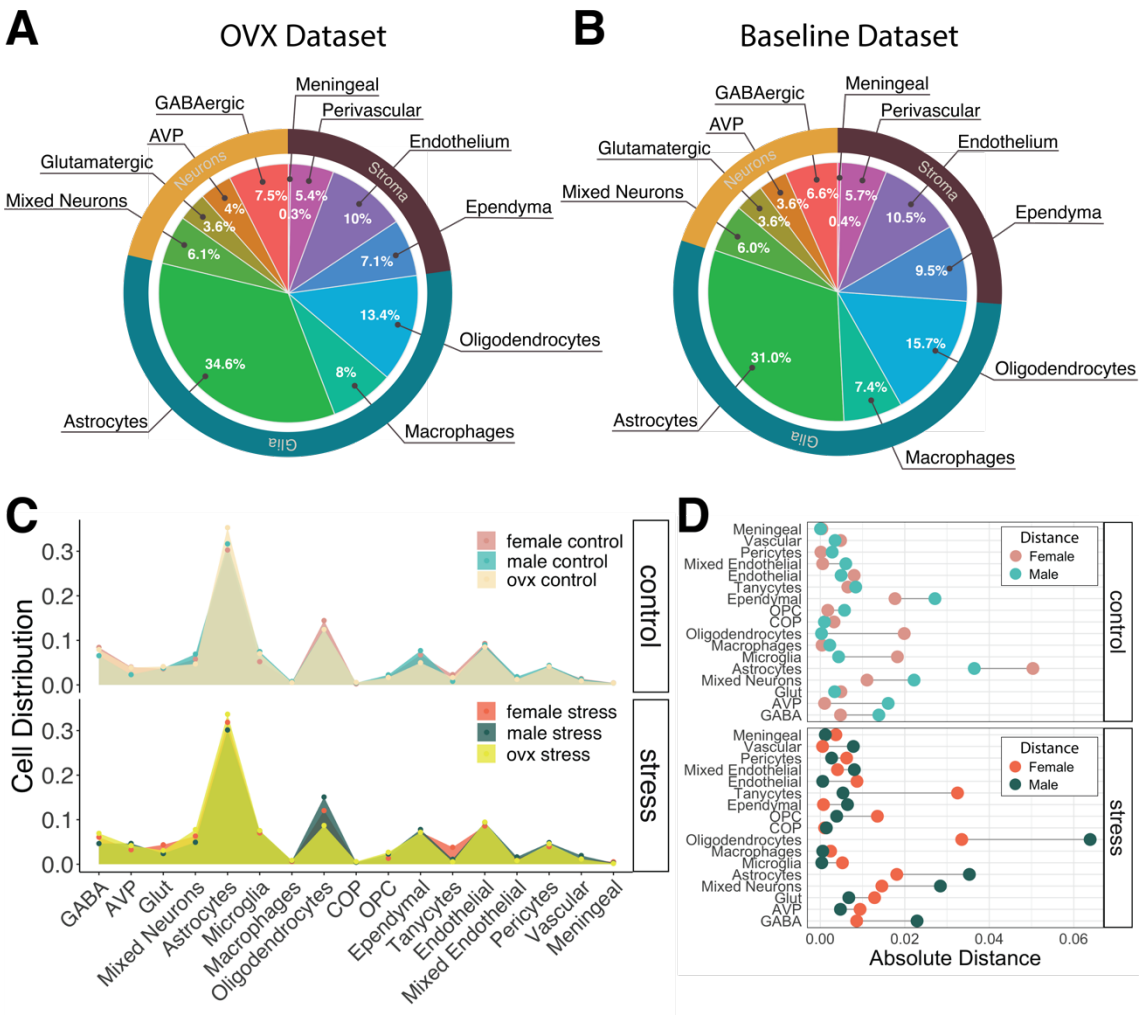
Supplementary Figure 11: Male and Female Tanycytes Distribution in the Two Control Replicates. Proportion of male and female cells across the 5 clusters of tanycytes. Two-way ANOVA, Tukey's post-hoc corrected p-values.



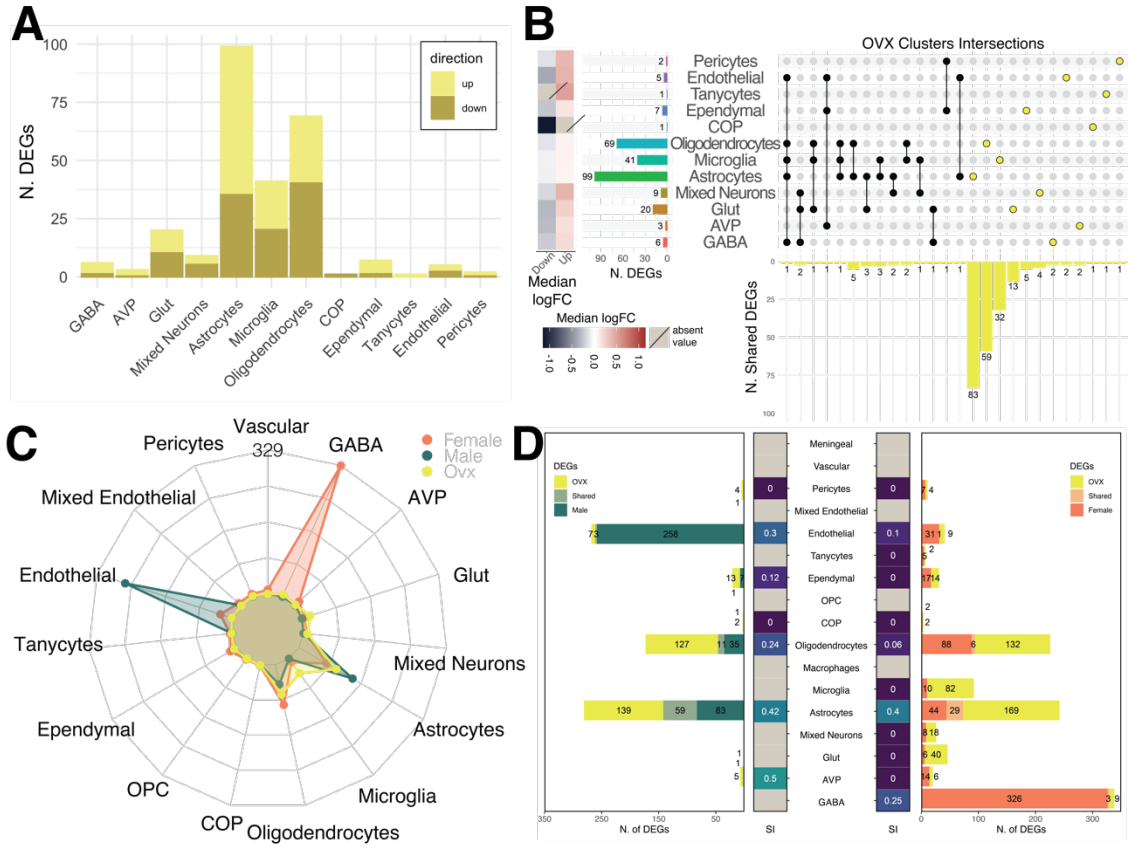
Supplementary Figure 12: Analysis on the Overlap between Male and Female ARS DEGs. The transcriptional response to ARS showed very limited overlap between males and females. (**A-B**) Females showed significantly more DEGs after ARS (Fisher's exact test). (**C**) Even increasing the threshold of significance to q < 0.1 does not increase the proportion of shared DEGs between males and females. (**D**) RRHO analysis corroborating the lack of transcriptional correlation between the sexes. (**E-F**) overlap between DEGs, non-DEGs and not present genes between males and females. (**E**) The biggest overlap between males and females is between non DEGs, followed by genes present in only one of the two sexes, but not DEGs. (**F**) Distribution of logFC for all genes present in both sexes divided by the type of overlap.



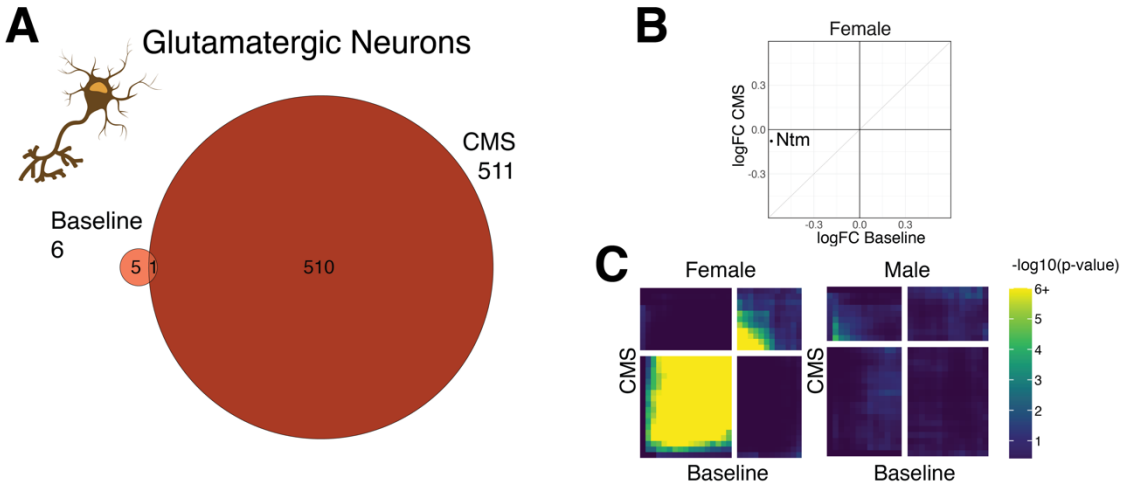
Supplementary Figure 13: Clustering Markers for the OVX scRNA-seq Dataset. Expression of the original markers used in the male-female scRNA-seq dataset for the label-transferred cell types in the OVX dataset. Expression patterns is very similar to the original dataset (**Supplementary Figure 8**, *page 134*).



Supplementary Figure 14: Cell Distribution of OVX scRNA-seq. (**A**-**B**) Distribution of cells among major cell types of (**A**) OVX cells resembles the one from (**B**) male-female Baseline scRNA-seq dataset. (**C** – **D**) Detailed exploration of distribution of cells among cell types shows that OVX has a different degree of similarity with male and female distribution based on cell types. (**C**) Distribution of cells of relative contribution across all cell types. (**D**) Absolute distance for each cell type with female (|Female – OVX|) and male (|Male – OVX|) samples. In controls, few cells show a more female-like phenotype (mixed, AVP, and GABAergic neurons), while other have a more male-like phenotype (astrocytes, microglia, oligodendrocytes).

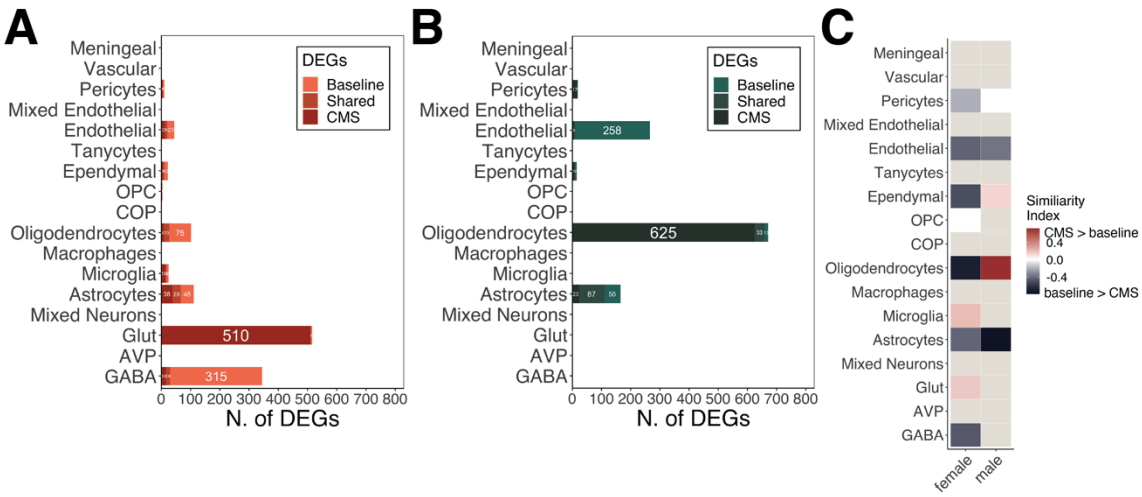


Supplementary Figure 15: Differential Gene Expression of OVX Females. Differential gene expression analysis between control and ARS OVX females identified several DEGs in many cell types. (**B**) Twelve cell types showed at least one DEGs and most of them had a combination of up- and down-regulated genes. (**B**) Most of the DEGs are unique to each cell type, similar to male and female samples. (**C**) The OVX response lost the female-specific strong response of GABAergic neurons, but also did not display the strong male response of endothelial cells. (**D**) Overlap analysis of OVX response to male and female responses shows similar overlap for cell types with low sex-specific responses such as astrocytes. Cell types with sex-specific responses such as oligodendrocytes show more similarity with one sex or the other. Central panels show the similarity index for both comparisons (Szymkiewicz–Simpson coefficient).

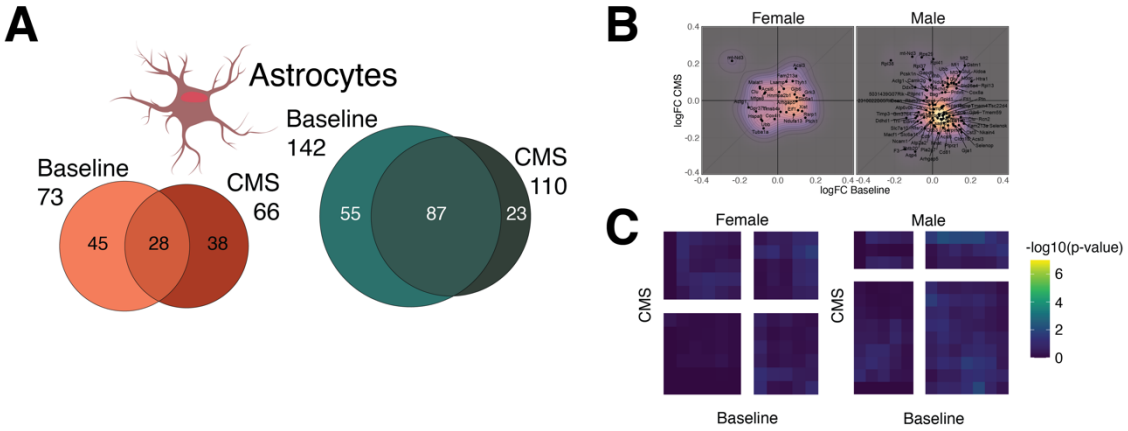


Supplementary Figure 16: ARS Response of Glutamatergic Neurons. Glutamatergic neurons showed female-specificity in their ARS response. No DEGs were found in males. (**A**) Overlap between

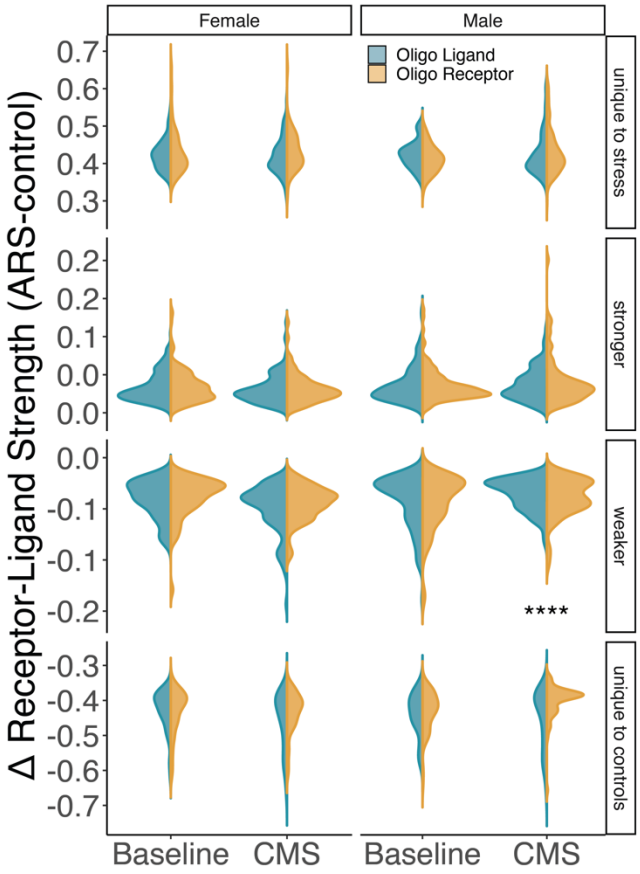
baseline and CMS ARS response for females. (**B**) Shared genes between baseline and CMS ARS responses in females represented by the logFC in each condition. Only one gene was found. (**C**) RRHO analysis showed a different pattern of correlation between baseline ARS and CMS responses for female and male samples. Each differentially expressed gene is expressed by its p-value • sign of logFC and ranked along the x and y axis.



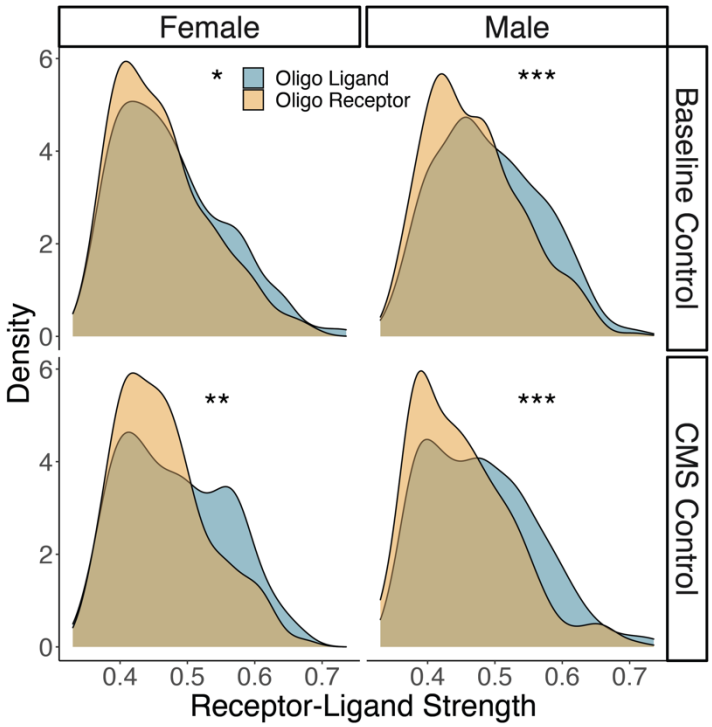
Supplementary Figure 17: Effect of Background Stress on the ARS Response. The change in background stress causes extensive changes in the DEGs after ARS. (**A**-**B**) Number of DEGs after ARS at Baseline and after CMS and their overlap for (**A**) females and (**B**) males. (**C**) Similarity index (Szymkiewicz–Simpson coefficient) for each cell types and sexes. The index grows with higher overlaps of DEGs between stress backgrounds. Positive values show that CMS ARS response bigger than Baseline ARS and negative values for responses bigger at Baseline than CMS.



Supplementary Figure 18: ARS Response of Astrocytes. Examples of a low susceptibility score cell-type. (**A**) Overlap between baseline and CMS ARS response for females and males showing an extensive overlap. (**B**) Shared genes between baseline and CMS ARS responses in females represented by the logFC in each condition. Background colours show gene density. (**C**) RRHO analysis showed a similar pattern of correlation between baseline ARS and CMS responses for female and male samples. Each differentially expressed gene is expressed by its p-value • sign of logFC and ranked along the x and y axis. Despite the high overlap in DEGs, a poor overall correlation was found.



Supplementary Figure 19: Distributions of the Change in Edge Weights for the Significantly Deregulated Receptor-Ligand Pairs by Type of Change. Distribution of the change in edge weights (ARS - control) for each condition and sex compared between the Oligo Ligand (blue, left side) and Oligo Receptor (yellow, right side) directions and divided by the type of change (unique to stress, stronger, weaker, unique to controls). Kruskal-Wallis with Dunn's test corrections.



Supplementary Figure 20: Distributions of Edge Weight for Significantly Deregulated Receptor-Ligand Pairs for Control Samples. Distribution of edge weight for each receptor-ligand pair within each Control conditions. For both control samples, Oligo Ligand pairs are stronger than Oligo Receptor. Three-way ANOVA, pairwise comparisons, Tukey's post-hoc corrected.

7.2 Supplementary Tables

Supplementary Table 1: Top 50 Markers of Sub-Cluster 1 of Tanycytes.

Gene Marker	Expressing Cells in Cluster 1	Expressing Cells Outside Cluster 1	logFC	Adjusted p-value
Atf3	0.836	0.121	1.98	1.06E-68
Klf2	0.862	0.156	1.99	2.78E-67
6330403K07Rik	0.995	0.932	1.21	3.47E-62
Fosb	0.918	0.286	1.48	5.61E-61
Meg3	0.979	0.601	1.84	7.26E-54
Nfatc2	0.636	0.059	1.47	2.20E-53
Marcksl1	0.846	0.207	1.14	2.57E-50
Maff	0.544	0.031	1.45	9.35E-49
Btg2	0.964	0.542	1.31	5.53E-47
Jund	0.995	0.896	0.89	2.32E-44
Socs3	0.913	0.333	1.19	4.18E-44
mt-Co1	1	0.991	0.55	2.16E-38
Zfp36	0.826	0.28	1.11	2.47E-38
H3f3b	0.979	0.806	0.93	5.00E-37
Creb5	0.508	0.059	1.07	2.52E-36
Ppp1r15a	0.708	0.172	1.02	4.88E-36
Klf4	0.749	0.218	1.22	6.01E-36
Egr1	0.944	0.522	1.01	2.58E-35
Klf6	0.846	0.341	1.11	4.00E-35
Nr4a1	0.703	0.185	1.05	2.28E-34
Junb	0.964	0.604	1.08	1.31E-31
Frzb	0.79	0.278	0.88	5.12E-31
Cyr61	0.882	0.441	1.32	5.93E-31
Col25a1	0.323	0.004	0.99	8.12E-31
Fos	1	0.756	0.85	2.56E-28
Prdx6	0.995	0.936	0.60	6.37E-28
Csrnp1	0.374	0.029	0.68	1.27E-27
Trib1	0.426	0.055	0.87	4.27E-27
mt-Co3	0.995	0.998	0.40	4.64E-27
Ptn	1	0.872	0.69	2.04E-26
Jun	0.985	0.784	0.80	4.74E-26
mt-Atp6	1	0.993	0.37	5.73E-26
Col23a1	0.938	0.535	0.71	8.56E-26
Tiparp	0.738	0.271	0.76	3.25E-25
Map1b	0.851	0.469	0.87	6.10E-25
Ubb	1	0.976	0.41	1.11E-24

Rpl22l1	0.99	0.852	0.53	1.43E-23
mt-Co2	0.995	0.965	0.40	2.17E-23
Ier2	0.918	0.487	0.81	2.63E-23
Ftl1	0.995	0.883	0.64	2.95E-23
Irf1	0.374	0.046	0.70	6.09E-23
Rhob	0.851	0.434	0.82	6.35E-23
Rcan1	0.395	0.062	0.81	1.11E-21
Irf2bpl	0.713	0.286	0.80	1.25E-21
Six6	0.308	0.024	0.62	1.27E-21
Cebpd	0.697	0.278	0.91	1.27E-21
Scn9a	0.236	0.002	0.46	1.77E-21
Lmo3	0.328	0.037	0.49	1.26E-19
Psmb8	0.256	0.013	0.50	1.29E-19
Crym	0.846	0.394	0.69	2.50E-19

Supplementary Table 2: Top 20 Enriched Pathway in Metascape Analysis for Female GABAergic Neurons After ARS.

Pathway	Term	Category	N. Present Genes/Total	log(q-value)
Oxidative phosphorylation	ko00190	KEGG Pathway	29/134	-20.530
Cellular responses to stress	R-MMU-2262752	Reactome Gene Sets	38/499	-12.424
Metabolism of RNA	R-MMU-8953854	Reactome Gene Sets	34/546	-8.459
Transmission across Chemical Synapses	R-MMU-112315	Reactome Gene Sets	20/188	-8.059
Glycolysis and gluconeogenesis	WP157	WikiPathways	12/51	-8.052
proton transmembrane transport	GO:1902600	GO Biological Processes	15/99	-7.749
Recycling pathway of L1	R-MMU-437239	Reactome Gene Sets	9/35	-6.114
Cytoplasmic ribosomal proteins	WP163	WikiPathways	13/95	-6.087
vesicle-mediated transport in synapse	GO:0099003	GO Biological Processes	20/256	-6.010
HSP90 chaperone cycle for steroid hormone receptors (SHR) in the presence of ligand	R-MMU-3371497	Reactome Gene Sets	10/50	-5.932

Signaling by Rho GTPases	R-MMU-194315	Reactome Gene Sets	31/652	-5.224
synapse organization	GO:0050808	GO Biological Processes	26/491	-5.015
protein folding	GO:0006457	GO Biological Processes	14/161	-4.559
Parvulin-associated pre-rRNP complex	CORUM:3047	CORUM	8/50	-4.025
RNA splicing	GO:0008380	GO Biological Processes	21/398	-3.975
regulation of cellular protein localization	GO:1903827	GO Biological Processes	25/563	-3.697
Glucagon signaling pathway	ko04922	KEGG Pathway	10/102	-3.493
proteolysis involved in cellular protein catabolic process	GO:0051603	GO Biological Processes	29/756	-3.313
positive regulation of protein depolymerization	GO:1901881	GO Biological Processes	5/18	-3.313
GABA synthesis, release, reuptake and degradation	R-MMU-888590	Reactome Gene Sets	5/18	-3.313

Supplementary Table 3: Top 20 Enriched Transcription Factors as Interactors of the 329 DEGs Found in Female GABAergic Neurons.

Transcription Factor	p-value	q-value
ESR1	3.88E-25	9.15E-23
HTT	3.82E-21	4.50E-19
ATF2	2.26E-14	1.78E-12
ILF3	1.60E-10	9.43E-09
MYC	2.12E-10	1.00E-08
NR3C1	5.01E-10	1.97E-08
POU5F1	4.94E-08	1.67E-06
NFKB1	2.39E-07	7.06E-06
ERG	2.93E-07	7.69E-06
HSF1	1.85E-06	4.36E-05
EED	2.50E-06	5.37E-05
CTNNB1	1.08E-05	0.0002
ESR2	1.10E-05	0.0002
NANOG	1.41E-05	0.00024
JUN	1.65E-05	0.00025
TP53	1.69E-05	0.00025
ILF2	5.61E-05	0.00078
SMC3	9.17E-05	0.00120
RAD21	0.000161	0.00197
TP63	0.000167	0.00197

Supplementary Table 4: List of Software and Coding Packages used.

Software/package	Version	References	Application
10x Genomics Cell Ranger software	v. 3.0.2		scRNA-seq pre-processing (Baseline background)

10x Genomics Cell Ranger software	v. 3.1.0		scRNA-seq pre-processing (CMS background)
R studio	v. 1.2.5033	[218]	Data analysis
R	v. 3.6.3	[203]	Data analysis
tidyverse	v. 1.3.0	[219]	Data handling
ggplot2	v. 3.3.0	[220]	Data plotting
viridis	v. 0.5.1	[221]	Data plotting
ComplexUpset	v. 1.2.0	[189], [190], [325]	Data plotting
eulerr	v. 6.1.0	[191], [192]	Data plotting
circlize	v 0.4.10	[199]	Data plotting
Seurat	v. 3.1.3	[183]	scRNA-seq data analysis
scran	v. 1.14.6	[185]	scRNA-seq data analysis
lme4	v. 1.1-26	[210]	Statistical analysis
nlme	v. 3.1-144	[211]	Statistical analysis
lmerTest	v. 3.1-3	[212]	Statistical analysis
emmeans	v. 1.5.4	[222]	Statistical analysis
RRHO2	v. 1.0	[194], [195]	Statistical analysis
monocle3	v. 0.2.1	[200]–[202]	scRNA-seq data analysis
ShinyCell	v. 2.1.0	[215]	Web app
shiny	v. 1.4.0.2	[216]	Web app
shinyhelper	v. 0.3.2	[217]	Web app
shinythemes	v. 1.2.0	[326]	Web app
fgsea	v. 1.12.0	[204]	Gene analysis
msigdbr	v. 7.0.1	[205]	Gene analysis
Metascape.org		[206]	gene analysis
Enrichr.com		[193]	gene analysis
Fiji ImageJ	v. 1.53c	[208]	Image processing
Simple Neurite Tracing	v. 3.1.7	[207]	Image processing
Sholl analysis	v. 4.0.1	[209]	Image processing
Solomon Coder	v. 17.03.22	[180]	Behavioural tracking
ANY-maze	v. 6.13	[327]	Behavioural tracking

Supplementary Table 5: List of Reagents.

Product	Supplier	Cat. N.	Application
Modified Wright-Giemsa stain	Sigma Aldrich	WG16	Estrus cycle monitoring
Ketamine hydrochloride injectable	Zoetis		Anesthesia
Xylazine hydrochloride	Sigma Aldrich	X1251	Anesthesia
NaCl 0.9%	Serumwerk	906388	Anesthesia
Metacam injectable 5 mg/ml	Boehringer Ingelheim Vetmedica		Anesthesia
Corticosterone Double Antibody RIA Kit	MP Biomedicals	0712010-CF	CORT measurements
NaCl	Carl Roth	9265.1	aCSF
KCl	Carl Roth	6781.1	aCSF
$NaH_2PO_4 \cdot H_2O$	Carl Roth	K300.2	aCSF
$NaHCO_3$	Merck Millipore	144-55-8	aCSF
D(+)-Glucose Monohydrate	Carl Roth	6887.1	aCSF
D(+)-Saccharose	Carl Roth	4621.1	aCSF
$MgCl \cdot 6H_2O$	Merck Millipore	7791-18-6	aCSF
$CaCl_2 \cdot 2H_2O$	Merck Millipore	10035-04-8	aCSF
Papain Dissociation System	Worthington BC	LK003163	scRNA-seq
Chromium™ Single Cell 3' Library Kit v2	10x Genomics	120234	scRNA-seq
Chromium™ Single Cell 3' Gel Bead Kit v2	10x Genomics	120235	scRNA-seq
Chromium™ Single Cell A Chip Kit	10x Genomics	1000009	scRNA-seq
Chromium™ i7 Multiplex Kit	10x Genomics	120262	scRNA-seq
AMPure XP beads	Beckman Coulter	A63881	scRNA-seq
Qubit® dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32854	scRNA-seq
Bioanalyzer High Sensitivity DNA Kit	Agilent	5067-4626	scRNA-seq

Paraformaldehyde	Carl Roth	0335.3	Immunofluorescence
Triton X-100	Sigma Aldrich	T-8787	Immunofluorescence
α-Tmem10, rabbit	Peles lab, Weizmann Institute of Science, Israel		Immunofluorescence
α-olig2, mouse	Millipore	MABN50	Immunofluorescence
α-Rb-Alexa Fluor 488, goat	Invitrogen	A32723	Immunofluorescence
α-M-Alexa Fluor 594, goat	Invitrogen	A32740	Immunofluorescence
Goat serum	Genetex	GTX73206	Immunofluorescence
DAPI Fluoromount-G	SouthernBiotech	0100-20	Immunofluorescence
DreamTaq DNA Polymerase	Thermo Fisher Scientific	EP0703	PCR
Deoxynucleoside Triphosphate Set	Sigma Aldrich	3622614001	PCR
UltraPure Agarose	Invitrogen	16500-500	PCR
Ethidium bromide solution 1 %	Carl Roth	2218.3	PCR

7.3 Peer-Reviewed Publications

7.3.1.1 Review Article

E. Brivio, J. P. Lopez, and A. Chen, "Sex differences: Transcriptional signatures of stress exposure in male and female brains," *Genes, Brain Behav.*, vol. 19, no. 3, pp. 1–22, Mar. 2020, doi: 10.1111/gbb.12643.

Received: 2 December 2019 | Revised: 13 January 2020 | Accepted: 22 January 2020
DOI: 10.1111/gbb.12643

Genes, Brain and Behavior

REVIEW

Sex differences: Transcriptional signatures of stress exposure in male and female brains

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Abstract

More than two-thirds of patients suffering from stress-related disorders are women but over two-thirds of suicide completers are men. These are just some examples of the many sex differences in the prevalence and manifestations of stress-related disorders, such as major depressive disorder, post-traumatic stress disorder, and anxiety disorders, which have been extensively documented in clinical research. Nonetheless, the molecular origins of this sex dimorphism are still quite obscure. In response to this lack of knowledge, the NIH recently advocated implementing sex as biological variable in the design of preclinical studies across disciplines. As a result, a newly emerging field within psychiatry is trying to elucidate the molecular causes underlying the clinically described sex dimorphism. Several studies in rodents and humans have already identified many stress-related genes that are regulated by acute and chronic stress in a sex-specific fashion. Furthermore, current transcriptomic studies have shown that pathways and networks in male and female individuals are not equally affected by stress exposure. In this review, we give an overview of transcriptional studies designed to understand how sex influences stress-specific transcriptomic changes in rodent models, as well as human psychiatric patients, highlighting the use of different methodological techniques. Understanding which mechanisms are more affected in males, and which in females, may lead to the identification of sex-specific mechanisms, their selective contribution to stress susceptibility, and their role in the development of stress-related psychiatric disorders.

KEYWORDS
mood disorders, psychiatry, rodents, sex differences, stress, transcription, transcriptome

1 | INTRODUCTION

Stress-related disorders, such as major depressive disorder (MDD), post-traumatic stress disorder (PTSD), and anxiety disorders, affect more than 500 million people worldwide.[1] Notably, women are two to three times more at risk to develop these disorders[1,2] and furthermore, the symptomatology, development, and responsiveness to treatment differ between genders.[3-5] For instance, women suffering from

depression have greater symptom severity and a higher chance of developing metabolic and sleep disturbances than men. On the other hand, men more often report symptoms of anger and aggression, and comorbidity with alcohol and substance abuse.[6,7] Finally, some evidence suggests that antidepressants' efficacy changes according to the sex of the patients.[8-11] Unfortunately, the current biological knowledge of the mechanisms behind this dimorphism is scarce compared with the abundant clinical evidence, which remains mostly unexplained. However, mood disorders, anxiety disorders, and trauma- and stress-related disorders have in common a strong association to stress exposure as a risk-factor.[12] Since sex has been shown to modulate the stress response and processing at multiple levels, studying how the male and female biological systems process stress might help to understand the origin for sex differences in psychiatric disorders.

The biological systems known to be activated by stressors include neurobiological systems, such as the hypothalamus-pituitary-adrenal (HPA) axis, the cortico-limbic, and the sympathetic adrenomedullary (SAM) systems, which interact with each other to coordinate the stress response.[13,14] Importantly, exposure to stress activates the paraventricular nucleus of the hypothalamus (PVN), which leads to a biological cascade that produces glucocorticoids, predominantly cortisol in humans and corticosterone (CORT), in rodents. These steroid hormones cross the blood-brain barrier thus acting directly on the brain, modulating its functions mostly through regulation of gene expression. Both the hypothalamus and the cortico-limbic system which includes the amygdala, the hippocampus, and the orbital/prefrontal cortex, have shown sexually dimorphic patterns of activation and morphology (Figure 1). In particular, women and female rodents have been shown to have higher HPA axis activation in response to stress and lower negative feedback.[15,16] Similarly, other regions, such as the hippocampus and the amygdala, have higher activation for women in response to negative emotions.[17,18] Many of these regions also show sex dimorphism in structure,[19-27] connectivity,[28] cell composition,[29,30] and transcriptional profile[31-36] (Figure 1).

The transcriptional profile or transcriptome of a tissue is the collection of gene transcripts present in its cells. Over the last decade, we have seen transcriptomic studies rising in popularity in several fields of biomedical research. This is mostly because different factors make the transcriptome an interesting and insightful target of research. First, the transcriptome provides a window on a tissue or cell phenotype and its molecular dynamics.[37] Second, the transcriptome is highly dynamic and reflects fast adaptation to the

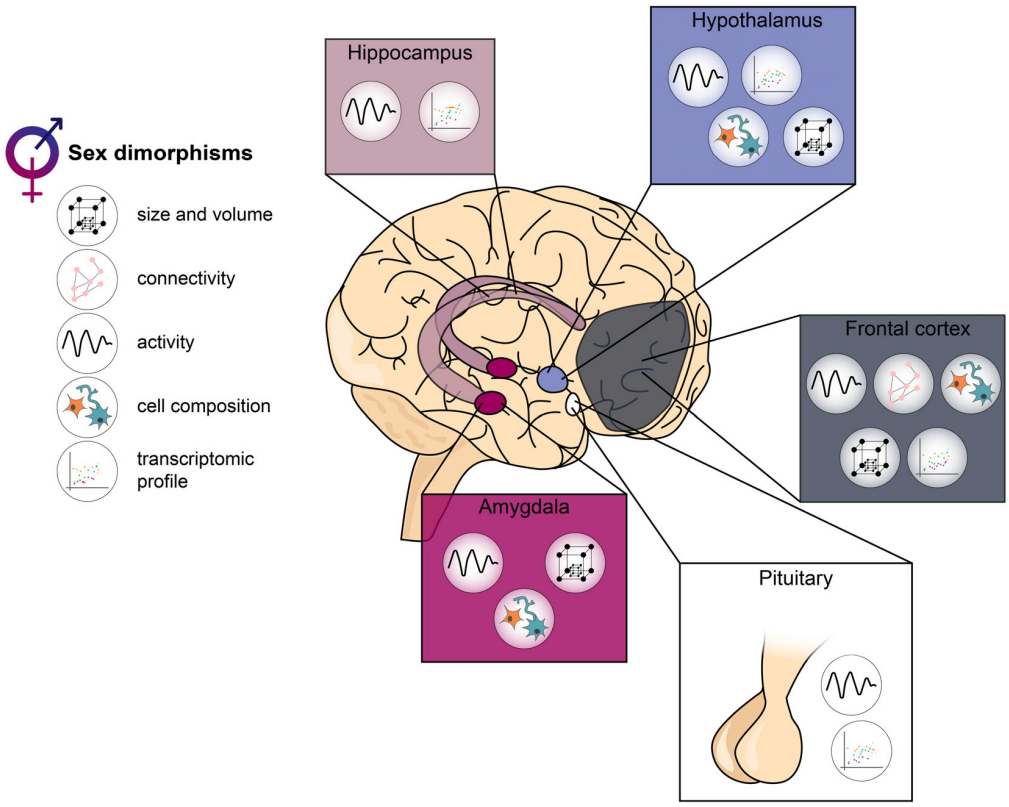

FIGURE 1 Sex dimorphism in the human brain stress system. Schematic representation of the main brain regions of the stress system that have been shown to be sexually dimorphic in adulthood. Dimorphism in size or volume has been found in the amygdala,[18-23,26] frontal cortex[18-23,26] and hypothalamus.[26] Connectivity has been shown to be different in the sexes in the frontal cortex,[28] whereas neuronal activity differs in the hippocampus,[17] hypothalamus,[15] frontal cortex,[17] amygdala,[17] and pituitary gland.[15] Cell composition of the frontal cortex,[29,30] amygdala,[29,30] and hypothalamus[29,30] and the transcriptional profile of the pituitary gland,[31,32] frontal cortex,[31-33,35] hippocampus,[31-33] hypothalamus,[31,32] and amygdala,[33] were also found to be sex specific

environment. For instance, transcriptomic changes on immediate early genes can be observed in a matter of minutes following a stimulus.[38] Third, a wide range of techniques for interrogating the transcriptomic state of a tissue or cell have been developed through the years.[39] These methods can be divided into two main categories: low- and high-throughput. To the first group classically belong techniques such as northern blot (NB), in situ hybridization (ISH), or quantitative real-time polymerase chain reaction (qPCR). The second group contains methods with wider targets such as microarrays and the so-called next-generation sequencing methods (NGS or RNA-sequencing). These approaches are characterized by increased sensitivity, higher throughput, and ability to detected novel transcripts.[40] Their differences in sensitivity are particularly relevant when comparing results across techniques. Analyzing these limitations in depth is outside the scope of the current review, but detailed discussions can be found in the following reviews.[40-42] Thanks to characteristics of the transcriptome, its study is particularly suitable to investigate the brain, a complex and dynamic organ with high sensitivity to the environment.[43] Transcriptomic studies have indeed already been insightful in the fields of neurobiology and neuroscience by elucidating molecular mechanisms behind diseases such as Alzheimer's disease[44] and alcohol addiction,[45] and basic molecular processes, such as the development of the central nervous system[46] and aging.[47,48] Moreover, some studies have already shown that the study of sex differences could benefit from using a transcriptomics approach.[49]

Understanding why and which molecular pathways are differentially regulated in response to stress in a sex-specific manner is crucial to understand the mechanisms involved in the etiology of stress-related psychiatric disorders. Most importantly, understanding these differences can lead to the development of sex-oriented approaches, both in diagnosis and treatment. In this review, we focus on how sex influences stress-specific transcriptomic changes in rodent models, as well as in psychiatric patients. We will discuss how different modalities of stress (acute or chronic) affect males and females differently. Furthermore, we will highlight the use of different methodological techniques used to address these changes and provide a general overview of the field and current status of the research.

2 | HUMAN STUDIES

Over the years, several studies have shown transcriptomic changes in post-mortem brains from psychiatric patients.[50-57] More specifically, these studies identified gene expression changes affecting different neurobiological systems in depressed and suicidal patients such as the GABAergic and glutamatergic systems, the monoaminergic system, the dopaminergic and reward system,[58] the brain-derived neurotrophic factor (BDNF) pathway, and the immune system[50] (for a comprehensive review see[59]). Gene expression changes in the somatostatin and acetylcholine systems, metallothionein proteins, metal-ion binding proteins, and the MAPK/ERK signaling have been, on the other hand, described in bipolar patients.[51,55,60] Finally, only few transcriptomic studies on PTSD patients can be found in literature and they point at mitochondrial disfunction[61,62] and alterations in the immune system[63] as PTSD transcriptional signatures. However, most of these studies have been focused solely on male patients or did not stratify by sex. Thus, very little information is available on how conserved these changes are in women or how sex modulates these transcriptional signatures. Surely several factors contribute to the scarce presence of sex as a biological variable in transcriptomic studies. We can hypothesize that the reasons contributing to the bias in preclinical research[64-67] are also, at least partially, the same for human studies as well. For instance, the misconception about the increased female[66,68,69] variability—often argued because of the fluctuating sex[64] hormones—and the misguided assumption that the biological sex does not influence the function of the central nervous system are among them, especially in the fields of neuroscience and psychiatry.[65] In addition, human brain samples are difficult samples to collect in big numbers,[53] especially from psychiatric patients.[70-72] Many of these samples come from patients who died from suicide[73] and men are twice as likely to be suicide completers.[74] As such, restricted sample availability and the limited statistical power and possibility of sex stratification that comes with it, together with the misconceptions might have contributed to the sex bias.

Nevertheless, there is a growing interest in analyzing sex as a biological variable to study transcriptional changes using both male and female psychiatric patients. As a result, new and interesting studies are emerging in literature. To date, however not all stress-related disorders have witnessed the same rate of inclusion of sex as a variable. For some of them, such as PTSD, no transcriptomic studies looking at sex differences have been published to the best of our knowledge. Instead, most of these emerging works have focused on MDD. The following sections of the review will reflect this trend in the literature, presenting mostly results from studies on MDD patients. Some of these studies have chosen a targeted approach focusing on a specific subset of genes. Others have started to explore the transcriptome at the genome-wide level, using high-throughput approaches. Both approaches are discussed below.

2.1 | Targeted studies

To date, only a handful of studies have shown gene expression changes in psychiatric patients in a sex-specific matter. These studies include changes in several systems, such as serotoninergic,[75] somatostatin,[76] and other less explored systems such as the galanin system.[77] Apart from neuropeptide systems, other candidate genes have been investigated and found to be regulated by stress and sex. Among them, the CRF system showed selective changes in the amygdala of bipolar male patients at the level of the CRF binding protein mRNA, but not in females nor MDD patients.[78] In addition, genes from the sex steroid hormone pathways have often been considered an interesting candidate to study sex differences. In fact, change of susceptibility to depressed mood, and fluctuation of neuropsychiatric symptoms across menstrual cycle and menopause have long pointed at a possible role of estrogens in depression and neuropsychiatric

disorders. In support of this idea, the levels of the estrogen receptor α (ERα, *ESR1*) in the post-mortem dorsolateral prefrontal cortex (DLPFC) of psychiatric patients were found upregulated in men, but in contrast downregulated in women, as compared with psychiatrically healthy controls.[79] The implication of ERα levels in stress susceptibility has also been suggested by a recent study in mice.[80] Finally, sexually dimorphic transcriptional changes can also be found in other less-explored regions such as the internal capsule, a bundle of white matter that participates in the corticostriatum-thalamic circuitry and is structurally altered in psychiatric patients.[81,82] Interestingly, Barley, Dracheva, and Byne[83] found evidence in this region for a sex-specific transcriptomic signature of oligodendrocytes in MDD and bipolar disorder. Overall, targeted studies have proven useful to explore specific candidate genes that were suspected to contribute to the sex dimorphism in psychiatry. Nevertheless, their low throughput and power is still a significant limitation for discovering novel genes and pathways involved in psychiatry.

2.2 | High-throughput studies

Genome-wide transcriptional studies investigating the role of sex in psychiatric disorders are also starting to emerge. Compared with targeted studies, high-throughput studies allow for a broader overview of the transcriptome landscape and thus the possibility to study transcriptional signatures in the context of pathways and networks. A representative example of the potentialities of this strategy is the work of Labonté et al.[84] Labonté et al studied sex-specific transcriptional signatures in the brains of depressed men and women as compared with healthy controls. The power of their study lies in the use of a large cohort of male and female human post-mortem brain samples, the inclusion of multiple brain regions, the advanced bioinformatic tools, as well as the comparison between clinical and preclinical samples. The six different regions analyzed show different degrees of overlap in gene expression patterns between patients and controls. More interestingly, their results show that the amount of MDD-related transcriptional changes in common between men and women depends on the region observed but is overall limited. In fact, only as little as 30% of differentially expressed genes (DEGs) are shared between men and women. Notably, this number drops further if the directionality of the change is taken into consideration. Moreover, gene network and gene ontology analyses showed that only a small percentage of the expression modules are present in both sexes with MDD, and they represent different pathways. This approach allowed the authors to identify new potential sex-specific players in depression. Similar results were obtained by Seney et al[85] with a large-scale gene expression meta-analysis across three corticolimbic structures of men and women MDD patients and controls. In accordance with Labonté et al, a small number of DEGs was shared among the sexes, but overall gene expression changes converged on similar pathways. Interestingly, the authors highlighted that these changes in the pathways are often in the opposite direction. For example, MDD men have decreased synapse-related genes, whereas women have an increased number. Notably, both studies identified a possible different involvement cell types in MDD between sexes. In addition to brain studies, genome-wide transcriptomic studies in peripheral blood samples of PTSD patients, such as by Breen et al,[86] have identified an analogous pattern of opposite gene expression changes between sexes and a possible involvement of different cell types.[87] Taken together, the high-throughput studies presented so far suggest that the male and female brains respond to stress in a different and region-specific way. In particular, pathway analysis indicates that synaptic function and structure might be differently affected by stress in the two sexes. Exploration of synaptic density and functionality especially across the corticolimbic structures would be an interesting and worthy path to analyze. Further studies might identify structural differences arising from stress exposure specific for one or the other sex and potentially identify new sex-specific therapeutic targets. In addition, inflammation seems to be regulated by a stress x sex interaction and suggests that different cell types might be involved in the stress response in the two sexes. Lastly, the nucleus accumbens (NAc) is the only region showing highly similar stress signatures between the sexes. However, the reward system, to which the NAc belongs, has been shown in human and animal models[88-90] to be affected by stress exposure in a sex-specific fashion. Investigating how similar gene expression changes might lead to divergent functional outcome may be of great interest to the field. Overall, high-throughput studies on human post-mortem samples indicate that male and female psychiatric patients do not differ only in clinical manifestations but also in their molecular organization.

Nevertheless, studies on human tissues are unfortunately strongly affected by unavoidable complications, like intrinsic variability because of treatment history, age, post-mortem indices, and processing. These factors are known to confound studies, especially when looking for transcriptional alterations.[91] For these limitations, preclinical work is a very valuable tool for studying the molecular consequences of stress, providing direct access to the brain and a high control over temporal resolution.

3 | RODENT STUDIES

Given the limitations associated with human samples, rodents are a proven useful tool to study the stress response.[92-96] Preclinical models of mice and rats have been developed to study both the acute and chronic stress response.

3.1 | Acute stress

Acute stressors are known to activate a biological response that culminates in the production of glucocorticoids. Prolonged high glucocorticoid levels are known to increase susceptibility to psychiatric conditions through the sustained activation of glucocorticoid receptors in the stress system.[13,97,98] Sex modulates the extent of this stress response, both in the corticolimbic structures and in the HPA

axis, but a thorough characterization of which exact molecular mechanisms are activated in the two sexes is still missing. The molecular mechanisms activated by acute stress have therefore become the focus of many researchers' interest and among them, many have tried to tackle this issue looking at the mRNA levels of various known mediators of the stress response after exposure to an acute stressor.

3.2 | Targeted studies

So far, rodent studies have employed different types of acute stressors, which can be divided into two main categories: physical and psychological stressors. Both types have shown to be informative in the research of sex differences. Physical stressors such as restraint, forced swim test or electroshock have been shown to alter gene expression in a sex-specific way in different brain regions (Tables 1 and 2). For instance, the glucocorticoid (*Nr3c1*, referred as GR) and mineralocorticoid (*Nr3c2*, referred as MR) receptors—the direct responders to CORT—are affected at the mRNA level by the combination of acute stress and sex in different brain areas. An overview of these changes in the hypothalamus, hippocampus, and pituitary of male and female rats after acute restraint can be found in the work of Karandrea, Kittas, and Kitraki.[99] Their data suggested that MR and GR plasticity to stress is modulated by sex and that the GR:MR ratio is adjusted in a sex-specific way in response to stress. Interestingly in a follow-up study, the same authors showed that the GR:MR ratio is regulated specifically according to the type of stressor.[100] In accordance with this idea, for example, GR levels in the hypothalamus were reported to be changed in an opposite direction in males and females after restraint,[99] but unchanged in both after footshock exposure.[101] It is interesting to note that GR knock-out animals show an alteration in the feedback inhibition on ACTH and CORT levels in response to an acute stressor only in males.[102] This reinforces the idea of a sex-specific mechanism of action for GR or MR regulation. On the other hand, other stress-associated genes, such as oxytocin (*Oxt*), arginine vasopressin (*Avp*), and corticotropin-releasing factor (*Crf*) have been also studied in recent works. Nonetheless, there is still a lack of agreement on how these gene changes are indeed regulated by sex and by the type of stressor. For instance, Lu et al[101] reported no sexually dimorphic changes for these genes after acute footshock in the hypothalamus, whereas, Guo et al reported male-specific increased levels of *Avp* after an acute footshock, in the same region.[103] Although they used the same animal model (Sprague Dawley rats), stress paradigm (footshock) and molecular assay (qPCR), Lu and colleagues[101] collected their samples within a bigger time window after stress compared with Guo et al.[103] Therefore, the timing of tissue collection might contribute to the observed inconsistency in these works. Other regions such as the central amygdala (CeA) have shown discrepant results in other stress-related genes such as *Crf*[104,105] (see Table 1 for more details). Specifically, the work from Sterrenburg et al shows upregulation in both sexes that is not found from Viau et al. It is important to notice that despite using the same molecular technique (ISH), the authors not only collected the samples at a different timepoint, but also used two different strains of rats (Wistar vs Sprague Dawley) and different durations for their restraint paradigms. Sterrenburg et al[104] used a longer stressor (1 hour instead of 30 minutes) and collected their results an extra hour after the ending of the paradigm, whereas Viau et al[105] had a shorter restraint session (30 minutes) and collected the sample immediately. The shorter stressor or the time of collection might have compromised the ability of the authors to induce or observe changes in *Crf* expression. Importantly, the two studies still agree on the absence of sex differences. Discrepancies in transcriptomic studies are likely to arise from differences in stress paradigms employed, molecular techniques, and timepoint of tissue collection. Further studies exploring these factors and aiming at replicating the current results are needed to give a clearer picture of sex differences and their source.

Furthermore, an interesting study by Iwasaki-Sekino et al[106] suggests that timing, at least for some genes and brain regions, might indeed play a role in finding sex dimorphism at the transcriptomic level. The authors showed that *Crf* mRNA levels after footshock change following different time course in the two sexes. Females had similar total change to males, but they achieved it an earlier time point in the PVN and it subsisted for longer both in the PVN and CeA.[106] A different kinetic in *cFos* levels upregulation was also found in the prefrontal cortex (PFC) of rats after an inescapable stressor.[107] In this study, however, female upregulation seemed slower and more persistent. These partially discordant results probably suggest that sex-specific stress responses at the transcriptomic level differ between regions not only for the genes involved but also for their temporal regulation. Currently, few other studies support the idea that the temporal dynamics of stress-response might differ between the sexes, in a region-specific fashion.[108-110] Further, it has been recently discovered that acute stress also elicits long-term alterations in neuronal function in mice,[111] which is reasonable to think could be associated with long-term alterations in the transcriptome. If so, these alterations might manifest in sex-specific ways too. Accordingly, the mRNA expression of *Avp* and *Oxt* is sexually dimorphic in the PVN and BNST even weeks after 3 days of defeat in mice[112-114] with *Avp* being downregulated in the PVN of males only and *Oxt* upregulated in the BNST of females only. Apart from the classic stress-related genes presented so far, other genes have been reported to modulate their expression in a sex-specific way. For example clock genes,[115] genes involved in the sex steroid system,[101,103] and genes encoding for epigenetic mediators.[104,116,117]

The gene expression changes described so far have been specifically observed in the context of physical stressors. In contrast, psychological stressors, such as footshock witnessing, have unfortunately received less attention. Nonetheless, the work from Iwasaki-Sekino et al[106] also suggests that the two types of stressors elicit a different stress response. This difference might originate from a different perception and process of the types of stress between the two sexes. In support of this idea, handling alone, which is recognized to be a mild stressor,[118,119] induced *cFos* transcription in the male hippocampus, but not in females.[109] Correspondingly, there is evidence that female and male perception of and susceptibility to psychological stressors

TABLE 1 Stress-related genes regulated by acute and subchronic stress in males and females

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
Nr3c1 (GR)	PIT	60 minutes restraint	/	Wistar rats	↑	—	NB	99
	HPT	60 minutes restraint	/	Wistar rats	↑	↓	NB	99
		Footshock	<30 minutes	Sprague Dawley rats	—	—	qPCR	101
		20 minutes FST	60 minutes	Wistar rats	↑	—	NB	100
	PFC	Footshock	5 minutes	Sprague Dawley rats	—	—	qPCR	103
		20 minutes FST	60 minutes	Wistar rats	↑	↓	NB	100
	HPC	60 minutes restraint	/	Wistar rats	—	—	NB	99
Nr3c2 (MR)	HPT	60 minutes restraint	/	Wistar rats	—	↓	NB	99
		20 minutes FST	60 minutes	Wistar rats	↑	—	NB	100
		Footshock	<30 minutes	Sprague Dawley rats	—	↓	qPCR	101
		Footshock	5 minutes	Sprague Dawley rats	—	—	qPCR	103
	HPC	60 minutes restraint	/	Wistar rats	—	—	NB	99
		20 minutes FST	60 minutes	Wistar rats	↓	—	NB	100
Crf	HPT	Footshock	30 minutes	Sprague Dawley rats	—	—	qPCR	101
		Footshock	5 minutes	Sprague Dawley rats	—	—	qPCR	103
	PVN	60 minutes footshock	30 minutes	Wistar rats	—	—	ISH	106
		60 minutes footshock	60, 120 minutes	Wistar rats	—	↑	ISH	106
		60 minutes footshock	90 minutes	Wistar rats	↑	↑	ISH	106
		60 minutes witnessing footshock	30, 60, 120 minutes	Wistar rats	—	—	ISH	106
		60 minutes witnessing footshock	90 minutes	Wistar rats	—	↑	ISH	106
		1 hour restraint	1 hour	Wistar rats	↑	—	ISH	104
		30 minutes restraint	/	Sprague Dawley rats	↑	—	ISH	105
		30 minutes restraint	/	Sprague Dawley rats	F > M[a]	F > M[a]	FISH	157
	CeA	60 minutes footshock	30, 60 minutes	Wistar rats	—	—	ISH	106
		60 minutes footshock	90 minutes	Wistar rats	↑	↑	ISH	106
		60 minutes footshock	120 minutes	Wistar rats	—	↑	ISH	106
		60 minutes witnessing footshock	30, 60, 120 minutes	Wistar rats	—	—	ISH	106
		60 minutes witnessing footshock	90 minutes	Wistar rats	↑	—	ISH	106
		1 hour restraint	1 hour	Wistar rats	↑	↑	ISH	104
		30 minutes restraint	/	Sprague Dawley rats	—	—	ISH	105
	BNSTov	1 hour restraint	1 hour	Wistar rats	↑	—	ISH	104
	BNSTfu	1 hour restraint	1 hour	Wistar rats	$\downarrow_t$	—	ISH	104
	MPOA	30 minutes restraint	/	Sprague Dawley rats	F > M[a]	F > M[a]	FISH	157
Avp	HPT	Footshock	<30 minutes	Sprague Dawley rats	—	—	qPCR	101
	HPT	Footshock	5 minutes	Sprague Dawley rats	↑	—	qPCR	103
	PVN	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	105
		Social defeat	2 weeks	California mice	↓	—	qPCR	113
Oxt	HPT	Footshock	<30 minutes	Sprague Dawley rats	—	—	qPCR	101
		Footshock	5 minutes	Sprague Dawley rats	—	—	qPCR	103
	PVN	Social defeat	2 weeks	California mice	—	—	qPCR	112
	BNST	Social defeat	2 weeks	California mice	—	↑	qPCR	112

Notes: Regions: PIT, pituitary gland; HPT, hypothalamus; PVN, paraventricular nucleus of the hypothalamus; PFC, prefrontal cortex; HPC, hippocampus; CeA, central amygdala; BNST, bed nucleus of the stria terminalis; MPOA, medial preoptic area. Paradigm: FST, forced swim test. Tissue collection: /, samples collected immediately at the end of the paradigm. Methods: NB, northern blot; ISH, in situ hybridization; FISH, fluorescent in situ hybridization; qPCR, quantitative PCR. ↓ down regulated; ↑ upregulated; t, trend; ?, unclear|discordant results; —, no differential expression.

[a]No control animals in the experiments.

TABLE 2 Nonstress-related genes regulated by acute and subchronic stress in males and females

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
Ar	HPT	Footshock	<30 minutes	Sprague Dawley rats	—	↓	qPCR	101
		Footshock	5 minutes	Sprague Dawley rats	—	↓	qPCR	103
Aro	HPT	Footshock	5 minutes	Sprague Dawley rats	↓	↑	qPCR	103
Esr1	HPT	Footshock	<30 minutes	Sprague Dawley rats	—	—	qPCR	101
		Footshock	5 minutes	Sprague Dawley rats	—	—	qPCR	103
	MeA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
Esr2	HPT	Footshock	<30 minutes	Sprague Dawley rats	—	—	qPCR	101
		Footshock	5 minutes	Sprague Dawley rats	—	↑	qPCR	103
	MeA	3 days social defeat	2 weeks	California mice	?	?	qPCR	116
cFos	PVN	30 minutes restraint	/	Sprague Dawley rats	F > M[a]	F > M[a]	ISH	157
		30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
		30 minutes restraint	30 minutes	Sprague Dawley rats	F = M	F = M	ISH	110
	PFC	100 minutes restraint + tailshock	/, 60 minutes	Sprague Dawley rats	↑	↑	ISH	107
		30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	cortex (different subregions)	30 minutes restraint	30 minutes	Sprague Dawley rats	F < M	F < M	ISH	110
	AC	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	MPOA	30 minutes restraint	/	Sprague Dawley rats	F > M[a]	F > M[a]	ISH	157
	BNSTav	30 minutes restraint	/	Sprague Dawley rats	F > M[a]	F > M[a]	ISH	157
	HPC	6 minutes cold swim stress	45 minutes	c57BL6 mice	↑	↑	qPCR	109
		6 minutes restraint	45 minutes	c57BL6 mice	—	↑	qPCR	109
		30 minutes restraint	30 minutes	Sprague Dawley rats	F < M[a]	F < M[a]	ISH	110
	MeA	30 minutes restraint	30 minutes	Sprague Dawley rats	F = M[a]	F = M[a]	ISH	110
	VO	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	RAI	30 minutes restraint	/	Sprague Dawley rats	—	—	ISH	115
	SCN	30 minutes restraint	/	Sprague Dawley rats	↑	—	ISH	115
	LS	30 minutes restraint	30 minutes	Sprague Dawley rats	F = M[a]	F = M[a]	ISH	110
Bdnf	PFC	100 minutes restraint + tailshock	/	Sprague Dawley rats	↑	—	ISH	107
		100 minutes restraint + tailshock	60 minutes	Sprague Dawley rats	—	—	ISH	107
	CeA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
	BLA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
	BNST	3 days social defeat	2 weeks	California mice	—	—	qPCR	114
Per1	PVN	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	PFC	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	AC	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	HPC	6 minutes cold swim stress	45 minutes	c57BL6 mice	↑	↑	qPCR	109
		6 minutes restraint	45 minutes	c57BL6 mice	↑	↑	qPCR	109
	SCN	30 minutes restraint	/	Sprague Dawley rats	—	—	ISH	115
	VO	30 minutes restraint	/	Sprague Dawley rats	↑	↑	ISH	115
	RAI	30 minutes restraint	/	Sprague Dawley rats	$\uparrow_t$	$\uparrow_t$	ISH	115

(Continues)

TABLE 2 (Continued)

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
Per2	PVN	30 minutes restraint	/	Sprague Dawley rats	—	↑	ISH	115
	VO	30 minutes restraint	/	Sprague Dawley rats	—	↑	ISH	115
		6 minutes restraint	45 minutes	c57BL6 mice	↑	↑	qPCR	109
Cbp	PVN	1 hour restraint	1 hour	Wistar rats	↑	—	qPCR	104
Dnmt1	CeA	3 days social defeat	2 weeks	California mice	—	↓	qPCR	116
	MeA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
	BLA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
Dnmt3a	CeA	3 days social defeat	2 weeks	California mice	?	—	qPCR	116
	MeA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
	BLA	3 days social defeat	2 weeks	California mice	—	—	qPCR	116
	NAc	6 days sCVS	4 hours, 24 hours	c57BL6 mice	↑	↑	qPCR	148
Cnr1	cerebellum	3 days tailshock + ARS	/	Sprague Dawley rats	↓	↓	qPCR	158
	brain stem	3 days tailshock + ARS	/	Sprague Dawley rats	—	—	qPCR	158
Cnr2	cerebellum	3 days tailshock + ARS	/	Sprague Dawley rats	—	—	qPCR	158
	brain stem	3 days tailshock + ARS	/	Sprague Dawley rats	—	—	qPCR	158

Notes: Regions: HPT, hypothalamus; PVN, paraventricular nucleus of the hypothalamus; PFC, prefrontal cortex; HPC, hippocampus; CeA, central amygdala; BLA, basolateral amygdala; MeA, medial amygdala; BNST, bed nucleus of the stria terminalis; LS, lateral septum; SCN, suprachiasmatic nucleus; AC, anterior cingulate; VO, ventro-orbital cortex; RAI, rostral agranular insula; MPOA, medial preoptic area. Paradigm: sCVS, subchronic variable stress; ARS, acute restraint stress. Tissue collection: /, collected right at the end of the paradigm. Methods: NB, northern blot; ISH, in situ hybridization; FISH, fluorescent in situ hybridization; qPCR, quantitative PCR. ↓ downregulated; ↑ upregulated; t, trend; ?, unclear|discordant results; —, no differential expression.

[a]No control animals were used in the experiments.

differ at the behavioral level.[120] Further studies are needed to elucidate if stress perception differs at the transcriptomic level between the sexes.

Based on this collection of evidence, we can speculate that many more regions and genes from the ones highlighted here might show sex-specific spatial and temporal regulation after stress. Overall, more comprehensive studies that include multiple regions and rigorous time points are needed to characterize the effects of sex on the temporal aspect of stress response. According to the studies reviewed here, the time point of observation after stress is probably a key factor for identifying and characterizing sex differences. This temporal factor might indeed account for the discrepancy found in literature.

3.3 | High-throughput studies

Given the fact that MR and GR are both two important transcription factors and that epigenetic players such as DNA methyltransferases seem to be modulated by sex in the context of stress,[116] it would not be surprising to find altered transcription levels on a more general scale. Unfortunately, large-scale approaches taking into consideration sex as a variable are still poorly represented in stress research.[121] Here, we review some studies that did investigate the transcriptional response to acute stress using high-throughput approaches and included sex as a biological variable in their design.

One of these studies, using RNA-sequencing on translating ribosome affinity purified (TRAP) pyramidal neurons of the hippocampus CA3, recently explored the actively translated immediate early genes in response to an acute forced swim test.[122] The authors found that while both males and females showed many DEGs (including the expected *cFos* and *Arc*), female DEGs were found to be almost three times more in number than the ones found in males. Interestingly, the number of DEGs with same directionality shared between the two sexes was found being less than 5%, similar to findings in humans.[84,85,123] Furthermore, the stress-affected pathways corresponded poorly between sexes and females had a higher number of involved pathways. Thus, males and females in response to the same acute stress showed not only different transcriptional plasticity but also unique responses. A second research group showed that altered gene expression after acute restraint stress in the hippocampus is correlated with the epigenetic marker 5hmC.[117,124] Interestingly, 25% of the genomic regions that are regulated by 5hmC after stress code for sex-specific DEGs. Moreover, the authors showed that other epigenetic regulators, such as *Dnmt3a*, *Hdac7*, and *Hdac10*, were altered in a sex-specific way. Overall their data corroborate the idea that epigenetic mechanisms can play a role in the sex-specific stress-induced transcriptomic alterations presented so far.

To summarize, the male and female response to acute stress seems to be processed in the brain differently (Figure 2, left panel). When looking at transcriptional profiles of stressed and control

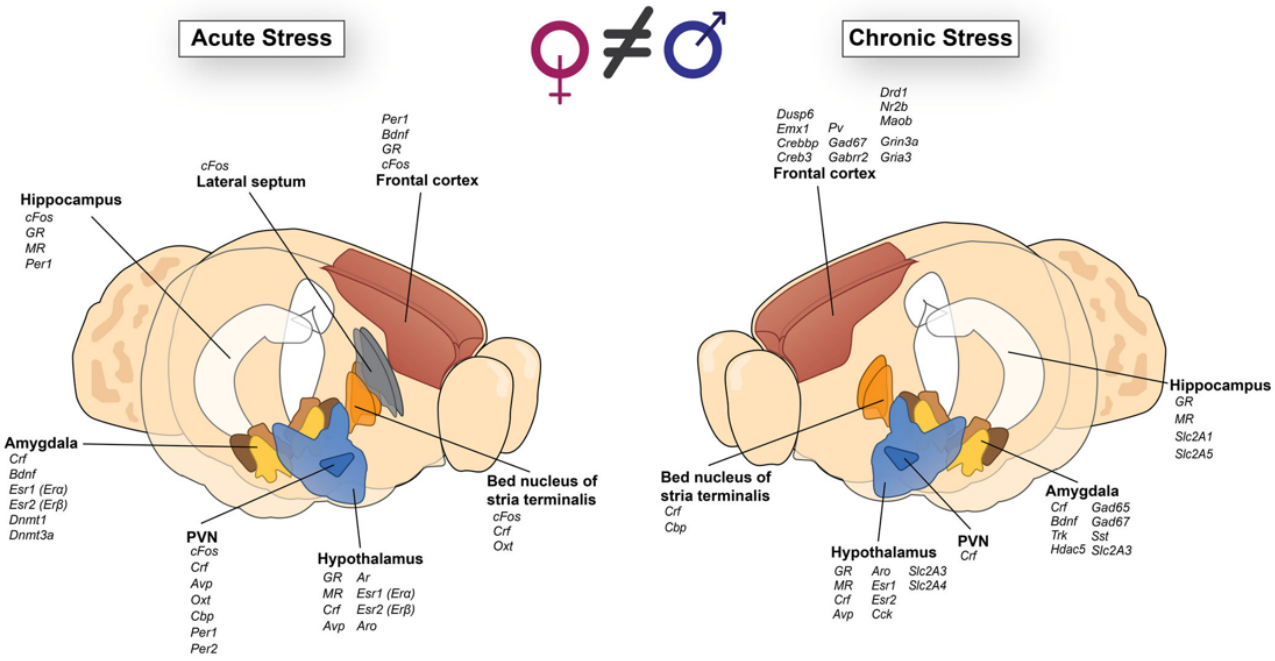

FIGURE 2 Genes differently affected by acute and chronic stress in male and female rodent brain regions. Schematic representation of genes affected by either acute (left panel) or chronic (right panel) stress in the rodent brain in a sex-specific fashion. Several stress-related genes such as *Nr3c1*, *Crf*, *Avp*, and activity-dependent genes such as *Bdnf* and *cFos* have been found to be regulated by acute stress in opposite directions in several brain regions of male and female rodents. The GABAergic system (*Pv*, *Gad65*, *Gad67*, and *Gabrr2*), the dopaminergic system (*Drd1*, *Nr2b*, and *Maob*) and stress-related genes (*Nr3c1*, *Nr3c2*, *Crf*, and *Avp*) seem to be regulated in opposite directions in the two sexes after chronic stress. A full list of genes regulated by acute and chronic stress can be found in Tables 1 and 2 and Tables 3 and 4, respectively

rodents, we can identify region-specific differences in stress-related genes and epigenetic players. Importantly, in the current literature is not uncommon to find discrepancy among studies. This inconsistency might arise from different technologies employed which have different sensitivity, but most importantly on the specific stress paradigm chosen and the time point of analysis.

4 | CHRONIC STRESS

Chronic exposure to stressors tunes the stress system and is recognized as a strong risk factor for the development of psychiatric disorders.[12] Thus, preclinical models of chronic stress exposure are currently studied to elucidate the biological processes underlining the pathogenesis of psychiatric disorders. Several of these models have also been used to study the role of sex in these processes, either exposing animals to single repeated stressors, or to more complex protocols, which include combinations of multiple stressors like the unpredictable chronic mild stress and its variant, the chronic variable stress.[125,126] Importantly, the classic paradigm of chronic social defeat stress, which has been widely used to study chronic stress exposure in male rodents, has recently been adapted for use in females of nonaggressive strains.[127-129] To the best of our knowledge, however, molecular studies with this paradigm are still lacking. Overall, these protocols have very different designs and limitations: an important factor to consider when trying to compare results from different studies. For example, exposing animals to the same stress across days may lead to stress habituation.[130] Given the fact that males and females differ in their molecular and behavioral coping strategies to stress exposure, stress habituation is potentially a sexually dimorphic process too.[131-133] Indeed, the HPA axis negative feedback and the process of adaptation to repeated homotypic stressors, such as restraint, have been shown to be influenced by estrogens.[130,134] However, information on how these differences happen at the level of gene expression are still lacking. Still, we can hypothesize that stress adaptation would show sex dimorphism also at the transcriptomic level. If this is correct, sex-specific transcriptional signatures observed after repeated stressors could result from the combination of stress and habituation responses, which would need to be taken into consideration when interpreting results. On the contrary, other more complex chronic stress paradigms, such as chronic mild stress, try to avoid the habituation process exposing the animals to various mild stressors across many days.[135] Nevertheless, no universal protocol for this paradigm exists, so a variety of stressor combinations, degrees of unpredictability and length can be found in literature. Importantly, chronic stress exposure is sometimes paired with tests to behaviorally assess the stress status of the animals. Exposure to commonly used tests such as the forced swim, tail suspension, or elevated plus maze, when not part of the chronic stress paradigm, can elicit an acute stress response. As a result, the observed

transcriptional signatures might combine chronic and acute responses. All these aspects need to be considered when trying to critically review the current literature and trying to understand discordant results. Finally, as with acute stress studies, different technologies can have a different degree of accuracy in quantifying gene expression. Here, we present studies that included sex as biological variable that either used a targeted or a high-throughput approach.

4.1 | Targeted studies

Targeted studies with chronic stress paradigms have been especially useful in elucidating how the interaction of sex and chronic stress affects the classic stress-related genes. The expression of genes such as MR and GR are, in fact, affected in a sex- and region-specific fashion not only by acute but also by chronic stress exposure. For instance, there is evidence in the literature that the response to chronic stress involves tuning the GR:MR system differently according to the region and sex.[99] For example, repeated restraint stress reduced the levels of GR in the hypothalamus in females, but not in males. In contrast, upon the same repeated restraint stress, MR is downregulated in the hippocampus of males, but upregulated in females. Importantly, exposing rats to a new stressor, such as the forced swim test, leads to different sex- and region-specific expression changes[100] (see Tables 3 and 4 for detailed description of the changes). It is therefore important to consider the selection of the type of stressor and the paradigm design when assessing sex differences as fundamental.

Exposure to chronic stress also modulates expression levels of other stress-related genes, such as the *Crf* system and the oxytocin-vasopressin pathway. Guo et al[103] showed that the combined exposure to chronic mild stress and an acute forced swim session led to a wide range of gene expression changes of stress-related genes in the hypothalamus in a different way between sexes. More specifically, the authors showed that *Crf*, *Avp*, *Oxt*, and *Esr1* are all upregulated in females, but are not changed in males. *Crf*, *Avp*, and *Oxt* were found increased specifically in females also when the mice were not further exposed to the forced swim test.[101] However, not surprisingly, not every work published agrees.[136,137] Other regions involved in the stress circuitry such as the CeA and basolateral amygdala (BLA), as well as the bed nucleus of the stria terminalis (BNST) show the different extent of sex-specific regulation of such genes and others, including the *Bdnf* cascade[137-140] (Tables 3 and 4).

Apart from the classical stress-related genes presented so far, psychopathologies are known to be characterized by an imbalance in several neurotransmitter pathways.[59] Some of these imbalances are reproduced in chronically stressed rodents and show patterns of sex dimorphism. For instance, the GABAergic pathway in corticolimbic structures seems to be affected in a sex-specific way in response to chronic stress. Parvalbumin mRNA levels in the PFC are upregulated in females, but are unchanged in males.[141,142] Other genes related to the GABAergic pathway such as *Gad67*, *Gad65*, and somatostatin (*Sst*) in the BLA are also strongly influenced by the interaction of stress exposure and sex.[139,143] For instance, using four core genotypes (FCG) mice, Puralewski et al[139] were able to dissect the role of chromosomal sex, gonadal sex, and circulating testosterone in shaping the stress response at the level of GABAergic circuitry. Despite not being able to directly compare controls and stressed animals, they identified some GABA-related genes, such as *Sst*, that do not show expression differences between sexes (either gonadal or chromosomal) at baseline but do after chronic mild stress. This argues for a potential sex × stress interaction on the GABAergic system, worthy of further studies. Similarly, stress-specific changes in genes belonging to the glutamate pathway were found to be sex-specific in different regions such as the hippocampus and the hypothalamus[144] (Table 4). In contrast, the dopaminergic/noradrenergic system in the locus coeruleus—the main source of noradrenaline in the brain—and the acetylcholine pathway are equally affected in both sexes in preclinical studies.[136,145] Importantly, these pathways do not work in isolation, rather they are strongly integrated among each other and across regions. Thus, observing more than one pathway at the same time might provide a more complete overview of the combined effect of stress and sex. Barko et al[146] attempted to tackle this issue by using a subset of genes of the GABA, glutamate and dopamine pathways in the PFC, BLA, and NAc. It is interesting to note that the three regions presented a different extent of overlap in gene expression changes after unpredictable chronic mild stress, similarly to the changes observed in MDD patients.[84] The authors further explored the sex dimorphism building a gene network across the three neurotransmitter pathways in the PFC. Surprisingly, already at baseline, the female network was more strongly coordinated than the male network and less stable against chronic stress. These results suggest that females might have a higher intrinsic transcriptional sensitivity to stress and that these three systems, the GABAergic, glutamatergic and dopaminergic/reward systems are potential sources of sex dimorphism in the stress response. However, it is difficult to conclude if these are overall features of the observed regions, in light of the small number of genes sampled (7-10 per neurotransmitter system). In contrast, high-throughput technologies such as next-generation RNA sequencing can test the whole transcriptome at once, allowing indeed to create a more complete view of stress-specific changes.

4.2 | High-throughput studies

High-throughput studies addressing the interaction between sex and chronic stress are slowly becoming more popular, even if still underrepresented. Thanks to these studies, an overview of differences in rodents is slowly building up allowing comparisons with evidence from psychiatric patients to be made. These high-throughput studies include both microarray[123,147] and RNA-sequencing[84,85,148-152] approaches. Both types of technologies allow for a genome-wide profiling of stress responses in the two sexes and the study of these responses in the context of pathways. For example, Karisetty et al[147] used mRNA microarrays to study the transcriptomic signatures of

TABLE 3 Stress-related genes regulated by chronic stress in males and females

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
Nr3c1 (GR)	PIT	14 days 60 minutes restraint	24 hours	Wistar rats	—	—	NB	99
		14 days 60 minutes restraint + ARS	/	Wistar rats	↑	—	NB	99
	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	—	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	↓	qPCR	101
		14 days 60 minutes restraint ± ARS	24 hours, 0 minute	Wistar rats	—	↓	NB	99, 100
		14 days 20 minutes FST	24 hours	Wistar rats	—	—	NB	100
		14 days 60 minutes restraint + FST	60 minutes	Wistar rats	↑	↓	NB	100
		14 days 60 minutes restraint + 13 days 20 minutes FST	24 hours	Wistar rats	—	—	NB	100
	HPC	14 days 60 minutes restraint ± ARS or ± FST	/, 24 hours	Wistar rats	↓	—	NB	99, 100
		14 days 20 minutes FST	24 hours	Wistar rats	↓	—	NB	100
Nr3c2 (MR)	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	—	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	↓	qPCR	101
		14 days 60 minutes restraint ± ARS	24 hours, /	Wistar rats	—	—	NB	99, 100
		14 days 20 minutes FST	24 hours	Wistar rats	—	—	NB	100
		14 days 60 minutes restraint + 20 minutes FST	60 minutes	Wistar rats	↑	—	NB	100
		14 days 60 minutes restraint + 13 days 20 minutes FST	24 hours	Wistar rats	—	—	NB	100
	HPC	14 days 60 minutes restraint	24 hours	Wistar rats	↓	↑	NB	99, 100
		14 days 60 minutes restraint + ARS or + 20 minutes FST	/, 60 minutes	Wistar rats	—	↑	NB	99, 100
		14 days 20 minutes FST	24 hours	Wistar rats	—	—	NB	100
		14 days 60 minutes restraint + 13 days 20 minutes FST	24 hours	Wistar rats	↓	↑	NB	100
Crf	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	—	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	↑	qPCR	101
	PVN	3 weeks CMS	3 days	Sprague Dawley rats	↑	—	ISH	136
		10 days CMS + EPM	30 minutes	Sprague Dawley rats	—	—	ISH	138
		2 weeks CMS	1 hour	Wistar rats	↑	—	ISH	137
	CeA	2 weeks CMS	1 hour	Wistar rats	$\downarrow_t$	—	ISH	137
		10 days CMS + EPM	30 minutes	Sprague Dawley rats	—	—	ISH	138
	BNSTov	2 weeks CMS	1 hour	Wistar rats	$\downarrow_t$	—	ISH	137
	BNSTfu	2 weeks CMS	1 hour	Wistar rats	$\downarrow_t$	$\uparrow_t$	ISH	137
Avp	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	↑	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	↑	— (↑ in diestrus)	qPCR	101

(Continues)

TABLE 3 (Continued)

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
		3 weeks CMS + EPM + OFT + FST	16—18 hours	c57BL6 mice	↑	—	qPCR	147
	PVN	10 days CMS + EPM	30 minutes	Sprague Dawley rats	—	—	ISH	138
Oxt	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	↑	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	—	qPCR	101
		3 weeks CMS + EPM + OFT + FST	16-18 hours	c57BL6 mice	—	↓	qPCR	147

Notes: Regions: HPT, hypothalamus; PVN, paraventricular nucleus of the hypothalamus; PFC, prefrontal cortex; HPC, hippocampus; CeA, central amygdala; BLA, basolateral amygdala; MeA, medial amygdala; BNST, bed nucleus of the stria terminalis; LS, lateral septum; SCN, suprachiasmatic nucleus; AC, anterior cingulate; VO, ventro-orbital cortex; RAI, rostral agranular insula; MPOA, medial preoptic area. Paradigm: CMS, chronic mild stress; FST, forced swim test; ARS, acute restraint stress; EPM, elevated plus maze test; OFT, open field test. Tissue collection: /, samples collected right at the end of the paradigm. Methods: NB, northern blot; ISH, in situ hybridization; FISH, fluorescent in situ hybridization; qPCR, quantitative PCR. ↓ downregulated; ↑ upregulated; t, trend; ?, unclear|discordant results; —, no differential expression.

chronic mild stress in the male and female hypothalamus. Several genome-wide stress-specific DEGs were identified in the male tissue and even a higher number in the female. Importantly, using in silico pathway analysis, the authors found these DEGs were enriched for "mood disorders" pathways and several other neuronal functions such as neuroendocrine peptides processing, synaptic transmission and transduction networks for both sexes. However, these pathways seemed to be altered at the level of different genes between males and females. For instance, within the "posttranslational processing of neuroendocrine peptides" pathway, males showed deregulation of *Avp* and cholecystokinin (*Cck*), whereas females had altered *Oxt* levels.[147] Studying gene alterations in the context of pathways can, therefore, help identifying which basic mechanisms are shared between the sexes and conversely how different gene expression changes can lead to similar outcomes.

On the other hand, RNA sequencing studies can achieve a further level of complexity: the discovery of a novel gene(s) of interest or the study of gene variants. Genes that have never been implicated in the stress response before, in fact, cannot be identified with targeted studies and only difficultly with microarrays. It is possible to find examples of the potentiality of this approach already in the current literature; for instance, in the work of Labontè et al.[84] Their study on male and female adult mice with chronic variable stress focused on two regions, the PFC and the NAc. Through a combination of network and pathway analyses, and the combination of human and rodent data, the authors were able to identify two different pathways, one in each sex, that were altered by the exposure to chronic stress. The stress-dependent deregulation of each of these pathways was shown to impact on neuronal activity selectively in one or the other sex. Importantly, the two hub genes of these two pathways, *Dusp6* and *Emx1*, were two genes not previously implicated in the stress response. This study showed how RNA-seq approach can help in the identification of novel sex-specific gene players. These types of studies can bring the field one step closer toward sex-specific treatments for stress-related disorders. In addition, when analyzing cell-type specific pathways of DEGs, the authors identified enrichment for different cell types in a sex-specific fashion. For instance, female PFC seemed to be mostly affected at the level of neurons, whereas the males were more affected in the endothelial pathways. Another study suggests that proliferation in the hippocampus is selectively affected in male rats, suggesting proliferative cells, such as glia or neuronal progenitors, are differentially affected in the two sexes.[151] Further studies at the single-cell level, however, are still necessary to help elucidate the origin of these differences.

It is also interesting to mention that RNA-sequencing approaches have been used to study the reported heightened susceptibility to chronic stress of females.[148,149] To study the molecular mechanisms that regulate the sex-specific susceptibility to stress, some groups have been using the subchronic variable stress paradigm. After 6 days of variable stress, in fact, only female mice develop a classic stressed phenotype of anhedonia and elevated CORT, whereas males cannot be differentiated from controls. Surprisingly, the authors found that the number of DEGs in the NAc was disproportionally higher in males than females. Furthermore, almost none of these genes were shared between them and the pathways enriched for these DEGs were not in common between males and females. Hence, in this work, subchronic stress was able to elicit a strong transcriptional response in males but failed to do the same in females. Considering that males appeared asymptomatic at this stage of the chronic paradigm and females did not, the data suggest the intriguing possibility that male rodents show an active resilience response that is not elicited in females. In the current literature, we can find extensive works about resilience in male animals, but a comparable line of research in females or comparing the sexes is still lacking. If replicated in further studies and different brain regions, these results might represent the first clue to find early-on differences between the sexes in response to prolonged stress. The authors might have identified the first manifestation of sex-dependent differences observed in chronic stress susceptibility and psychiatric disorders and it is therefore worthy of further investigation.

TABLE 4 Nonstress-related genes regulated by chronic stress in males and females

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
Ar	HPT	3 weeks CMS	24 hours	Sprague Dawley rats	—	—	qPCR	101
Aro	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	↓	—	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	—	qPCR	101
Esr1	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	↑	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	—	qPCR	101
Esr2	HPT	3 weeks CMS + FST	5 minutes	Sprague Dawley rats	—	—	qPCR	103
		3 weeks CMS	24 hours	Sprague Dawley rats	—	— (↑ in diestrus)	qPCR	101
Bdnf	BLA	8 weeks CMS	/	FCG mice	[a]	[a]	qPCR	139
Trkb	BLA	8 weeks CMS	/	FCG mice	[a]	[a]	qPCR	139
Cbp	PVN, CeA	2 weeks CMS	1 hour	Wistar rats	—	—	qPCR	137
	BNST	2 weeks CMS	1 hour	Wistar rats	—	↑	qPCR	137
Hdac3	PVN, BNST, CeA	2 weeks CMS	1 hour	Wistar rats	—	—	qPCR	137
Hdac4	PVN, BNST, CeA	2 weeks CMS	1 hour	Wistar rats	—	—	qPCR	137
Hdac5	PVN, BNST	2 weeks CMS	1 hour	Wistar rats	—	—	qPCR	137
	CeA	2 weeks CMS	1 hour	Wistar rats	↓	—	qPCR	137
Pcaf	PVN, BNST, CeA	2 weeks CMS	1 hour	Wistar rats	—	—	qPCR	137
Cck	HPT	3 weeks CMS + EPM + OFT + FST	16-18 hours	c57BL6 mice	↑	—	qPCR	147
Dusp6	PFC	CVS	*na*	c57BL6 mice	—	↓	RNA-seq	84
Emx1	PFC	CVS	*na*	c57BL6 mice	↑	—	RNA-seq	84
GABAergic system								
Sst	BLA	8 weeks CMS	/	FCG mice	[a]	[a]	qPCR	139
Gad65	BLA	8 weeks CMS	/	FCG mice	[a]	[a]	qPCR	139
Gad67	PFC	2 weeks CMS + FST	48 hours	c57bl6	$\uparrow_t$	—	qPCR	141
		4 weeks CMS + FST	48 hours	c57bl6	—	—	qPCR	141
	BLA	8 weeks CMS	/	FCG mice	[a]	[a]	qPCR	139
Gabra2	PFC	2|4 weeks CMS + FST	48 hours	c57bl6	—	—	qPCR	141
		8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Gabra5	PFC	8 weeks CMS	*na*	FCG mice	↓	↓	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↓	↓	qPCR	146
Gabrr2	PFC	8 weeks CMS	*na*	FCG mice	XY —	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Gphn	PFC, BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146

(Continues)

TABLE 4 (Continued)

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
Gat1	PFC, BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Gabarap	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Gabarapl1	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Grin2b	DLPFC	4 weeks CMS + FST	48 hours	Balb|c mice	—	—	qPCR	142
Glutamatergic system								
Gria1	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Gria3	PFC	8 weeks CMS	*na*	FCG mice	XY —	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Grin1	PFC	4 weeks CMS + FST	48 hours	Balb|c mice	—	—	qPCR	142
Grin2a	PFC	4 weeks CMS + FST	48 hours	Balb|c mice	—	—	qPCR	142
Grin2b	PFC	4 weeks CMS + FST	48 hours	Balb|c mice	$\downarrow_t$	—	qPCR	142
Grm1	PFC, BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Grik3	PFC, BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Grin3a	PFC	8 weeks CMS	*na*	FCG mice	XY —	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Slc25a22	PFC	8 weeks CMS	*na*	FCG mice	↓	↓	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Grip1	PFC	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA, NAc	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Slc2a1	HPT, PFC, AMY	6 days social defeat + 6 days ARS	3 days	Wistar rats	—	—	qPCR	144
	HPC	6 days social defeat + 6 days ARS	3 days	Wistar rats	↑	—	qPCR	144
Slc2a3	HPT	6 days social defeat + 6 days ARS	3 days	Wistar rats	—	↓	qPCR	144
	PFC, HPC	6 days social defeat + 6 days ARS	3 days	Wistar rats	—	—	qPCR	144
	AMY	6 days social defeat + 6 days ARS	3 days	Wistar rats	↑	—	qPCR	144
Slc2a4	HPT	6 days social defeat + 6 days ARS	3 days	Wistar rats	↓	—	qPCR	144

(Continues)

TABLE 4 (Continued)

Gene	Region	Paradigm	Tissue collection (time after last stressor)	Animal model	M stress vs ctrl	F stress vs ctrl	Method	References
	PFC	6 days social defeat + 6 days ARS	3 days	Wistar rats	—	—	qPCR	144
Slc2a5	PFC, AMY	6 days social defeat + 6 days ARS	3 days	Wistar rats	—	—	qPCR	144
	HPC	6 days social defeat + 6 days ARS	3 days	Wistar rats	—	↑	qPCR	144
Dopaminergic system								
Th	LC	3 weeks CMS	3 days	Sprague Dawley rats	—	—	ISH	136
Drd1	PFC	8 weeks CMS	*na*	FCG mice	XY —	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Drd2	PFC, BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Drd5	PFC	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Comt	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Maoa	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Maob	PFC	8 weeks CMS	*na*	FCG mice	XY ↓	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Creb1	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
Creb3	PFC	8 weeks CMS	*na*	FCG mice	XY —	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Crebbp	PFC	8 weeks CMS	*na*	FCG mice	XY —	XX ↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
	NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
Ddc	PFC, NAc	8 weeks CMS	*na*	FCG mice	↑	↑	qPCR	146
	BLA	8 weeks CMS	*na*	FCG mice	—	—	qPCR	146
HCNP-pp	HPC	4 weeks CMS	*na*	C57bl6 mice	$\uparrow_t$	$\uparrow_t$	qPCR	145

Notes: Regions: HPT, hypothalamus; PVN, paraventricular nucleus of the hypothalamus; PFC, prefrontal cortex; HPC, hippocampus; AMY, amygdala; CeA, central amygdala; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis. Paradigm: CMS, chronic mild stress; CVS, chronic variable stress; FST, forced swim test; ARS, acute restraint stress; EPM, elevated plus maze test; OFT, open field test. For FCG mice, four core genotypes mice, XY or XX have been specified when gene expression changes were observed for chromosomal sex. Tissue collection: /, samples collected right at the end of the paradigm; na, information not available. Methods: ISH, in situ hybridization; qPCR, quantitative PCR. ↓ downregulated; ↑ upregulated; t, trend; ?, unclear| discordant results; —, no differential expression.

[a]No direct comparison stress vs controls.

TABLE 5 Nonstress-related genes regulated by psychopathologies in men and women

Gene	Region	Condition	M stress vs ctrl	F stress vs ctrl	Method	References
DUSP6	PFC	MDD	—	↓	RNA-seq	84
EMX1	PFC	MDD	↑	—	RNA-seq	84
ARPP21	AMY	MDD	—	↑	qPCR	85
P2RY12	ACC	MDD	—	$\downarrow_t$	Microarray	85
	AMY	MDD	—	↓	qPCR	85
MTHFR	ACC	MDD	$\downarrow_t$	—	Microarray	85
	AMY	MDD	—	—	qPCR	85
SLCO1A2	ACC	MDD	—	↓	Microarray	85
ARHGEF3	ACC	MDD	—	↑	Microarray	85
GABRD	ACC	MDD	$\downarrow_t$	—	Microarray	85
CAMK2B	ACC	MDD	↓	—	Microarray	85
CACNA1I	ACC	MDD	↓	—	Microarray	85
NOL3	ACC	MDD	↓	↓	Microarray	85
NUB1	ACC	MDD	↑	↑	Microarray	85
PSMA3	ACC	MDD	↓	↓	Microarray	85
GRIA1	DLPFC	MDD	—	—	qPCR	159
GRIA2	DLPFC	MDD	—	↑	qPCR	159
GRIA3	DLPFC	MDD	—	↑	qPCR	159
GRIA4	DLPFC	MDD	—	↑	qPCR	159
GRIN1	DLPFC	MDD	—	↑	qPCR	159
GRIN2A	DLPFC	MDD	—	↑	qPCR	159
GRIN2B	DLPFC	MDD	—	↑	qPCR	159
GRIN2C	DLPFC	MDD	—	↑	qPCR	159
GRIN2D	DLPFC	MDD	—	↑	qPCR	159
GRIN3A	DLPFC	MDD	—	—	qPCR	159
		Suicide	—	↑	qPCR	159
GRM1	DLPFC	MDD	—	↑	qPCR	159
GRM2	DLPFC	MDD	—	—	qPCR	159
		Suicide	—	↑	qPCR	159
GRM3	DLPFC	MDD	—	—	qPCR	159
GRM4	DLPFC	MDD	—	↑	qPCR	159
GRM5	DLPFC	MDD	↓	↑	qPCR	159
GRM7	DLPFC	MDD	—	↑	qPCR	159
GRIK1	DLPFC	MDD	—	↑	qPCR	159
GRIK2	DLPFC	MDD	—	↑	qPCR	159
GRIK3	DLPFC	MDD	—	—	qPCR	159
		Suicide	↑	—	qPCR	159
HCNP-pp	AMY	MDD	—	↑	qPCR	145
CRF-BP	BLA, lateral AMY	MDD	—	—	ISH	78
	BLA, lateral AMY	BPD	↓	—	ISH	78
IL-4	OFC	Suicide	—	↑	qPCR	160
IL-13	OFC	Suicide	↑	—	qPCR	160
TNFa	OFC	Suicide	—	$\uparrow_t$	qPCR	160

Notes: Regions: PIT, pituitary; HPT, hypothalamus; PVN, paraventricular nucleus of the hypothalamus; PFC, prefrontal cortex; OFC, orbitofrontal cortex; HPC, hippocampus; CeA, central amygdala; BLA, basolateral amygdala; MeA, medial amygdala; BNST, bed nucleus of the stria terminalis; AMY, amygdala; NAc, nucleus accumbens; LC, locus ceruleus. Condition: MDD, major depressive disorder; BPD, bipolar disorder. Methods: NB, northern blot; ISH, in situ hybridization; FISH, fluorescent in situ hybridization; qPCR, quantitative PCR. ↓, downregulated; ↑, upregulated; t, trend; —, no differential expression.

The evidence reviewed here suggests that there are profound transcriptomic differences in response to chronic stress across males and females in several brain regions of the rodent brain (Figure 2, right panel). Females that look behaviorally more susceptible to chronic stress display a higher number of deregulated genes and often more deregulated pathways. Furthermore, it is not uncommon to identify genes and pathways affected by stress uniquely in one sex. These genes and pathway could be involved in the sex dimorphism of psychiatric disorders or become novel targets for treatment. Finally, a preliminary study suggests that females and males differ already at the level of molecular signatures of resilience after subchronic stress. Further studies are needed to assess if these gene expression changes are indeed associated to resilience and to potentially develop early-on treatments.

5 | CONCLUSIONS

Stress is processed in the brain by a network of regions interacting with each other, including the PVN, hippocampus, amygdala, PFC and other nuclei. Their response to stress is mediated by a set of transcriptional adaptations in several gene networks and recent studies have highlighted sex as a modulator factor in these processes. Well-known stress-related genes such as MR, GR, CRF, AVP, and OXT (Tables 1 and 3) are differentially regulated after acute or chronic stress in a sex-specific way. However, sex-mediated differences in transcriptional signatures of stress can be found also in other genes not classically associated with stress-related pathways (Figure 2). These include genes involved in neuronal function and architecture, proliferation and immune system regulation. Some of these genes and pathways look already like promising candidates to further explore sex differences, such as the GR, MR or the GABAergic system (Tables 1–5). Yet, future studies should carefully select not only the region to analyze but also the stress paradigm and the time point of observation. As discussed earlier, the current literature supports the idea that the kinetics of transcriptional signatures in response to stress might be different between the sexes. On a more global scale, females show an overall higher transcriptional plasticity to stress compared with males. This holds true for acute and chronic stress, but might not apply to subchronic stress exposure. For subchronic variable stress, males show an active resilience transcriptional response, which seems to be lacking in females. Further genome-wide studies would help in elucidating this and if these features are broadly shared by all brain regions or rather region-specific. With the development of modified chronic social defeat paradigms applicable to females,[127-129] it will be interesting to see if behaviorally resilient individuals can be identified among females and investigate their transcriptional profile as has already been done for males.[80,91] Other stress paradigms applied in other life phases (perinatality, adolescence) that in the past have shown to generate resilient and susceptible phenotypes such as early life stress will also be a powerful way to further address the matter of sex difference in stress resilience.[153-155] Identifying differences in stress resilience and when they emerge is a key point to dissect the origin of sex differences in stress response and susceptibility to psychopathologies, since, for many of these disorders, differences start to emerge after puberty (for a review see[156]). Moreover, future studies should also try to address how transcriptional changes in response to acute stress contribute to behavioral susceptibility to chronic stress. In turn, more studies are needed to understand how the changes elicited by chronic stress contribute to the development of psychopathologies in humans. Finally, there is also some evidence pointing at the involvement of different cell types on the pathophysiology of stress response between the sexes. Using emerging technologies, such as single-cell RNA sequencing, future studies should be better suited to further understand these differences at a higher resolution.

Studying these sex-specific differences at the transcriptional level will enable the identification of the underlying mechanisms engaged in response to a stressful stimulus. Understanding which mechanisms are more affected in males, and which in females, may lead to the identification of sex-specific key players, their selective contribution to stress susceptibility, and the development of stress-related psychiatric disorders. Ultimately, it will help to understand why treatments have different efficiency between the two sexes and eventually lead to the development of better treatment options.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. World Health Organization. *Depression and Other Common Mental Disorders: Global Health Estimates*. Geneva: World Health Organization; 2017 http://www.who.int/mental_health/management/depression/prevalence_global_health_estimates/en/.
2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005;62(6):593-602. https://doi.org/10.1001/archpsyc.62.6.593.
3. Young LJ, Pfaff DW. Sex differences in neurological and psychiatric disorders. *Front Neuroendocrinol*. 2014;35(3):253-254. https://doi.org/10.1016/j.yfrne.2014.05.005.
4. Altemus M, Sarvaiya N, Neill Epperson C. Sex differences in anxiety and depression clinical perspectives. *Front Neuroendocrinol*. 2014;35(3):320-330. https://doi.org/10.1016/j.yfrne.2014.05.004.
5. Kuehner C. Why is depression more common among women than among men? *Lancet Psychiatry*. 2017;4(2):146-158. https://doi.org/10.1016/S2215-0366(16)30263-2.
6. Martin LA, Neighbors HW, Griffith DM. The experience of symptoms of depression in men vs women: analysis of the national comorbidity survey replication. *JAMA Psychiat*. 2013;70(10):1100-1106. https://doi.org/10.1001/jamapsychiatry.2013.1985.
7. Marcus SM, Young EA, Kerber KB, et al. Gender differences in depression: findings from the STAR*D study. *J Affect Disord*. 2005;87(2–3):141-150. https://doi.org/10.1016/j.jad.2004.09.008.
8. Kokras N, Dalla C. Preclinical sex differences in depression and antidepressant response: implications for clinical research. *J Neurosci Res*. 2017;95(1–2):731-736. https://doi.org/10.1002/jnr.23861.
9. Kornstein SG, Schatzberg AF, Thase ME, et al. Gender differences in treatment response to sertraline versus imipramine in chronic depression. *Am J Psychiatry*. 2000;157(9):1445-1452. https://doi.org/10.1176/appi.ajp.157.9.1445.
10. Khan A, Brodhead AE, Schwartz KA, Kolts RL, Brown WA. Sex differences in antidepressant response in recent antidepressant clinical trials. *J Clin Psychopharmacol*. 2005;25(4):318-324. https://doi.org/10.1097/01.jcp.0000168879.03169.ce.
11. Sramek JJ, Murphy MF, Cutler NR. Sex differences in the psychopharmacological treatment of depression. *Dialogues Clin Neurosci*. 2016;18(4):447-457.
12. Kessler RC. The effects of stressful life events on depression. *Annu Rev Psychol*. 1997;48(1):191-214. https://doi.org/10.1146/annurev.psych.48.1.191.
13. Herman JP, McKlveen JM, Ghosal S, et al. Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Comprehensive Physiology*. Vol 6. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2016:603-621. https://doi.org/10.1002/cphy.c150015.
14. Gunnar M, Quevedo K. The neurobiology of stress and development. *Annu Rev Psychol*. 2007;58(1):145-173. https://doi.org/10.1146/annurev.psych.58.110405.085605.
15. Kudielka BM, Kirschbaum C. Sex differences in HPA axis responses to stress: a review. *Biol Psychol*. 2005;69:113-132. https://doi.org/10.1016/j.biopsycho.2004.11.009.
16. Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. *Front Neuroendocrinol*. 2014;35(3):303-319. https://doi.org/10.1016/j.yfrne.2014.03.008.
17. Stevens JS, Hamann S. Sex differences in brain activation to emotional stimuli: a meta-analysis of neuroimaging studies. *Neuropsychologia*. 2012;50(7):1578-1593. https://doi.org/10.1016/j.neuropsychologia.2012.03.011.
18. Goldstein JM, Jerram M, Abbs B, Whitfield-Gabrieli S, Makris N. Sex differences in stress response circuitry activation dependent on female hormonal cycle. *J Neurosci*. 2010;30(2):431-438. https://doi.org/10.1523/JNEUROSCI.3021-09.2010.
19. Dachtler J, Fox K. Do cortical plasticity mechanisms differ between males and females? *J Neurosci Res*. 2017;95(1–2):518-526. https://doi.org/10.1002/jnr.23850.
20. Luders E, Gaser C, Narr KL, Toga AW. Why sex matters: brain size independent differences in Gray matter distributions between men and women. *J Neurosci*. 2009;29(45):14265-14270. https://doi.org/10.1523/JNEUROSCI.2261-09.2009.
21. Joel D, Berman Z, Tavor I, et al. Sex beyond the genitalia: the human brain mosaic. *Proc Natl Acad Sci USA*. 2015;112(50):15468-15473. https://doi.org/10.1073/pnas.1509654112.
22. Chekroud AM, Ward EJ, Rosenberg MD, Holmes AJ. Patterns in the human brain mosaic discriminate males from females. *Proc Natl Acad Sci USA*. 2016;113(14):E1968. https://doi.org/10.1073/pnas.1523888113.
23. Anderson NE, Harenski KA, Harenski CL, et al. Machine learning of brain gray matter differentiates sex in a large forensic sample. *Hum Brain Mapp*. 2019;40(5):1496-1506. https://doi.org/10.1002/hbm.24462.
24. Kurth F, Jancke L, Luders E. Sexual dimorphism of Broca's region: more gray matter in female brains in Brodmann areas 44 and 45. *J Neurosci Res*. 2017;95(1–2):626-632. https://doi.org/10.1002/jnr.23898.
25. Davatzikos C, Resnick SM. Sex differences in anatomic measures of interhemispheric connectivity: correlations with cognition in women but not men. *Cereb Cortex*. 1998;8(7):635-640. https://doi.org/10.1093/cercor/8.7.635.
26. Goldstein JM. Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cereb Cortex*. 2001;11(6):490-497. https://doi.org/10.1093/cercor/11.6.490.
27. Gur RC, Turetsky BI, Matsui M, et al. Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. *J Neurosci*. 1999;19(10):4065-4072. https://doi.org/10.1523/JNEUROSCI.19-10-04065.1999.
28. Ingalhalikar M, Smith A, Parker D, et al. Sex differences in the structural connectome of the human brain. *Proc Natl Acad Sci USA*. 2014;111(2):823-828. https://doi.org/10.1073/pnas.1316909110.
29. Kim Y, Yang GR, Pradhan K, et al. Brain-wide maps reveal stereotyped cell-type-based cortical architecture and subcortical sexual dimorphism. *Cell*. 2017;171(2):456-469.e22. https://doi.org/10.1016/j.cell.2017.09.020.
30. Rosinger ZJ, Jacobskind JS, de Guzman RM, Justice NJ, Zuloaga DG. A sexually dimorphic distribution of corticotropin-releasing factor receptor 1 in the paraventricular hypothalamus. *Neuroscience*. 2019;409:195-203. https://doi.org/10.1016/j.neuroscience.2019.04.045.
31. Naqvi S, Godfrey AK, Hughes JF, Goodheart ML, Mitchell RN, Page DC. Conservation, acquisition, and functional impact of sex-biased gene expression in mammals. *Science (80-)*. 2019;365:eaaw7317. https://doi.org/10.1126/science.aaw7317.
32. Trabzuni D, Ramasamy A, Imran S, et al. Widespread sex differences in gene expression and splicing in the adult human brain. *Nat Commun*. 2013;4(1):2771. https://doi.org/10.1038/ncomms3771.
33. Shi L, Zhang Z, Su B. Sex biased gene expression profiling of human brains at major developmental stages. *Sci Rep*. 2016;6(1):21181. https://doi.org/10.1038/srep21181.
34. Reinius B, Saetre P, Leonard JA, et al. An evolutionarily conserved sexual signature in the primate brain. *PLoS Genet*. 2008;4(6):e1000100. https://doi.org/10.1371/journal.pgen.1000100.
35. Xu H, Wang F, Liu Y, Yu Y, Gelernter J, Zhang H. Sex-biased methylome and transcriptome in human prefrontal cortex. *Hum Mol Genet*. 2014;23(5):1260-1270. https://doi.org/10.1093/hmg/ddt516.

36. Gegenhuber B, Tollkuhn J. Signatures of sex: sex differences in gene expression in the vertebrate brain. *WIREs Dev Biol*. 2020;9(1):348. https://doi.org/10.1002/wdev.348.
37. Schwanhäusser B, Busse D, Li N, et al. Global quantification of mammalian gene expression control. *Nature*. 2011;473(7347):337-342. https://doi.org/10.1038/nature10098.
38. Tyssowski KM, DeStefino NR, Cho JH, et al. Different neuronal activity patterns induce different gene expression programs. *Neuron*. 2018;98(3):530-546.e11. https://doi.org/10.1016/j.neuron.2018.04.001.
39. Stark R, Grzelak M, Hadfield J. RNA sequencing: the teenage years. *Nat Rev Genet*. 2019;20:631-656. https://doi.org/10.1038/s41576-019-0150-2.
40. van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C. Ten years of next-generation sequencing technology. *Trends Genet*. 2014;30(9):418-426. https://doi.org/10.1016/j.tig.2014.07.001.
41. Head SR, Komori HK, LaMere SA, et al. Library construction for next-generation sequencing: Overviews and challenges. *Biotechniques*. 2014;56:61-64. https://doi.org/10.2144/000114133.
42. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 2009;10(1):57-63. https://doi.org/10.1038/nrg2484.
43. Rubin TG, Gray JD, McEwen BS. Experience and the ever-changing brain: what the transcriptome can reveal. *Bioessays*. 2014;36(11):1072-1081. https://doi.org/10.1002/bies.201400095.
44. Zhang B, Gaiteri C, Bodea L-G, et al. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell*. 2013;153(3):707-720. https://doi.org/10.1016/j.cell.2013.03.030.
45. Farris SP, Dayne Mayfield R. RNA-Seq reveals novel transcriptional reorganization in human alcoholic brain. *Int Rev Neurobiol*. 2014;116:275-300. https://doi.org/10.1016/B978-0-12-801105-8.00011-4.
46. Zhu Y, Silbereis JC, Pochareddy S, Li M, Sestan N. The molecular landscape of the developing human central nervous system. In: Lehner T, Miller BL, State MW, eds. *Genomics, Circuits, Pathways Clin Neuropsychiatry*. New York: Academic Press; 2016:203-220. https://doi.org/10.1016/B978-0-12-800105-9.00013-5.
47. Dulin JN, Antunes-Martins A, Chandran V, et al. Transcriptomic approaches to neural repair. *J Neurosci*. 2015;35(41):13860-13867. https://doi.org/10.1523/JNEUROSCI.2599-15.2015.
48. Miller JA, Guillozet-Bongaarts A, Gibbons LE, et al. Neuropathological and transcriptomic characteristics of the aged brain. *Elife*. 2017;6:e31126. https://doi.org/10.7554/eLife.31126.
49. Gershoni M, Pietrokovski S. The landscape of sex-differential transcriptome and its consequent selection in human adults. *BMC Biol*. 2017;15(1):7. https://doi.org/10.1186/s12915-017-0352-z.
50. Turecki G. The molecular bases of the suicidal brain. *Nat Rev Neurosci*. 2014;15(12):802-816. https://doi.org/10.1038/nrn3839.
51. Cruceanu C, Tan PPC, Rogic S, et al. Transcriptome sequencing of the anterior cingulate in bipolar disorder: dysregulation of G protein-coupled receptors. *Am J Psychiatry*. 2015;172(11):1131-1140. https://doi.org/10.1176/appi.ajp.2015.14101279.
52. Lutz P-E, Tanti A, Gasecka A, et al. Association of a History of child abuse with impaired myelination in the anterior cingulate cortex: convergent epigenetic, transcriptional, and morphological evidence. *Am J Psychiatry*. 2017;174(12):1185-1194. https://doi.org/10.1176/appi.ajp.2017.16111286.
53. Almeida D, Turecki G. A slice of the suicidal brain: what have post-mortem molecular studies taught us? *Curr Psychiatry Rep*. 2016;18(11):98. https://doi.org/10.1007/s11920-016-0736-8.
54. Mann JJ. Neurobiology of suicidal behaviour. *Nat Rev Neurosci*. 2003;4(10):819-828. https://doi.org/10.1038/nrn1220.
55. Konradi C, Sillivan SE, Clay HB. Mitochondria, oligodendrocytes and inflammation in bipolar disorder: evidence from transcriptome studies points to intriguing parallels with multiple sclerosis. *Neurobiol Dis*. 2012;45(1):37-47. https://doi.org/10.1016/j.nbd.2011.01.025.
56. Seney ML, Tripp A, McCune S, Lewis A. D, Sibille. *E Laminar and Cellular Analyses of Reduced Somatostatin Gene Expression in the Subgenual Anterior Cingulate Cortex in Major Depression Neurobiol Dis*. 2015;73:213-219. https://doi.org/10.1016/j.nbd.2014.10.005.
57. Iwamoto K, Kakiuchi C, Bundo M, Ikeda K, Kato T. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol Psychiatry*. 2004;9(4):406-416. https://doi.org/10.1038/sj.mp.4001437.
58. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci*. 2013;14(9):609-625. https://doi.org/10.1038/nrn3381.
59. Villas Boas GR, Boerngen de Lacerda R, Paes MM, et al. Molecular aspects of depression: a review from neurobiology to treatment. *Eur J Pharmacol*. 2019;851:99-121. https://doi.org/10.1016/j.ejphar.2019.02.024.
60. Seifuddin F, Pirooznia M, Judy JT, Goes FS, Potash JB, Zandi PP. Systematic review of genome-wide gene expression studies of bipolar disorder. *BMC Psychiatry*. 2013;13(1):213. https://doi.org/10.1186/1471-244X-13-213.
61. Su YA, Wu J, Zhang L, et al. Dysregulated mitochondrial genes and networks with drug targets in postmortem brain of patients with posttraumatic stress disorder (PTSD) revealed by human mitochondria-focused cDNA microarrays. *Int J Biol Sci*. 2008;4:223-235. https://doi.org/10.7150/ijbs.4.223.
62. Zhang L, Li H, Hu X, et al. Mitochondria-focused gene expression profile reveals common pathways and CPT1B dysregulation in both rodent stress model and human subjects with PTSD. *Transl Psychiatry*. 2015;5(6):e580-e580. https://doi.org/10.1038/tp.2015.65.
63. Morrison FG, Miller MW, Wolf EJ, et al. Reduced interleukin 1A gene expression in the dorsolateral prefrontal cortex of individuals with PTSD and depression. *Neurosci Lett*. 2019;692:204-209. https://doi.org/10.1016/j.neulet.2018.10.027.
64. Shansky RM. Are hormones a "female problem" for animal research? *Science (80-)*. 2019;364(6443):825-826. https://doi.org/10.1126/science.aaw7570.
65. Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev*. 2011;35(3):565-572. https://doi.org/10.1016/j.neubiorev.2010.07.002.
66. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobehav Rev*. 2014;40:1-5. https://doi.org/10.1016/j.neubiorev.2014.01.001.
67. Cahill L. Why sex matters for neuroscience. *Nat Rev Neurosci*. 2006;7(6):477-484. https://doi.org/10.1038/nrn1909.
68. Itoh Y, Arnold AP. Are females more variable than males in gene expression? Meta-analysis of microarray datasets. *Biol Sex Differ*. 2015;6(1):1-9. https://doi.org/10.1186/s13293-015-0036-8.
69. Beery AK. Inclusion of females does not increase variability in rodent research studies. *Curr Opin Behav Sci*. 2018;23:143-149. https://doi.org/10.1016/j.cobeha.2018.06.016.
70. de Lange GM. Understanding the cellular and molecular alterations in PTSD brains: the necessity of post-mortem brain tissue. *Eur J Psychotraumatol*. 2017;8(1):10-12. https://doi.org/10.1080/20008198.2017.1341824.
71. Deep-Soboslay A, Iglesias B, Hyde TM, et al. Evaluation of tissue collection for postmortem studies of bipolar disorder. *Bipolar Disord*. 2008;10(7):822-828. https://doi.org/10.1111/j.1399-5618.2008.00607.x.
72. Deep-Soboslay A, Benes FM, Haroutunian V, Ellis JK, Kleinman JE, Hyde TM. Psychiatric brain banking: three perspectives on current trends and future directions. *Biol Psychiatry*. 2011;69(2):104-112. https://doi.org/10.1016/j.biopsych.2010.05.025.
73. Zhou Y, Lutz P-E, Ibrahim EC, et al. Suicide and suicide behaviors: a review of transcriptomics and multiomics studies in psychiatric

disorders. *J Neurosci Res*. 2018;00:1-15. https://doi.org/10.1002/jnr.24367.
74. World Health Organization. *World Health Statistics 2018*. Geneva: World Health Organization; 2018 http://apps.who.int/iris/bitstream/handle/10665/272596/9789241565585-eng.pdf?ua=1.
75. Goswami DB, May WL, Stockmeier CA, Austin MC. Transcriptional expression of serotonergic regulators in laser-captured microdissected dorsal raphe neurons of subjects with major depressive disorder: sex-specific differences. *J Neurochem*. 2010;112(2):397-409. https://doi.org/10.1111/j.1471-4159.2009.06462.x.
76. Tripp A, Kota RS, Lewis DA, Sibille E. Reduced somatostatin in subgenual anterior cingulate cortex in major depression. *Neurobiol Dis*. 2011;42(1):116-124. https://doi.org/10.1016/j.nbd.2011.01.014.
77. Barde S, Rüegg J, Prud'homme J, et al. Alterations in the neuropeptide galanin system in major depressive disorder involve levels of transcripts, methylation, and peptide. *Proc Natl Acad Sci USA*. 2016;113(52):E8472-E8481. https://doi.org/10.1073/pnas.1617824113.
78. Herringa RJ, Roseboom PH, Kalin NH. Decreased amygdala CRF-binding protein mRNA in post-mortem tissue from male but not female bipolar and schizophrenic subjects. *Neuropsychopharmacology*. 2006;31(8):1822-1831. https://doi.org/10.1038/sj.npp.1301038.
79. Perlman WR, Tomaskovic-Crook E, Montague DM, et al. Alteration in estrogen receptor α mRNA levels in frontal cortex and Hippocampus of patients with major mental illness. *Biol Psychiatry*. 2005;58(10):812-824. https://doi.org/10.1016/j.biopsych.2005.04.047.
80. Lorsch ZS, Loh Y-HE, Purushothaman I, et al. Estrogen receptor α drives pro-resilient transcription in mouse models of depression. *Nat Commun*. 2018;9(1):1116. https://doi.org/10.1038/s41467-018-03567-4.
81. Barysheva M, Jahanshad N, Foland-Ross L, Altshuler LL, Thompson PM. White matter microstructural abnormalities in bipolar disorder: a whole brain diffusion tensor imaging study. *NeuroImage Clin*. 2013;2(1):558-568. https://doi.org/10.1016/j.nicl.2013.03.016.
82. Coloigner J, Batail J-M, Commowick O, et al. White matter abnormalities in depression: a categorical and phenotypic diffusion MRI study. *NeuroImage Clin*. 2019;22:101710. https://doi.org/10.1016/J.NICL.2019.101710.
83. Barley K, Dracheva S, Byne W. Subcortical oligodendrocyte- and astrocyte-associated gene expression in subjects with schizophrenia, major depression and bipolar disorder. *Schizophr Res*. 2009;112(1–3):54-64. https://doi.org/10.1016/J.SCHRES.2009.04.019.
84. Labonté B, Engmann O, Purushothaman I, et al. Sex-specific transcriptional signatures in human depression. *Nat Med*. 2017;23:1102-1111. https://doi.org/10.1038/nm.4386.
85. Seney ML, Huo Z, Cahill K, et al. Opposite molecular signatures of depression in men and women. *Biol Psychiatry*. 2018;84(1):18-27. https://doi.org/10.1016/j.biopsych.2018.01.017.
86. Breen MS, Tylee DS, Maihofer AX, et al. PTSD blood Transcriptome mega-analysis: shared inflammatory pathways across biological sex and modes of trauma. *Neuropsychopharmacology*. 2018;43(3):469-481. https://doi.org/10.1038/npp.2017.220.
87. Neylan TC, Sun B, Rempel H, et al. Suppressed monocyte gene expression profile in men versus women with PTSD. *Brain Behav Immun*. 2011;25(3):524-531. https://doi.org/10.1016/j.bbi.2010.12.001.
88. Zhang S, Zhang H, Ku SM, et al. Sex differences in the Neuroadaptations of reward-related circuits in response to subchronic variable stress. *Neuroscience*. 2018;376:108-116. https://doi.org/10.1016/j.neuroscience.2018.02.021.
89. Becker JB, Chartoff E. Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology*. 2019;44(1):166-183. https://doi.org/10.1038/s41386-018-0125-6.
90. Rubinow DR, Schmidt PJ. Sex differences and the neurobiology of affective disorders. *Neuropsychopharmacology*. 2019;44(1):111-128. https://doi.org/10.1038/s41386-018-0148-z.
91. Bagot RC, Cates HM, Purushothaman I, et al. Circuit-wide transcriptional profiling reveals brain region-specific gene networks regulating depression susceptibility. *Neuron*. 2016;90(5):969-983. https://doi.org/10.1016/j.neuron.2016.04.015.
92. Krishnan V, Nestler EJ. Animal models of depression: molecular perspectives. *Curr Top Behav Neurosci*. 2011;7:121-147. https://doi.org/10.1007/7854_2010_108.
93. Nestler EJ, Gould E, Manji H, et al. Preclinical models: status of basic research in depression. *Biol Psychiatry*. 2002;52(6):503-528.
94. Palanza P. Animal models of anxiety and depression: how are females different? *Neurosci Biobehav Rev*. 2001;25(3):219-233.
95. Wang Q, Timberlake MA, Prall K, Dwivedi Y. The recent progress in animal models of depression. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2017;77:99-109. https://doi.org/10.1016/j.pnpbp.2017.04.008.
96. Gururajan A, Kos A, Lopez JP. Preclinical stress research: where are we headed? An early career investigator's perspective. *Stress*. 2018;21:1-5. https://doi.org/10.1080/10253890.2018.1446519.
97. Gass P, Reichardt HM, Strekalova T, Henn F, Tronche F. Mice with targeted mutations of glucocorticoid and mineralocorticoid receptors: models for depression and anxiety? *Physiol Behav*. 2001;73(5):811-825. https://doi.org/10.1016/S0031-9384(01)00518-2.
98. Barden N. Implication of the hypothalamic-pituitary-adrenal axis in the physiopathology of depression. *J Psychiatry Neurosci*. 2004;29(3):185-193.
99. Karandrea D, Kittas C, Kitraki E. Contribution of sex and cellular context in the regulation of brain corticosteroid receptors following restraint stress. *Neuroendocrinology*. 2000;71(6):343-353. https://doi.org/10.1159/000054555.
100. Karandrea D, Kittas C, Kitraki E. Forced swimming differentially affects male and female brain corticosteroid receptors. *Neuroendocrinology*. 2002;75(4):217-226. https://doi.org/10.1159/000054713.
101. Lu J, Wu X-Y, Zhu Q-B, et al. Sex differences in the stress response in SD rats. *Behav Brain Res*. 2015;284:231-237. https://doi.org/10.1016/j.bbr.2015.02.009.
102. Solomon MB, Loftspring M, De Kloet AD, et al. Neuroendocrine function after hypothalamic depletion of glucocorticoid receptors in male and female mice. *Endocrinology*. 2015;156(8):2843-2853. https://doi.org/10.1210/en.2015-1276.
103. Guo L, Chen Y-X, Hu Y-T, et al. Sex hormones affect acute and chronic stress responses in sexually dimorphic patterns: consequences for depression models. *Psychoneuroendocrinology*. 2018;95:34-42. https://doi.org/10.1016/j.psyneuen.2018.05.016.
104. Sterrenburg L, Gaszner B, Boerrigter J, et al. Sex-dependent and differential responses to acute restraint stress of corticotropin-releasing factor-producing neurons in the rat paraventricular nucleus, central amygdala, and bed nucleus of the stria terminalis. *J Neurosci Res*. 2012;90(1):179-192. https://doi.org/10.1002/jnr.22737.
105. Viau V, Bingham B, Davis J, Lee P, Wong M. Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology*. 2005;146(1):137-146. https://doi.org/10.1210/en.2004-0846.
106. Iwasaki-Sekino A, Mano-Otagiri A, Ohata H, Yamauchi N, Shibasaki T. Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological. *Psychoneuroendocrinology*. 2009;34(2):226-237. https://doi.org/10.1016/j.psyneuen.2008.09.003.
107. Bland ST, Schmid MJ, Der-Avakian A, Watkins LR, Spencer RL, Maier SF. Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable

stress. *Brain Res*. 2005;1051(1–2):90-99. https://doi.org/10.1016/j.brainres.2005.05.065.

108. Babb JA, Masini CV, Day HEW, Campeau S. Sex differences in activated corticotropin-releasing factor neurons within stress-related neurocircuitry and hypothalamic–pituitary–adrenocortical axis hormones following restraint in rats. *Neuroscience*. 2013;234:40-52. https://doi.org/10.1016/j.neuroscience.2012.12.051.
109. Bohacek J, Manuella F, Roszkowski M, Mansuy IM. Hippocampal gene expression induced by cold swim stress depends on sex and handling. *Psychoneuroendocrinology*. 2015;52:1-12. https://doi.org/10.1016/j.psyneuen.2014.10.026.
110. Figueiredo HF, Dolgas CM, Herman JP. Stress activation of cortex and Hippocampus is modulated by sex and stage of estrus. *Endocrinology*. 2002;143(7):2534-2540. https://doi.org/10.1210/endo.143.7.8888.
111. Musazzi L, Tomese P, Sala N, Popoli M. Acute stress is not acute: sustained enhancement of glutamate release after acute stress involves readily releasable pool size and synapsin I activation. *Mol Psychiatry*. 2017;22(9):1226-1227. https://doi.org/10.1038/mp.2016.175.
112. Steinman MQ, Duque-Wilckens N, Greenberg GD, et al. Sex-specific effects of stress on oxytocin neurons correspond with responses to intranasal oxytocin. *Biol Psychiatry*. 2016;80(5):406-414. https://doi.org/10.1016/j.biopsych.2015.10.007.
113. Steinman MQ, Laredo SA, Lopez EM, et al. Hypothalamic vasopressin systems are more sensitive to the long term effects of social defeat in males versus females. *Psychoneuroendocrinology*. 2015;51:122-134. https://doi.org/10.1016/J.PSYNEUEN.2014.09.009.
114. Greenberg GD, Laman-Maharg A, Campi KL, et al. Sex differences in stress-induced social withdrawal: role of brain derived neurotrophic factor in the bed nucleus of the stria terminalis. *Front Behav Neurosci*. 2014;7:223. https://doi.org/10.3389/fnbeh.2013.00223.
115. Chun LE, Christensen J, Woodruff ER, Morton SJ, Hinds LR, Spencer RL. Adrenal-dependent and -independent stress-induced Per1 mRNA in hypothalamic paraventricular nucleus and prefrontal cortex of male and female rats. *Stress*. 2018;21(1):69-83. https://doi.org/10.1080/10253890.2017.1404571.
116. Wright EC, Johnson SA, Hao R, et al. Exposure to extrinsic stressors, social defeat or bisphenol a, eliminates sex differences in DNA methyltransferase expression in the amygdala. *J Neuroendocrinol*. 2017;29:1-9. https://doi.org/10.1111/jne.12475.
117. Papale LA, Li S, Madrid A, et al. Sex-specific hippocampal 5-hydroxymethylcytosine is disrupted in response to acute stress. *Neurobiol Dis*. 2016;96:54-66. https://doi.org/10.1016/j.nbd.2016.08.014.
118. Towers AE, Oelschlager ML, Lorenz M, et al. Handling stress impairs learning through a mechanism involving caspase-1 activation and adenosine signaling. *Brain Behav Immun*. 2019;80:763-776. https://doi.org/10.1016/j.bbi.2019.05.025.
119. Balcombe JP, Barnard ND, Sandusky C. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci*. 2004;43(6):42-51.
120. Avgustinovich DF, Kovalenko IL. Gender-related characteristics of responding to prolonged Psychoemotional stress in mice. *Neurosci Behav Physiol*. 2010;40(3):257-262. https://doi.org/10.1007/s11055-010-9252-1.
121. Joel D, McCarthy MM. Incorporating sex as a biological variable in neuropsychiatric research: where are we now and where should we be? *Neuropsychopharmacology*. 2017;42(2):379-385. https://doi.org/10.1038/npp.2016.79.
122. Marrocco J, Petty GH, Ríos MB, et al. A sexually dimorphic pre-stressed translational signature in CA3 pyramidal neurons of BDNF Val66Met mice. *Nat Commun*. 2017;8(1):808. https://doi.org/10.1038/s41467-017-01014-4.
123. Seney ML, Chang L-C, Oh H, et al. The role of genetic sex in affect regulation and expression of GABA-related genes across species. *Front Psych*. 2013;4:104. https://doi.org/10.3389/fpsyt.2013.00104.
124. Li S, Papale LA, Zhang Q, et al. Genome-wide alterations in hippocampal 5-hydroxymethylcytosine links plasticity genes to acute stress. *Neurobiol Dis*. 2016;86:99-108. https://doi.org/10.1016/J.NBD.2015.11.010.
125. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)*. 1997;134(4):319-329. https://doi.org/10.1007/s002130050456.
126. Franceschelli A, Herchick S, Thelen C, Papadopoulou-Daifoti Z, Pitychoutis PM. Sex differences in the chronic mild stress model of depression. *Behav Pharmacol*. 2014;25(5–6):1-383. https://doi.org/10.1097/FBP.0000000000000062.
127. Iñiguez SD, Flores-Ramirez FJ, Riggs LM, et al. Vicarious social defeat stress induces depression-related outcomes in female mice. *Biol Psychiatry*. 2018;83(1):9-17. https://doi.org/10.1016/j.biopsych.2017.07.014.
128. Sial OK, Warren BL, Alcantara LF, Parise EM, Bolaños-Guzmán CA. Vicarious social defeat stress: bridging the gap between physical and emotional stress. *J Neurosci Methods*. 2016;258:94-103. https://doi.org/10.1016/J.JNEUMETH.2015.10.012.
129. Newman EL, Covington HE, Suh J, et al. Fighting females: neural and behavioral consequences of social defeat stress in female mice. *Biol Psychiatry*. 2019;86:657-668. https://doi.org/10.1016/j.biopsych.2019.05.005.
130. Grissom N, Bhatnagar S. Habituation to repeated stress: get used to it. *Neurobiol Learn Mem*. 2009;92(2):215-224. https://doi.org/10.1016/j.nlm.2008.07.001.
131. Radley J, Morilak D, Viau V, Campeau S. Chronic stress and brain plasticity: mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders. *Neurosci Biobehav Rev*. 2015;58:79-91. https://doi.org/10.1016/j.neubiorev.2015.06.018.
132. Goel N, Bale TL. Examining the intersection of sex and stress in modelling neuropsychiatric disorders. *J Neuroendocrinol*. 2009;21(4):415-420. https://doi.org/10.1111/j.1365-2826.2009.01843.x.
133. Zavala JK, Fernandez AA, Gosselink KL. Female responses to acute and repeated restraint stress differ from those in males. *Physiol Behav*. 2011;104(2):215-221. https://doi.org/10.1016/j.physbeh.2011.03.022.
134. Lunga P, Herbert J. 17Beta-oestradiol modulates glucocorticoid, neural and behavioural adaptations to repeated restraint stress in female rats. *J Neuroendocrinol*. 2004;16(9):776-785. https://doi.org/10.1111/j.1365-2826.2004.01234.x.
135. Willner P. The chronic mild stress (CMS) model of depression: history, evaluation and usage. *Neurobiol Stress*. 2017;6:78-93. https://doi.org/10.1016/j.ynstr.2016.08.002.
136. Dunćko R, Kiss A, Škultétyová I, Rusnák M, Ježová D. Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. *Psychoneuroendocrinology*. 2001;26(1):77-89. https://doi.org/10.1016/S0306-4530(00)00040-8.
137. Sterrenburg L, Gaszner B, Boerrigter J, et al. Chronic stress induces sex-specific alterations in methylation and expression of Corticotropin-releasing factor gene in the rat. *PLoS One*. 2011;6(11):e28128. https://doi.org/10.1371/journal.pone.0028128.
138. Lan N, Hellemans KGC, Ellis L, Weinberg J. Exposure to chronic mild stress differentially alters Corticotropin-releasing hormone and arginine vasopressin mRNA expression in the stress-responsive Neurocircuitry of male and female rats prenatally exposed to alcohol. *Alcohol Clin Exp Res*. 2015;39(12):2414-2421. https://doi.org/10.1111/acer.12916.
139. Puralewski R, Vasilakis G, Seney ML. Sex-related factors influence expression of mood-related genes in the basolateral amygdala

differentially depending on age and stress exposure. *Biol Sex Differ*. 2016;7(1):50. https://doi.org/10.1186/s13293-016-0106-6.
140. Girgenti MJ, Wohleb ES, Mehta S, Ghosal S, Fogaca MV, Duman RS. Prefrontal cortex interneurons display dynamic sex-specific stress-induced transcriptomes. *Transl Psychiatry*. 2019;9(1):292. https://doi.org/10.1038/s41398-019-0642-z.
141. Shepard R, Page CE, Coutellier L. Sensitivity of the prefrontal GABAergic system to chronic stress in male and female mice: relevance for sex differences in stress-related disorders. *Neuroscience*. 2016;332:1-12. https://doi.org/10.1016/j.neuroscience.2016.06.038.
142. Shepard R, Coutellier L. Changes in the prefrontal Glutamatergic and Parvalbumin Systems of Mice Exposed to unpredictable chronic stress. *Mol Neurobiol*. 2018;55(3):2591-2602. https://doi.org/10.1007/s12035-017-0528-0.
143. Seney ML, Ekong KI, Ding Y, Tseng GC, Sibille E. Sex chromosome complement regulates expression of mood-related genes. *Biol Sex Differ*. 2013;4(1):20. https://doi.org/10.1186/2042-6410-4-20.
144. Kelly SD, Harrell CS, Neigh GN. Chronic stress modulates regional cerebral glucose transporter expression in an age-specific and sexually-dimorphic manner. *Physiol Behav*. 2014;126:39-49. https://doi.org/10.1016/j.physbeh.2013.12.002.
145. Bassi S, Seney ML, Argibay P, Sibille E. Elevated hippocampal cholinergic Neurostimulating peptide precursor protein (HCNP-pp) mRNA in the amygdala in major depression. *J Psychiatr Res*. 2015;63:105-116. https://doi.org/10.1016/j.jpsychires.2015.02.006.
146. Barko K, Paden W, Cahill KM, Seney ML, Logan RW. Sex-specific effects of stress on mood-related gene expression. *Mol Neuropsychiatry*. 2019;5(3):162-176. https://doi.org/10.1159/000499105.
147. Karisetty BC, Khandelwal N, Kumar A, Chakravarty S. Sex difference in mouse hypothalamic transcriptome profile in stress-induced depression model. *Biochem Biophys Res Commun*. 2017;486(4):1122-1128. https://doi.org/10.1016/j.bbrc.2017.04.005.
148. Hodes GE, Pfau ML, Purushothaman I, et al. Sex differences in nucleus Accumbens Transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress. *J Neurosci*. 2015;35(50):16362-16376. https://doi.org/10.1523/JNEUROSCI.1392-15.2015.
149. Pfau ML, Purushothaman I, Feng J, et al. Integrative analysis of sex-specific microRNA networks following stress in mouse nucleus Accumbens. *Front Mol Neurosci*. 2016;9:144. https://doi.org/10.3389/fnmol.2016.00144.
150. Daskalakis NP, Cohen H, Cai G, Buxbaum JD, Yehuda R. Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. *Proc Natl Acad Sci USA*. 2014;111(37):13529-13534. https://doi.org/10.1073/pnas.1401660111.
151. Mychasiuk R, Muhammad A, Kolb B. Chronic stress induces persistent changes in global DNA methylation and gene expression in the medial prefrontal cortex, orbitofrontal cortex, and hippocampus. *Neuroscience*. 2016;322:489-499. https://doi.org/10.1016/j.neuroscience.2016.02.053.
152. Lobentanzer S, Hanin G, Klein J, Soreq H. Integrative Transcriptomics reveals sexually dimorphic control of the cholinergic/Neurokine Interface in schizophrenia and bipolar disorder. *Cell Rep*. 2019;29(3):764-777.e5. https://doi.org/10.1016/j.celrep.2019.09.017.
153. Peña CJ, Kronman HG, Walker DM, et al. Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science (80-)*. 2017;356(6343):1185-1188. https://doi.org/10.1126/science.aan4491.
154. Peña CJ, Smith M, Ramakrishnan A, et al. Early life stress alters transcriptomic patterning across reward circuitry in male and female mice. *Nat Commun*. 2019;10(1):5098. https://doi.org/10.1038/s41467-019-13085-6.
155. Candemir E, Post A, Dischinger US, et al. Limited effects of early life manipulations on sex-specific gene expression and behavior in adulthood. *Behav Brain Res*. 2019;369:111927. https://doi.org/10.1016/j.bbr.2019.111927.
156. Bale TL, Epperson CN. Sex differences and stress across the lifespan. *Nat Neurosci*. 2015;18(10):1413-1420. https://doi.org/10.1038/nn.4112.
157. Babb JA, Masini CV, Day HEW, Campeau S. Stressor-specific effects of sex on HPA axis hormones and activation of stress-related neurocircuitry. *Stress*. 2013;16(6):664-677. https://doi.org/10.3109/10253890.2013.840282.
158. Xing G, Carlton J, Zhang L, et al. Cannabinoid receptor expression and phosphorylation are differentially regulated between male and female cerebellum and brain stem after repeated stress: implication for PTSD and drug abuse. *Neurosci Lett*. 2011;502(1):5-9. https://doi.org/10.1016/j.neulet.2011.05.013.
159. Gray AL, Hyde TM, Deep-Soboslay A, Kleinman JE, Sodhi MS. Sex differences in glutamate receptor gene expression in major depression and suicide. *Mol Psychiatry*. 2015;20(9):1057-1068. https://doi.org/10.1038/mp.2015.91.
160. Tonelli LH, Stiller J, Rujescu D, et al. Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. *Acta Psychiatr Scand*. 2008;117(3):198-206. https://doi.org/10.1111/j.1600-0447.2007.01128.x.

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7.3.1.2 First-Author Research Article

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eLife RESEARCH ARTICLE

Social dominance mediates behavioral adaptation to chronic stress in a sex-specific manner

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Abstract Sex differences and social context independently contribute to the development of stress-related disorders. However, less is known about how their interplay might influence behavior and physiology. Here we focused on social hierarchy status, a major component of the social environment in mice, and whether it influences behavioral adaptation to chronic stress in a sex-specific manner. We used a high-throughput automated behavioral monitoring system to assess social dominance in same-sex, group-living mice. We found that position in the social hierarchy at baseline was a significant predictor of multiple behavioral outcomes following exposure to chronic stress. Crucially, this association carried opposite consequences for the two sexes. This work demonstrates the importance of recognizing the interplay between sex and social factors and enhances our understating of how individual differences shape the stress response.

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Introduction

Stress-related psychopathologies, such as mood and anxiety disorders, show a pronounced gender bias in their prevalence, severity, age-of-onset, and most common comorbidities (***Altemus et al., 2014***; ***Bangasser and Valentino, 2014***; ***Kessler et al., 2005***; ***Young and Pfaff, 2014***). For example, the latest studies estimate the prevalence of major depressive disorder among women as 1.5 times higher than in men (***World Health Organization, 2017***). In addition, in women major depression is characterized by increased symptom severity (***Martin et al., 2013***) and is more commonly comorbid with anxiety disorders, eating disorders, and sleep disturbances, while men with major depression are more prone to develop aggression, alcohol or substance abuse, and suicidal ideation (***Marcus et al., 2005***; ***Martin et al., 2013***).

Despite these observations and the documented examples of sexual dimorphism in human stress response (***Bangasser and Valentino, 2012***), the biological mechanisms that give rise to sex differences in stress response are not well understood (***Beery and Zucker, 2011***; ***Joel and McCarthy, 2017***). The symptomatology of stress-related pathologies and the biological response to stress span several domains of functioning including energy metabolism, mood, and sociability. Recent studies in rodent models of stress-related psychopathologies have already identified several differences across molecular, behavioral, and metabolic levels (***Bangasser and Wicks, 2017***; ***Brivio et al., 2020***; ***Hodes, 2018***; ***Hodes and Epperson, 2019***; ***Young and Pfaff, 2014***). Very few studies, however, have looked into the interaction between pre-existing differences in social behavior between the sexes and stress. Considering that abnormalities in social functioning are an essential part of the symptomatology of stress-related disorders, differences in social behavior and social cognition prior to disorder onset are likely to contribute to disorder susceptibility. Here, we explored how social

eLife digest Most people experience chronic stress at some point in their life, which may increase their chances of developing depression or anxiety. There is evidence that chronic stress may more negatively impact the well-being of women, placing them as higher risk of developing these mental health conditions. The biological factors that underlie these differences are not well understood, which leaves clinicians and scientists struggling to develop and provide effective treatments.

The social environment has a powerful influence on how people experience and cope with stress. For example, a person's social and socioeconomic status can change their perception of and reaction to everyday stress. Researchers have found differences in how men and women relate to their social standing. One way for scientists to learn more about the biological processes involved is to study the effect of social standing and chronic stress in male and female mice.

Now, Karamihalev, Brivio et al. show that social status influences the behavior of stressed mice in a sex-specific way. In the experiments, an automated observation system documented the behavior of mice living in all female or male groups. Karamihalev, Brivio et al. determined where each animal fit into the social structure of their group. Then, they exposed some groups of mice to mild chronic stress and compared their behaviors to groups of mice housed in normal conditions. They found that both the sex and social status of each played a role in how they responded to stress. For example, subordinate males displayed more anxious behavior under stressful circumstances, while dominant females acted bolder and less anxious.

More studies in mice are needed to understand the biological basis of these social- and sex-based differences in stress response. Learning more may help scientists understand why some individuals are more susceptible to the effects of stress and lead to the development of personalized prevention or treatment strategies for anxiety and depression.

context shapes the response to chronic stress. We focused specifically on social dominance, an essential characteristic of rodent social groups.

Wild and laboratory rodents form complex and dynamic social structures which typically involve the formation of dominance hierarchies (***Kondrakiewicz et al., 2019***). These have been observed in the lab in group sizes ranging from three to over a dozen individuals (***Horii et al., 2017***; ***Varholick et al., 2019***; ***Wang et al., 2014***). Hierarchies are thought to improve social stability and reduce severe conflicts and aggression (***Curley, 2016***). As a consequence, an individual's position in the dominance hierarchy has important consequences, including preferential access to food, shelter, and mates (***Drews, 1993***). Social rank within male hierarchies is also known to influence health, hormonal profile, brain function, metabolism, and mortality (***Pallé et al., 2019***; ***Razzoli et al., 2018***). For instance, subordinate individuals display increased anxiety-like behavior, a suppressed immune response, higher basal corticosterone levels, and reduced life span (***Bartolomucci, 2007***). These types of relationships have classically been studied in male animals, as female mice have usually appeared more communal and displayed limited aggression (***König and Lindholm, 2012***). Recent work, however, has demonstrated that female laboratory mice also form hierarchies that appear quite similar to those seen in males, accompanied by some of the same dominance-related physiological markers, such as differences in corticosterone levels (***Schuhr, 1987***; ***van den Berg et al., 2015***; ***Varholick et al., 2019***; ***Varholick et al., 2018***; ***Williamson et al., 2019***). Thus, we examined social dominance status as a putative mediator of sex differences in the response to adverse events.

To do so, we took advantage of a high-throughput automated behavioral monitoring system (the Social Box, SB) to assess and better understand the hierarchies of groups of male or female mice (***Forkosh et al., 2019***; ***Shemesh et al., 2013***). We then exposed mice to a well-established chronic stress procedure, the chronic mild stress (CMS) paradigm, and evaluated its effects using a series of standard behavioral and physiological readouts. Finally, we used social dominance status at baseline to predict behavioral outcomes following CMS. We hypothesized that an individual's standing in the social hierarchy would be a predictor of behavior upon stress exposure, and that this relationship would differ between the sexes.

Results

Male and female dominance hierarchies

We first explored the hierarchical structure of grouped CD-1 mice over four days of baseline monitoring as well as the stability of hierarchies following an acute stressor (15 min of restraint stress). Social dominance was assessed by calculating the David's Score (DS), an established method for inferring social hierarchies (**David, 1987**; **Gammell et al., 2003**). We based the DS on the numbers and directionality of chases between each pair of individuals in a group. A cumulative DS for the four baseline days of the SB assessment was used as a final measure of social dominance. In line with previous studies (**Schuhr, 1987**; **van den Berg et al., 2015**; **Varholick et al., 2019**; **Varholick et al., 2018**; **Williamson et al., 2019**), we were able to detect some stability in the hierarchies of both sexes (**Figure 1a**).

We further calculated several properties of male and female hierarchies to explore potential differences in their characteristics. Namely, we calculated: (1) steepness – a measure of social distance between each individual in the hierarchy, (2) despotism – a measure of the extent to which the top-ranking individuals dominate over the rest of the group, (3) directional consistency – the extent to which the directionality of the interactions follow the expected direction from higher to lower rank, and (4) Landau's modified h' – a measure of hierarchy linearity (**de Vries, 1995**; **Landau, 1951**; **Figure 1—figure supplement 1**). We found that male hierarchies were steeper, more linear, more despotic, and had higher directional consistency than those of females. Interestingly, mice housed in larger groups show analogous relationships between sexes (**Williamson et al., 2019**).

To investigate if social rank carries any sex-specific implications for overall behavior, we analyzed the correlation structure between an individual's DS and 59 behavioral readouts recorded post-habituation (days 2–4) in the SB within each sex (briefly described in Methods. For a detailed list of behaviors and how they are computed, see **Forkosh et al., 2019**). Thirty of the fifty-nine behavioral readouts tested (50.84%) showed significant correlations with cumulative baseline DS in at least one of the sexes (**Figure 1—figure supplement 2**, $q < 0.05$, Spearman's rank correlation, Benjamini-Hochberg adjustment within each sex). While the overall association pattern was quite similar between male and female mice, there were several correlations seen in males that were absent in females (**Figure 1b** and **Figure 1—figure supplement 2**). These included measures of overall locomotion, such as *Distance Outside* (Male $r_s = 0.478$, $n = 40$ mice, p = 0.00355. Female $r_s = -0.253$, $n = 48$ mice, p = 0.137), and *Fraction of Time Outside* – the mean proportion of time a mouse spent outside the nest (Male $r_s = 0.523$, $n = 40$ mice, p = 0.00125. Female $r_s = -0.144$, $n = 48$ mice, p = 0.402), as well as two related measures of roaming entropy, which assess the predictability of how an individual explores their environment – *Entropy* and *Grid Entropy [6 × 6]* (for brevity we only report the latter, Male $r_s = 0.531$, $n = 40$ mice, p = 0.00123. Female $r_s = 0.00677$, $n = 48$ mice, p = 0.694). These correlations indicate that overall locomotion and exploration of the home environment may be more strongly connected to social rank in male groups, while being seemingly independent of social status in females. Interestingly, no correlations were present in females but absent in males. Altogether these findings suggest that male and female social dominance hierarchies, despite having a similar structure, have different relationships to overall behavior.

Next, we estimated DS stability over time by examining the frequency of rank change events and comparing those to the chance-level expectation. Briefly, normalized daily DS values were ranked for each group to create a four-rank hierarchy: α (most dominant), β, γ, and δ (most subordinate) and each mouse was assigned a single rank based on its four-day cumulative DS. For each pair of consecutive days, we observed how many individuals maintained the same rank they had been assigned on the previous day. We then calculated the rank maintenance odds for animals in each final rank category relative to the expected chance-level (**Figure 1c**). The true probability of rank maintenance in our data was higher than chance in α-females and in all male ranks (one-tailed binomial tests against the rank maintenance probability of 25%, α-females: 21/36 successes, $p = 2.1\times10^{-5}$, α-males: 22/30 successes, $p = 3.7\times10^{-8}$, β- *and* γ-males: 13/30 successes each, p = 0.0216, δ-males: 19/30 successes, $p = 1.02\times10^{-5}$). These results indicate that the highest rank in a hierarchy is often occupied by the same individual over time in both sexes, while the lower ranks appeared to be stable in males only (**Figure 1c**).

In addition to stability over time during baseline recordings, individual DS also remained stable following acute restraint stress (Pearson's correlation between cumulative baseline DS and DS

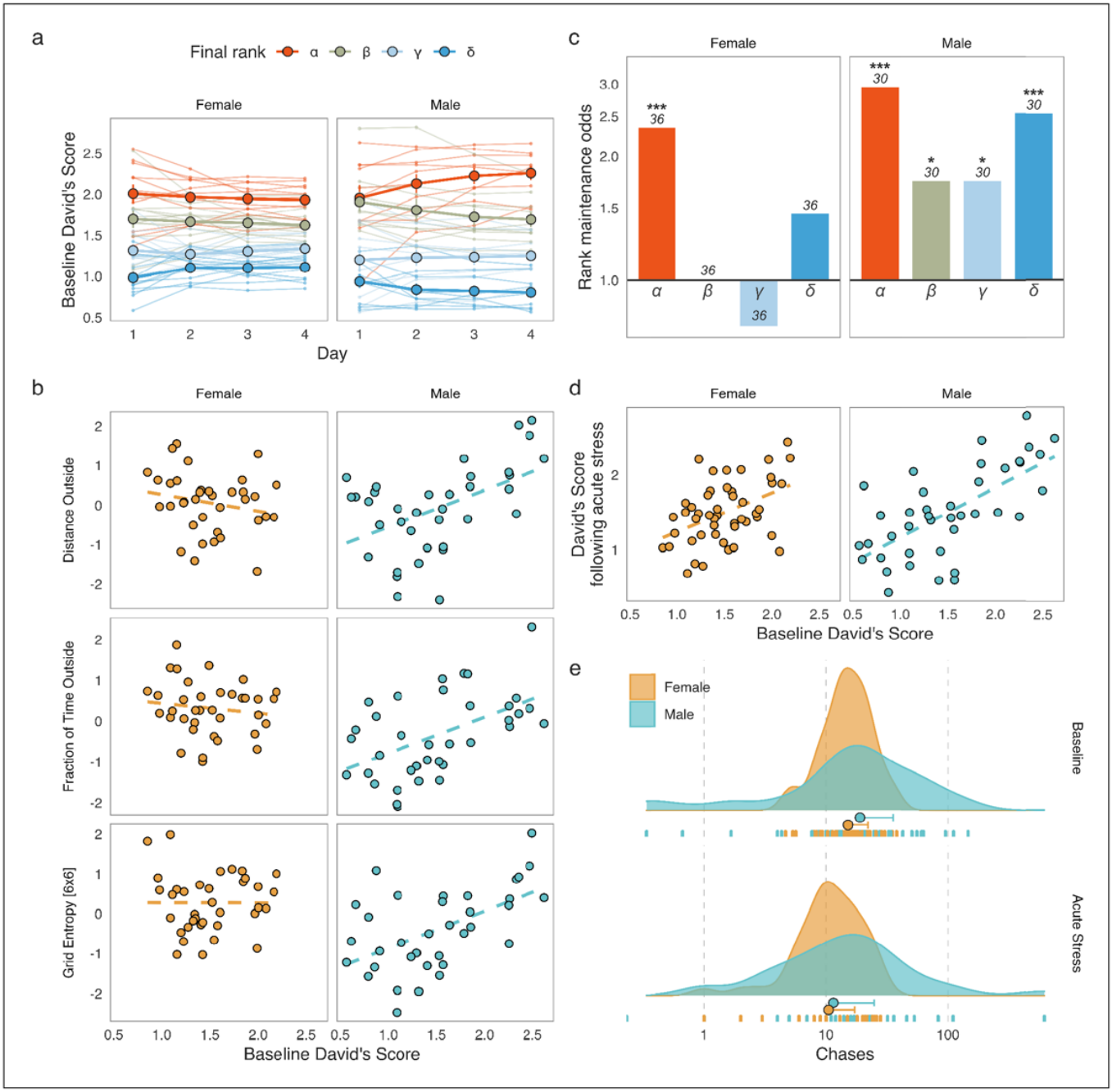

Figure 1. Social dominance hierarchies in males and females. (**a**) David's Scores (DS) based on chases during the four baseline days of Social Box (SB) assessment show relatively stable social hierarchies in both male and female groups (each line represents an individual, colors represent the cumulative social rank on day 4, points are mean values for each rank ± standard error of the mean). (**b**) Male-specific associations between social dominance scores and behaviors related to locomotion and exploration. Dominant males had increased overall locomotion, spend more time outside the nest, and moved through the SB environment in a more unpredictable manner. These associations were not found in females. (**c**) Rank maintenance odds over the four-day baseline period. Depicted are odds of maintaining the same rank between consecutive days relative to chance-level (25%). Data is summarized according to the cumulative social rank on day 4; numbers indicate the number of individuals per rank. (**d**) Baseline DS predicts DS following acute restraint stress in both sexes, indicating that social dominance hierarchies may be relatively robust against acute stress. (**e**) Numbers of chases in male and female groups at baseline as well as following acute restraint stress. Both sexes display significantly fewer chases following an acute physiological stressor. The x-axis shows the absolute number of chases between pairs of mice. Dot: median, whisker: 1.5 x IQR.
The online version of this article includes the following figure supplement(s) for figure 1:

Figure 1 continued on next page

Figure 1 continued

Figure supplement 1. Social hierarchy properties in male and female groups.
Figure supplement 2. Associations between David's Scores (DS) and other behaviors measured in the Social Box (SB).

following acute restraint on day 5, ***Figure 1d*** and ***Figure 1—figure supplement 1***). Both males (r = 0.6639, n = 40 mice, p = 3×10^{-6}) and females (r = 0.446, n = 48 mice, p = 0.000149) showed significant DS correlations from baseline to acute restraint. Finally, we investigated the possibility of differential effects of acute restraint on the behavior used to produce the DS – numbers of chase events (***Figure 1e***). Repeated-measures ANOVA on log-transformed chase numbers showed that the number of chases decreased significantly following acute restraint stress (F(1, 86) = 29.04, n = 88 mice, p = 6.11×10^{-7}), however the extent of this decrease did not differ between the sexes (Sex x Stage interaction, F(1, 86) = 1.053, n = 88 mice, p = 0.301).

The apparent robustness of social hierarchies over time and in response to acute stress suggested that predictions from the baseline assessment may carry information that would still be relevant to behavioral outcomes following a long-term intervention. More specifically, we hypothesized that occupancy of the highest-ranking positions in the social hierarchy in both sexes and additionally the lowest in males might be sufficiently stable to allow for long-term predictions.

Effects of CMS on behavior and physiology

To investigate the effects of pre-existing social dominance status on the behavioral response to chronic stress, we employed a CMS protocol adapted for group-housed animals.

In short, groups were exposed to a weekly schedule of two daily randomly combined mild stressors (e.g. wet bedding, tilted cage, overcrowding) for a total of three weeks. Six groups of each sex (n = 24 per sex) were randomly assigned to receive CMS, while the rest of the groups (six groups of females and four groups of males) were assigned to the control condition. The 21-day CMS procedure was followed by a behavioral test battery for both control and CMS mice, which included tests previously shown to capture the effects of chronic stress (***Figure 2a***). This included, among others, classical tests of locomotion (open field test, OFT), anhedonia (sucrose preference test, SPT), anxiety-like behavior (elevated plus maze, EPM), and stress coping (tail suspension test, TST). Additionally, we assessed several physiological indicators of stress level (***Figure 2b–e***). All the physiological and behavioral outcome variables following CMS were collected into a single dataset. Since the full experiment was run in two batches, all outcome variables were adjusted for batch effect (*see* Methods). To improve readability, we report the batch-adjusted values relative to the mean of female control mice.

As expected, we found that both bodyweight change and cumulative coat quality were significantly reduced following CMS in both males and females (***Figure 2b–c***, Bodyweight: F(1, 82) = 7.394, p = 0.00798, Coat quality: KW test, χ^2(1) = 18.586, p = 1.6×10^{-5}), although post-hoc pairwise comparisons indicated a bodyweight difference in females only (females: t(43.784) = 3.9447, p = 0.000285, males: t(36.937) = 1.1064, p = 0.27). Bodyweight-adjusted adrenal weights were increased after CMS in males only (***Figure 2d and*** -way ANOVA, sex by condition interaction, F(1, 80) = 4.42, p = 0.039, followed by pairwise within-sex 2-sided t-tests: males: t(27.03) = −3.143, p = 0.004; Females: t(41.18) = 0.0726, p = 0.94). For all further analyses, these physiological outcomes were combined with the behavioral ones in a single dataset.

Sex-specific effects of dominance on CMS outcomes

To explore how exposure to chronic stress shapes behavior in groups of mice, we investigated the major drivers of variance in the dataset containing all behavioral and physiological readouts following CMS using principal components analysis (PCA, ***Figure 3a–d***). The first principal component (PC1), explained approximately 21.6% of the variance in the outcome data (***Figure 3a***). To our surprise, neither sex nor condition (CMS vs controls) appeared to capture variance contained in PC1 (***Figure 3b***, condition effect: F(1, 82) = 0.608, p = 0.44). Instead, sex and condition were associated with PC2 and PC3 respectively (***Figure 3—figure supplement 1***). Since none of the expected variables (sex, condition, or their interaction) contributed to the main source of variance in the dataset, we investigated whether social dominance was a contributing factor. We tested the association

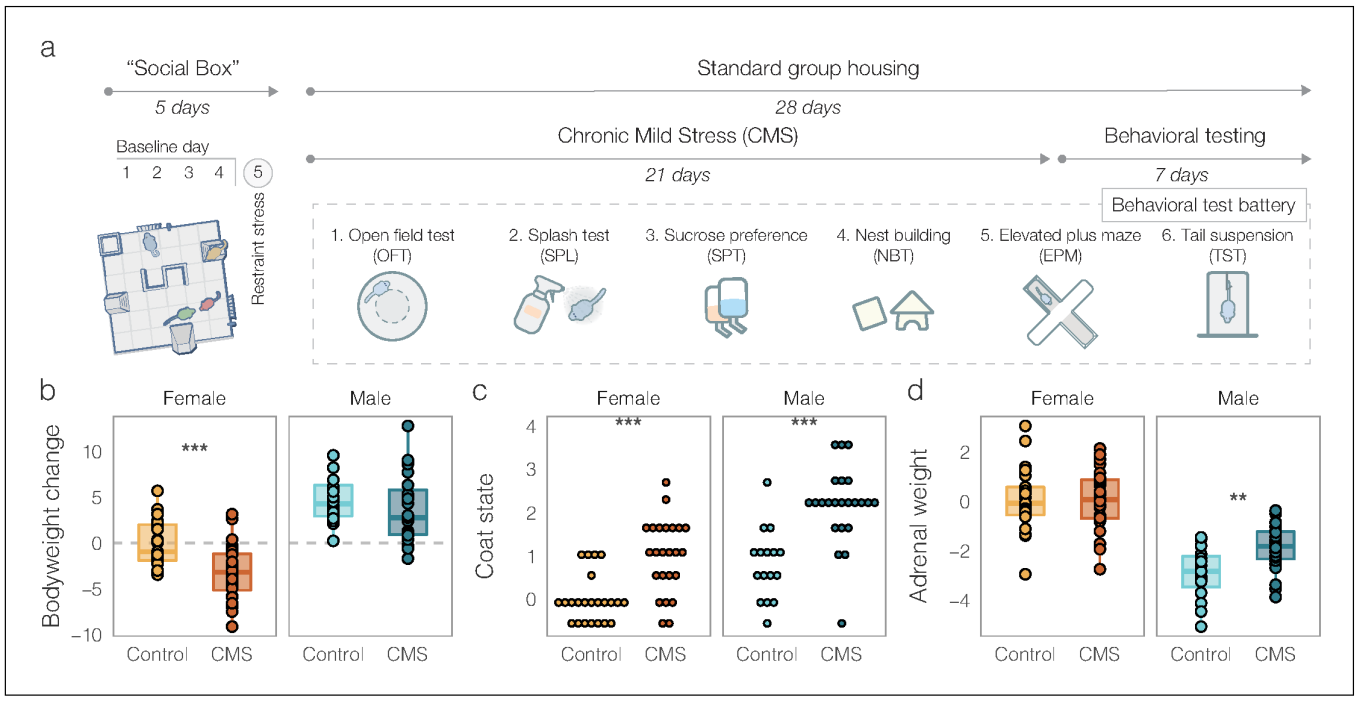

Figure 2. Outcomes of chronic mild stress (CMS) in males and females. (**a**) Experimental timeline. All groups underwent five days of Social Box (SB) monitoring. This consisted of four days of baseline monitoring followed by a 15-min acute restraint stress for all individuals prior to being re-introduced into the SB for a final 12-h dark phase monitoring period (day 5). After the SB, groups received three weeks of either control treatment (bodyweight and fur quality assessments two times a week) or CMS (see Materials and methods for details). The following week, all groups underwent a behavioral test battery in the order depicted. (**b**) Batch-adjusted bodyweight change following three weeks of CMS. Both male and female CMS mice showed significantly reduced weight compared to controls. (**c**) Batch-adjusted coat state scores (higher means poorer fur quality) following CMS. Male and female CMS groups showed significant deterioration of their coat. (**d**) Batch- and initial bodyweight-adjusted adrenal weights. CMS increased adrenal size in males, but not in females. Boxplots: line – median, box limits – 1st and 3rd quartile, whiskers – 1.5 x IQR. Data is presented relative to female controls. Number of mice per condition: Female Control = 23, Female CMS = 24, Male Control = 16, Male CMS = 23. (*p < 0.05, **p < 0.01, ***p < 0.001).

between PC1 scores and DS (***Figure 3c***). Remarkably, baseline DS significantly predicted scores on PC1 in CMS individuals only and this association was in opposite directions between the two sexes (sex by DS interaction: $F(1, 43) = 6.016$, $p = 0.0183$). Thus, the principal source of variation in the outcome dataset contained an interaction between baseline dominance scores and sex in the CMS mice.

To better assess the set of behaviors responsible for this association, we correlated PC1 scores with all the input features from the behavioral and physiological readouts (***Figure 3d***). We found that seventeen readouts were significantly correlated with PC1 scores in this dataset (Spearman's rank correlation, Bonferroni-adjusted $p < 0.05$). Among the strongest correlates of PC1 were measures derived from the OFT and EPM, and specifically features related to locomotion and anxiety-like behavior, such as distance traveled and visits to the anxiogenic regions of test chambers. Interestingly, these behaviors do not typically differentiate CMS and control individuals. Instead, CMS exposure appeared to create relationships between dominance and the outcome variables that were not present in controls (the top examples from the OFT and EPM are depicted in ***Figure 3e–f***, correlations between individual readouts and DS within each sex and condition are available in ***Figure 3—figure supplement 2***). To conclude, we were able to narrow down a portion of the variance in a broad range of behavioral and physiological outcomes following CMS to an interaction between dominance and sex with more subordinate CMS males showing apparent increases in measures of overall activity (distance/speed in the OFT and EPM) and more subordinate males and more dominant females showing an apparent reduction in anxiety-like behavior. Thus, we were able to identify

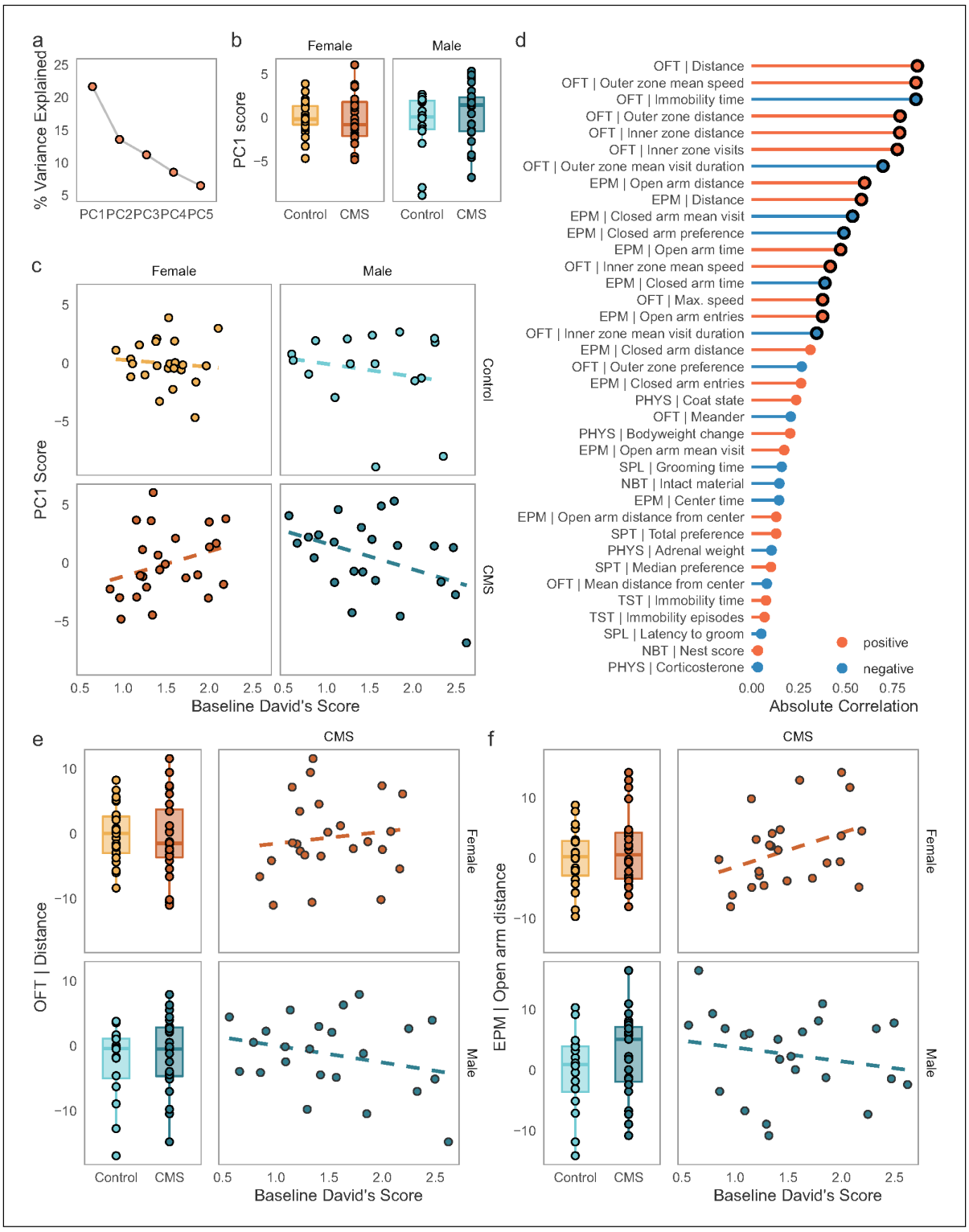

Figure 3. Opposing effects of baseline social dominance scores on behavioral outcomes following CMS. (**a**) Percentage of variance explained by the first five components of a principal components analysis conducted on the batch-adjusted behavioral and physiological outcome data. PC1 explains ca. 21% of the variance in this dataset. (**b**) PC1 is not significantly different between sexes or conditions, indicating that this component did not capture variance associated with either variable. (**c**) Association between baseline David's Scores and PC1 in control and CMS individuals. Baseline dominance

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Figure 3 continued

predicted scores on PC1, the major source of variance in the outcome data, in a sex-specific manner in the CMS group, but not in the control group. (**d**) Spearman's rank correlations between PC1 and the physiological and behavioral outcome variables. The strongest associations for PC1 are variables derived from the open field test (OFT) and elevated plus maze (EPM). Black circles around points identify associations significant at $p < 0.05$ after adjustment for multiple testing (Bonferroni correction). (**e-f**) Examples of interactions between baseline dominance and sex on CMS behavioral outcomes. Males and females show significant opposite correlations between dominance and example of locomotion and anxiety-like behavior. (Boxplots: line – median, box limits – 1st and 3rd quartile, whiskers – 1.5 x IQR. Scales for behavioral outcomes are relative to female controls).
The online version of this article includes the following figure supplement(s) for figure 3:

Figure supplement 1. PC2 and PC3 capture variance associated with sex and condition.
Figure supplement 2. Correlations between behaviors and baseline dominance scores.

a novel role for social rank belonging in modulating behavior following chronic stress in a sexually dimorphic way.

Discussion

Social behavior in general and social dominance in particular are important contributors to individual differences (***Forkosh et al., 2019***). As such, they may also shape how individuals respond to environmental challenges. In humans, different types of social hierarchies coexist in complex structures and they influence an individual's behavior and health (***Sapolsky, 2005***). Mouse social dominance hierarchies are considerably simpler, however parallels between the two species can be drawn in particular with regard to human socioeconomic status (SES). Both objective and perceived SES impact human health, mortality, morbidity, and susceptibility to psychiatric disorders such as depression or anxiety (***Farah, 2017***; ***Freeman et al., 2016***; ***Hoebel et al., 2017***; ***McEwen and Gianaros, 2010***; ***Shaked et al., 2016***; ***Wetherall et al., 2019***). These outcomes resemble findings related to social dominance in mice (***Bartolomucci, 2007***; ***Pallé et al., 2019***; ***Razzoli et al., 2018***). Intriguingly, some human studies have identified sex differences in the relationship between SES and stress-related psychopathologies (***Kosidou et al., 2011***; ***Mwinyi et al., 2017***; ***Peplinski et al., 2018***). All this suggests that social status and the stress and health gradient that characterize human social structures (***McEwen and Gianaros, 2010***) likely have close analogues in other mammals with well-defined social structures.

Here, we have demonstrated that both male and female socially housed mice establish social dominance hierarchies, which are relatively stable over time and resistant to acute perturbations. In agreement with previous work, female hierarchies were less despotic and had lower directional consistency (***Williamson et al., 2019***), suggesting that females may be maintaining a less rigid structure compared to males. This is supported by our finding that only the top rank in females showed significant stability over time, whereas in males both subordinate and dominant ranks appeared stable. Further research is needed to ascertain if this observation is limited to our paradigm and specific set of measurements (same-sex groups of four individuals), or if it represents a true sex difference in social dominance hierarchies.

Additionally, our data suggest that an individual's position in the hierarchy carries different implications for overall behavior in each sex. While we are unable to assess whether social rank belonging should be considered a cause or consequence of these behavioral differences, we have observed an interesting sex difference in this relationship. For groups living in the enriched environment of the SB apparatus, dominance in males but not in females was associated with overall locomotion, proportion of time spent outside the nest, and exploration entropy. These associations likely reflect territorial or patrolling behavior in males, which may be less relevant to female social hierarchies.

As hypothesized, occupancy of different positions in the social hierarchy conferred varying levels of responsiveness to the challenges posed by chronic stress. Previous investigations have rarely found associations between social dominance and response to chronic stress (***Larrieu and Sandi, 2018***). An important exception is a recent study by ***Larrieu et al., 2017*** in groups of male mice exposed to chronic social defeat stress. The authors found increased susceptibility to chronic social defeat for dominant males, but in contrast to our results, no real behavioral alterations for subordinates. It is important to note, however, that chronic social defeat and CMS are profoundly different paradigms. Social defeat is strongly tied to social dominance and might be perceived as loss of

status more than other stressors (**Larrieu and Sandi, 2018**). Our use of CMS allowed us to investigate both sexes under comparable levels of stress. Nevertheless, both the study from Larrieu and colleagues, and ours highlight that social status can influence an individual's response to long-term adverse life events.

Importantly, we demonstrated that the effects of preexisting dominance on stress outcomes were sexually divergent, such that the association between dominance and anxiety-like and locomotor behavior following CMS was in opposite directions between males and females. Specifically, subordinate males appeared to display hyperlocomotion, while dominant females displayed increased boldness (reduced anxiety-like behavior) compared to non-CMS controls. Overall, our data indicate that an individual's position within a social structure can influence their behavioral response to chronic stress in a sex-specific fashion.

These findings suggest an intriguing possibility. Given that male social hierarchies are likely antagonistic, we speculate that social living carries an especially high cost for subordinate males, who are the recipients of most antagonistic interactions. Conversely, female hierarchies may contribute to more affiliative social interactions, and thus social context may carry a net benefit for females, with the highest benefit gained by the dominant females. We speculate that this positioning as the most advantaged and disadvantaged individuals may confer higher behavioral flexibility and results in the strongest behavioral change upon exposure to environmental challenges.

Crucially, since we decided to maintain social context throughout our experimental design, the current work did not allow for the assessment of the effect of group- versus single-housing on CMS outcomes. Given this constraint, we were not able to confidently assess the difference in how CMS was experienced by each sex in groups as opposed to if they had been single-housed. However, since we were interested in the prediction from baseline dominance, we did not wish to remove the salience and thereby the effect of social context. Likewise, in naturalistic conditions, mice are found in mixed-sex groups (**Kondrakiewicz et al., 2019**). Working with same-sex groups provided us with a more controlled environment, preventing confounding by mating behavior and pregnancy. However, this was at the expense of the ethological validity of our findings. Further research is needed to understand if and how mixed-sex social structures may differ in their impact on stress outcomes.

Moreover, while CMS produced some of the expected physiological changes (i.e., reduction of bodyweight gain, reduced coat quality, adrenal weight increase), we did not observe several of the behavioral phenotypes often found using similar protocols (e.g., hyperlocomotion, anhedonia, passive coping, **Franceschelli et al., 2014**). While we have sufficient evidence that CMS individuals experienced significant amounts of stress, we are not able to determine if the absence of some of these behavioral signatures of CMS is a result of the maintenance of social context throughout the protocol or if it is due to other unknown factors. We are, however, not the first to observe no change in adrenal size or sucrose preference in female CD1 mice (**Dadomo et al., 2018**). Additionally, we did not observe any changes in basal corticosterone levels. This is probably be due to the fact that our blood sampling was performed one week after the end of the CMS paradigm, allowing enough time for corticosterone levels to return to normal.

Finally, we employed the David's Score as a continuous linear indicator of social dominance for the additional statistical power that this approach provides. Dominance hierarchies, however, are more commonly thought of as ordinal, and we lacked sufficient sample sizes per rank and condition to be able to reliably quantify the contribution of each rank to the behavioral outcomes of chronic stress. Further research is needed to replicate and extend these findings to specific social ranks.

While were not able to directly compare between single- and group-housed animals, our data suggest that the existence of a social hierarchy in groups of mice might contribute to increased variability in behavioral outcomes after chronic treatment generating rank-specific responses. Moreover, this effect could be especially relevant when studying sex differences. Often, housing conditions (single vs. group) are not taken into consideration as a variable of interest. Based on the findings reported here, we speculate that housing conditions might have contributed to discordant behavioral findings in studies of stress and sex (**Franceschelli et al., 2014**). Our results argue for considering group-derived individual differences and, in particular, dominance status, in the design of experiments, especially when investigating the contribution of sex differences to stress response.

Taken together, this work suggests that social dominance might influence the perception of and reaction to chronic stress differently for male and female mice. While there has been some work looking into the effects of dominance on stress susceptibility in males (**Larrieu et al., 2017**), very

little is known about female social dominance and its contribution to stress coping. Our findings emphasize the need for exploring the stress response in the presence of conspecifics in a more naturalistic manner and the importance of recognizing that the same social factors may carry divergent consequences for the behavior of males and females.

Materials and methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Commercial assay or kit	Corticosterone Double Antibody RIA Kit	MP Biomedicals	SKU 0712010-CF	
Software, algorithm	*Tidyverse*, ecosystem of packages	***R Core Development Team, 2013*** doi:10.21105/joss.01686		R version 4.0.2
Software, algorithm	*'RNOmni* R package	***McCaw, 2019***	v 0.7.1	R version 4.0.2
Software, algorithm	*'cowplot'* R package	***Wilke, 2019***	v 1.0.0	R version 4.0.2
Software, algorithm	*'compete'* R package	***Curley et al., 2015***	v 0.1	R version 4.0.2
Software, algorithm	*'steepness'* R package	***Leiva and de Vries, 2014***	v 0.2–2	R version 4.0.2

Animal housing and care

Male and female ICR CD-1 mice at 7–9 months old were employed for all experiments (Charles River, Sulzfeld, Germany). Mice were housed in groups of four in the animal facilities of the Max Planck Institute of Psychiatry in Munich, Germany, from weaning and were maintained under standard conditions (12L:12D light cycle, lights on at 07:00 AM, temperature 23 ± 2˚C) with food and water available ad libitum. All experiments were approved by and conducted in accordance with the regulations of the local Animal Care and Use Committee (Government of Upper Bavaria, Munich, Germany), under licenses Az.: 55.2-1-54-2532-148-2012, Az.:55.2-1-54-2532-32-2016 and ROB-55.2–2532.Vet_02-18-50. The fur of all mice was marked using four different colors under mild isoflurane anesthesia and mice were left to recover for several days before the start of the experiment. On day 1, animals were transferred to the SB (see 'The 'Social Box' paradigm' section), for a total of 5 days (five light periods and five dark periods). On day 6, animals were removed from the SB and placed in their original cage under standard housing conditions for the rest of the experimental procedure (see 'Chronic mild stress protocol' and 'Behavioral battery' sections).

Behavior in a semi-naturalistic environment

The 'Social Box' paradigm

The 'Social Box' is a behavioral arena wherein groups of mice live under continuous observation over a period of several days (***Forkosh et al., 2019***; ***Shemesh et al., 2013***). Mouse identities are maintained using fur markings in four different colors (***Shemesh et al., 2020***; ***Forkosh et al., 2019***). The entire SB observation period was recorded using cameras mounted above each arena. Videos of the dark periods of the light cycle (7:00 PM to 7:00 AM) were then compressed and analyzed using a custom automated tracking system which determines mouse locations over time based on color segmentation (***Shemesh et al., 2013***; ***Shemesh et al., 2020***). From the location data we inferred agonistic interactions as well a variety of other behavioral readouts as described in ***Forkosh et al., 2019***. Briefly, we used the absolute locations and smoothed movements of individuals as well as location and movement with respect to regions of interest in the SB to compute readouts related to overall locomotion, feeding/drinking, etc. (e.g., distance and speed outside the nest, distance from walls,

distance from the nest, time spent in the feeders, on ramps, in the S-wall). Exploratory behavior is assessed by estimating the unpredictability of movement (entropy) outside the nest using spatial bins of either the regions of interest or a 6 by 6 10 cm grid overlaid on the SB. We model social interactions using a Hidden Markov Model which takes into consideration the relative trajectories and distances between pairs of mice and determines who initiates a contact, its progression and its properties using a simple topology states (idle, approach/avoid, contact, follow/avoid, described in detail in **Forkosh et al., 2019**).

Social dominance was assessed using the David's Score (DS), a measure based on the pairwise directionalities and numbers of agonistic interactions in a group (**David, 1987**). Chases during the four days of the baseline period were used to build the DS, which was then normalized to group number (n = 4), creating a continuous range between 0 (least dominant) and 3 (most dominant). The steepness of the social hierarchy was characterized as described in **de Vries et al., 2006** by using the slope of a line fitted to the DS from a ranked DS using Ordinary Least Squares regression. We used an implementation of this procedure made available in the open-source '*steepness*' R package (**Leiva and de Vries, 2014**), whose output ranges between 0 and 1, with one meaning a very steep hierarchy in which power is unequally distributed between dominant and subordinate individuals. Despotism was defined as the fraction of the group's total number of chases that were initiated by the highest-ranking individual. Its values range as well between 0 and 1, in which one represents the presence of a very strong alpha who initiate all chases. Directional consistency was calculated using the average fraction of pairwise social interactions that occur in the direction from the individual who displayed more instances of an agonistic behavior to the individual who displayed fewer instances (**van Hooff and Wensing, 1987**; **Williamson et al., 2016**). A directional consistency equal to one indicates that all agonist interactions are directed from an individual with a higher DS to one with a lower DS. Finally, we used Landau's modified h' to assess the linearity of a social hierarchy, as described in **de Vries, 1995**, in which hierarchies which are fully linearly ordered are assigned a value of 1. We calculated both directional consistency and Landau's modified h' using functions made available in the R package '*compete*' (**Curley et al., 2015**).

Acute restraint

Before the beginning of the fifth night in the SB, mice were removed from the SB and restrained in a ventilated tube for 15 min. To account for the smaller size of females, we employed a smaller sized ventilated tube to ensure the same degree of movement restriction between sexes. At the end of the acute restraint, groups of mice were put back in their original SB and tracked for additional 12 hr.

CMS protocol

Two separate batches of mice were exposed to three weeks of CMS prior to the behavioral test battery. A random combination of two stressors per day (one in the a.m. and one in the p.m. hours) was chosen among the followings: acute restraint in the dark (15 min), acute restraint in bright light (15 min, ~200 lux), acute restraint witnessing (half of the group at a time was restrained and placed inside the cage, 15 min each), removal of nesting material (24 hr), cage-tilt 30˚ along the vertical axis (6 hr), no bedding or nesting material (8 hr), wet bedding (6 hr), water avoidance (15 min), cage change (fresh cage every 30 min for a total of 4 hr), cage switching (mice are assigned the cage of another group of the same sex), overcrowding (eight mice per cage, 1 hr). For the water avoidance stress, an empty rat cage (395 × 346 cm) was filled with room temperature water. Mice were placed on a platform (10 × 12 cm), 2 cm above the water level, for 15 min.

On days 1, 3, 7, 10, 14, 17, and 21 both CMS and control mice were weighed. During the weighing session, their coat state was scored on a scale 0 to 3 according to the following criteria:

1. Bright and well-groomed coat. Clean eyes. No wounds.
2. Less shiny and less groomed coat OR unclean eyes. No wounds.
3. Dirty and dull coat and/or small wounds and not clear eyes.
4. Extensive piloerection OR alopecia with crusted eyes OR extensive wounds.

Cumulative coat state was calculated as the sum of the seven daily scores.

Control mice were kept in an adjacent room to the stressed mice and handled twice per week to obtain weight and coat scores.

Behavioral battery

The day after the last stressor, mice started a behavioral test battery consisting of the OFT, 2-hr SPT, grouped SPT, the splash test (SPL), the nest building test (NBT), the EPM, a grouped sucrose preference, and the TST. Throughout the testing period, mice were maintained in their original groups and habituated to the testing room for at least one hour prior the start of the test. Forty-eight hours after the last test, mice were terminally anesthetized in isoflurane and sacrificed. Terminal bodyweight, plasma, adrenal glands, and thymus were collected. Adrenal glands and thymus were cleaned from fat tissue and weighed. Absolute values were adjusted to bodyweight using the bodyweights collected on day 1. Tissue weighing, corticosterone measurement, and behavioral scoring were performed by an experimenter blind to sex, condition, and social rank.

Open field test

On the day following the last stressors (day 22), mice locomotor activity and exploratory behavior were assessed in the OFT for 10 min. The apparatus consisted in round arenas (diameter 38 cm) made of black polyvinylchloride (PVC) under dim illumination (3 lux). Mice were automatically tracked with ANYmaze Video Tracking System 6.13 (Stoelting, IL, USA). The space was virtually divided in an inner zone (diameter 16 cm) and an outer zone. Total distance traveled, distance from the center, speed, and turn angle were calculated across the full 10 min. In addition, distance traveled, speed, visits, and time spent in each of the subdivisions were used as parameters. Preference was calculated as follows: $\frac{outer\ zone\ time}{inner\ zone\ time}$.

Two-hour daily sucrose preference test

Twenty-four hours after the OFT, the anhedonia phenotype was tested with a modified version of the SPT. Each group was assigned a test cage containing one water bottle and one bottle with 2% sucrose. One mouse per group at a time was placed in the test cage for two hours, across three consecutive days during the light phase (days 23, 24, and 25). At the end of each session, the bottles were weighed. At the end of the test the amounts of water and sucrose consumed were summed across the three sessions. Sucrose preference was calculated as $\frac{sucrose}{water+sucrose} * 100$.

Grouped sucrose preference test

On day 27, sucrose preference was tested at a group level. Each group was given a bottle of water and a bottle of 2% sucrose within their home-cage. Their sucrose preference was calculated after 24 hr as above. A grouped sucrose preference value was obtained for each group.

Splash test

On day 24, during the dark period, mice were tested in the splash test under dim light (3 lux). Mice were placed in their test cage for 5 min prior being sprayed on their dorsal coat twice (approximately 1 ml) with 10% sucrose solution. Mice were recorded for 5 min and total time spent grooming, and latency to the first grooming bout was manually scored using Solomon Coder 17.03.32 (https://solomon.andraspeter.com/).

Nest building test

During the third day of the 2-hr sucrose preference, mice in the test cage were given a small square cotton pad of approximately 23 g. The cotton pad was weighed at the beginning of the test and at the end of the two hours and the percentage of intact material was calculated. The built nest was scored from 0 to 4 according to the following criteria:

1. Material untouched.
2. Material partially torn (50–90% remaining intact).
3. Material mostly shredded but often no identifiable nest site/scattered around.
4. Material accumulated in an identifiable nest site, but the nest is flat.
5. A (near) perfect nest: material fine shredded, doughnut like with walls higher than the mouse.

For nests matching only partially the description (e.g., identifiable flat nest, but less than 50% of torn material), half points were assigned.

Elevated plus maze

On day 26, during the light phase, anxiety phenotype was assessed using the EPM test. An apparatus composed of four arms made of gray polyvinylchloride (PVC), two open without walls, two enclosed by 14 cm walls and a central platform (5 × 5 cm) was used. The apparatus was placed 33 cm from the ground under dim illumination (3 lux). Mice were placed on the central platform facing the open arms and let free to explore the apparatus for 10 min. Mice were automatically tracked using ANYmaze Video Tracking System 6.13 (Stoelting, IL, USA). Number of entries in each arms, time, and distance were calculated. In addition, closed arm preference was calculated as $\frac{time\ in\ closed\ arms}{time\ in\ closed\ +\ time\ in\ open\ arms}$.

Tail suspension test

Stress coping behavior was assessed using the TST on day 28. Mice were hung by their tail 50 cm above the surface and their behavior recorded for 6 min. Immobility was automatically scored using ANYmaze Video Tracking System 6.13 (Stoelting, IL, USA) and number of immobility episodes and total time immobile were used as parameters.

Corticosterone assessment

At sacrifice, trunk blood was collected in EDTA-coated tubes. Blood was centrifuged at 1,000 g for 15 min at 4˚C. Plasma was retrieved and corticosterone levels were measured using [^{125}I] radioimmunoassay kit (MP Biomedicals), according to the manufacturer's instructions.

Data analysis

Quality control and outlier removal

Low-quality tracks from the SBs were labeled by estimating the number of large (>100 pixels) interruptions in the mouse trajectories as well as the fraction of time mice were spotted outside the nest. Tracks where a mouse had more than 200 trajectory interruptions or was found outside the nest for less than 2% of the total monitoring time were excluded from further analyses. Additionally, all the tracks of an individual were excluded in cases when more than two of the four baseline day recordings of a mouse did not pass quality control thresholds. Based on these criteria, the complete SB data of four mice was excluded. A single value from the corticosterone outcomes was labeled as outlier and removed (453.4 ng/ml, >3.5 standard deviations away from the mean of the appropriate sex and condition grouping). The results of the EPM test for one mouse and the ones from the splash test for two mice were excluded due to a technical recording failure and the nest building test 'percent intact' value for one mouse was lost due to experimental failure.

Statistical testing

All statistical analyses were performed in R version 4.0.2 assisted by the '*Tidyverse*' ecosystem of packages (***R Core Development Team, 2013***; ***Wickham et al., 2019***). The tests employed for each specific analysis are reported in the Results section. All inverse rank-transformed behavioral outcomes (Blom transform, RNOmni R package) (***McCaw, 2019***) were adjusted for batch effects using the standardized residuals of a linear model with each variable of interest as outcome and batch as a factorial predictor. Figure panels were assembled with the help of the '*cowplot*' R package (***Wilke, 2019***). Outcome data distributions were tested for deviations from normality (Shapiro-Wilk test) and heteroscedasticity (Levene's test). Whenever normality was violated and the data could not be transformed to fit a normal distribution, non-parametric tests were employed. Violations of homogeneity of variances are reported with each test. As this was an exploratory set of experiments without an a priori hypothesis regarding the association between dominance, stress response, and sex, no power calculations were performed, and sample sizes were chosen based published work with CMS interventions.

Principal component analysis

Principal component analysis (PCA) was employed to explore the sources of variance in our multidimensional behavioral dataset. Specifically, we performed PCA using singular value decomposition on scaled and centered data from the behavioral and physiological outcomes following CMS. Prior

to decomposition, missing data points (for a maximum of two values per individual) were replaced with the median of the respective outcome. The principal components (PCs) obtained were ranked by the total amount of variance explained. The top 3 PCs, namely PC1, PC2, and PC3, contained most of the variance in our dataset and were thus used to investigate the effects of known variables (sex, stress condition, social dominance status). To evaluate the influence of social dominance we assessed the association between PC1 and the David's Score within each experimental group.

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Additional files

Supplementary files

- Transparent reporting form

Data availability

All data used to support the findings of this work and the code used in performing the analyses and producing the figures for this manuscript is freely accessible in a GitHub repository: https://stoyokaramihalev.github.io/CMS_Dominance/ copy archived at (https://archive.softwareheritage.org/swh:1:dir:31e8482879f9eb61cf9341e95ccca36991847ba3). The MATLAB-based mouse tracking system is available at https://en.bio-protocol.org/prep207.

References

Altemus M, Sarvaiya N, Neill Epperson C. 2014. Sex differences in anxiety and depression clinical perspectives. *Frontiers in Neuroendocrinology* **35**:320–330. DOI: https://doi.org/10.1016/j.yfrne.2014.05.004, PMID: 24887405

Bangasser DA, Valentino RJ. 2012. Sex differences in molecular and cellular substrates of stress. *Cellular and Molecular Neurobiology* **32**:709–723. DOI: https://doi.org/10.1007/s10571-012-9824-4, PMID: 22488525

Bangasser DA, Valentino RJ. 2014. Sex differences in stress-related psychiatric disorders: neurobiological perspectives. *Frontiers in Neuroendocrinology* **35**:303–319. DOI: https://doi.org/10.1016/j.yfrne.2014.03.008, PMID: 24726661

Bangasser DA, Wicks B. 2017. Sex-specific mechanisms for responding to stress. *Journal of Neuroscience Research* **95**:75–82. DOI: https://doi.org/10.1002/jnr.23812, PMID: 27870416

Bartolomucci A. 2007. Social stress, immune functions and disease in rodents. *Frontiers in Neuroendocrinology* **28**:28–49. DOI: https://doi.org/10.1016/j.yfrne.2007.02.001, PMID: 17379284

Beery AK, Zucker I. 2011. Sex Bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews* **35**:565–572. DOI: https://doi.org/10.1016/j.neubiorev.2010.07.002, PMID: 20620164

Brivio E, Lopez JP, Chen A. 2020. Sex differences: transcriptional signatures of stress exposure in male and female brains. *Genes, Brain and Behavior* **19**:1–22. DOI: https://doi.org/10.1111/gbb.12643

Curley JP, Shen K, Huang Z. 2015. compete: Analyzing competitive interaction data. v0.1. R package. https://github.com/jalapic/compete

Curley JP. 2016. Temporal pairwise-correlation analysis provides empirical support for attention hierarchies in mice. *Biology Letters* **12**:0192. DOI: https://doi.org/10.1098/rsbl.2016.0192, PMID: 27194290

Dadomo H, Gioiosa L, Cigalotti J, Ceresini G, Parmigiani S, Palanza P. 2018. What is stressful for females? differential effects of unpredictable environmental or social stress in CD1 female mice. *Hormones and Behavior* **98**:22–32. DOI: https://doi.org/10.1016/j.yhbeh.2017.11.013, PMID: 29187314

David HA. 1987. Ranking from unbalanced paired-comparison data. *Biometrika* **74**:432–436. DOI: https://doi.org/10.1093/biomet/74.2.432

de Vries H. 1995. An improved test of linearity in dominance hierarchies containing unknown or tied relationships. *Animal Behaviour* **50**:1375–1389. DOI: https://doi.org/10.1016/0003-3472(95)80053-0

de Vries H, Stevens JMG, Vervaecke H. 2006. Measuring and testing the steepness of dominance hierarchies. *Animal Behaviour* **71**:585–592. DOI: https://doi.org/10.1016/j.anbehav.2005.05.015

Drews C. 1993. The concept and definition of dominance in animal behaviour. *Behaviour* **125**:283–313. DOI: https://doi.org/10.1163/156853993X00290

Farah MJ. 2017. The neuroscience of socioeconomic status: correlates, causes, and consequences. *Neuron* **96**: 56–71. DOI: https://doi.org/10.1016/j.neuron.2017.08.034, PMID: 28957676

Forkosh O, Karamihalev S, Roeh S, Alon U, Anpilov S, Touma C, Nussbaumer M, Flachskamm C, Kaplick PM, Shemesh Y, Chen A. 2019. Identity domains capture individual differences from across the behavioral repertoire. *Nature Neuroscience* **22**:2023–2028. DOI: https://doi.org/10.1038/s41593-019-0516-y, PMID: 31686022

Franceschelli A, Herchick S, Thelen C, Papadopoulou-Daifoti Z, Pitychoutis PM. 2014. Sex differences in the chronic mild stress model of depression. *Behavioural Pharmacology* **25**:372–383. DOI: https://doi.org/10.1097/FBP.0000000000000062, PMID: 25025701

Freeman A, Tyrovolas S, Koyanagi A, Chatterji S, Leonardi M, Ayuso-Mateos JL, Tobiasz-Adamczyk B, Koskinen S, Rummel-Kluge C, Haro JM. 2016. The role of socio-economic status in depression: results from the COURAGE (aging survey in Europe). *BMC Public Health* **16**:1–8. DOI: https://doi.org/10.1186/s12889-016-3638-0

Gammell MP, de Vries H, Jennings DJ, Carlin CM, Hayden TJ. 2003. David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Animal Behaviour* **66**:601–605. DOI: https://doi.org/10.1006/anbe.2003.2226

Hodes GE. 2018. A primer on sex differences in the behavioral response to stress. *Current Opinion in Behavioral Sciences* **23**:75–83. DOI: https://doi.org/10.1016/j.cobeha.2018.03.012

Hodes GE, Epperson CN. 2019. Sex differences in vulnerability and resilience to stress across the life span. *Biological Psychiatry* **86**:421–432. DOI: https://doi.org/10.1016/j.biopsych.2019.04.028, PMID: 31221426

Hoebel J, Maske UE, Zeeb H, Lampert T. 2017. Social inequalities and depressive symptoms in adults: the role of objective and subjective socioeconomic status. *PLOS ONE* **12**:e0169764. DOI: https://doi.org/10.1371/journal.pone.0169764, PMID: 28107456

Horii Y, Nagasawa T, Sakakibara H, Takahashi A, Tanave A, Matsumoto Y, Nagayama H, Yoshimi K, Yasuda MT, Shimoi K, Koide T. 2017. Hierarchy in the home cage affects behaviour and gene expression in group-housed C57BL/6 male mice. *Scientific Reports* **7**:6991. DOI: https://doi.org/10.1038/s41598-017-07233-5, PMID: 28765614

Joel D, McCarthy MM. 2017. Incorporating sex as a biological variable in neuropsychiatric research: where are we now and where should we be? *Neuropsychopharmacology* **42**:379–385. DOI: https://doi.org/10.1038/npp.2016.79, PMID: 27240659

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Archives of General Psychiatry* **62**:593. DOI: https://doi.org/10.1001/archpsyc.62.6.593, PMID: 15939837

Kondrakiewicz K, Kostecki M, Szadzińska W, Knapska E. 2019. Ecological validity of social interaction tests in rats and mice *genes. Brain and Behavior* **18**:e12525. DOI: https://doi.org/10.1111/gbb.12525

König B, Lindholm AK. 2012. The complex social environment of female house mice (Mus domesticus). In: Macholan M, Baird S. J. E, Munclinger P (Eds). *Evolution of the House Mouse*. Cambridge: Cambridge University Press. p. 114–134. DOI: https://doi.org/10.1017/CBO9781139044547

Kosidou K, Dalman C, Lundberg M, Hallqvist J, Isacsson G, Magnusson C. 2011. Socioeconomic status and risk of psychological distress and depression in the Stockholm public health cohort: a population-based study. *Journal of Affective Disorders* **134**:160–167. DOI: https://doi.org/10.1016/j.jad.2011.05.024, PMID: 21665286

Landau HG. 1951. On dominance relations and the structure of animal societies: ii. some effects of possible social factors. *The Bulletin of Mathematical Biophysics* **13**:245–262. DOI: https://doi.org/10.1007/BF02477920

Larrieu T, Cherix A, Duque A, Rodrigues J, Lei H, Gruetter R, Sandi C. 2017. Hierarchical status predicts behavioral vulnerability and nucleus accumbens metabolic profile following chronic social defeat stress. *Current Biology* **27**:2202–2210. DOI: https://doi.org/10.1016/j.cub.2017.06.027, PMID: 28712571

Larrieu T, Sandi C. 2018. Stress-induced depression: is social rank a predictive risk factor? *BioEssays* **40**:1800012. DOI: https://doi.org/10.1002/bies.201800012

Leiva D, de Vries H. 2014. Steepness: Testing steepness of dominance hierarchies. 0.2-2. R package version. https://rdrr.io/cran/steepness/

Marcus SM, Young EA, Kerber KB, Kornstein S, Farabaugh AH, Mitchell J, Wisniewski SR, Balasubramani GK, Trivedi MH, Rush AJ. 2005. Gender differences in depression: findings from the STAR*D study. *Journal of Affective Disorders* **87**:141–150. DOI: https://doi.org/10.1016/j.jad.2004.09.008, PMID: 15982748

Martin LA, Neighbors HW, Griffith DM. 2013. The experience of symptoms of depression in men vs women. *JAMA Psychiatry* **70**:1100. DOI: https://doi.org/10.1001/jamapsychiatry.2013.1985

McCaw Z. 2019. RNOmi: Rank Normal Transformation Omnibus Test. 0.7.1. R package. https://rdrr.io/cran/RNOmni/
McEwen BS, Gianaros PJ. 2010. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Annals of the New York Academy of Sciences* **1186**:190–222. DOI: https://doi.org/10.1111/j.1749-6632.2009.05331.x, PMID: 20201874
Mwinyi J, Pisanu C, Castelao E, Stringhini S, Preisig M, Schiöth HB. 2017. Anxiety disorders are associated with low socioeconomic status in women but not in men. *Women's Health Issues* **27**:302–307. DOI: https://doi.org/10.1016/j.whi.2017.01.001, PMID: 28215982
Pallé A, Zorzo C, Luskey VE, McGreevy KR, Fernández S, Trejo JL. 2019. Social dominance differentially alters gene expression in the medial prefrontal cortex without affecting adult hippocampal neurogenesis or stress and anxiety-like behavior. *The FASEB Journal* **33**:6995–7008. DOI: https://doi.org/10.1096/fj.201801600R, PMID: 30857420
Peplinski B, McClelland R, Szklo M. 2018. Associations between socioeconomic status markers and depressive symptoms by race and gender: results from the Multi-Ethnic study of atherosclerosis (MESA). *Annals of Epidemiology* **28**:535–542. DOI: https://doi.org/10.1016/j.annepidem.2018.05.005, PMID: 29934242
R Core Development Team. 2013. A language and environment for statistical computing. 2.6.2. Vienna, Austria, R Foundation for Statistical Computing. https://www.R-project.org/
Razzoli M, Nyuyki-Dufe K, Gurney A, Erickson C, McCallum J, Spielman N, Marzullo M, Patricelli J, Kurata M, Pope EA, Touma C, Palme R, Largaespada DA, Allison DB, Bartolomucci A. 2018. Social stress shortens lifespan in mice. *Aging Cell* **17**:e12778. DOI: https://doi.org/10.1111/acel.12778, PMID: 29806171
Sapolsky RM. 2005. The influence of social hierarchy on primate health. *Science* **308**:648–652. DOI: https://doi.org/10.1126/science.1106477, PMID: 15860617
Schuhr B. 1987. Social structure and plasma corticosterone level in female albino mice. *Physiology & Behavior* **40**:689–693. DOI: https://doi.org/10.1016/0031-9384(87)90269-1, PMID: 3671537
Shaked D, Williams M, Evans MK, Zonderman AB. 2016. Indicators of subjective social status: differential associations across race and sex. *SSM - Population Health* **2**:700–707. DOI: https://doi.org/10.1016/j.ssmph.2016.09.009, PMID: 29349181
Shemesh Y, Sztainberg Y, Forkosh O, Shlapobersky T, Chen A, Schneidman E. 2013. High-order social interactions in groups of mice. *eLife* **2**:e00759. DOI: https://doi.org/10.7554/eLife.00759, PMID: 24015357
Shemesh Y, Forkosh O, Chen A, Schneidman E. 2020. Mouse tracking. *Bio-Protocol*. prep207. https://en.bio-protocol.org/prep207
van den Berg WE, Lamballais S, Kushner SA. 2015. Sex-specific mechanism of social hierarchy in mice. *Neuropsychopharmacology* **40**:1364–1372. DOI: https://doi.org/10.1038/npp.2014.319, PMID: 25469681
van Hooff J, Wensing J. 1987. Dominance and its behavioral measures in a captive wolf pack. In: Frank H (Ed). *Perspectives in Vertebrate Science*. Dordrecht, Netherlands: Dr W Junk Publishers. p. 219–252.
Varholick JA, Bailoo JD, Palme R, Würbel H. 2018. Phenotypic variability between social dominance ranks in laboratory mice. *Scientific Reports* **8**:6593. DOI: https://doi.org/10.1038/s41598-018-24624-4, PMID: 29700322
Varholick JA, Pontiggia A, Murphy E, Daniele V, Palme R, Voelkl B, Würbel H, Bailoo JD. 2019. Social dominance hierarchy type and rank contribute to phenotypic variation within cages of laboratory mice. *Scientific Reports* **9**:13650. DOI: https://doi.org/10.1038/s41598-019-49612-0, PMID: 31541122
Wang F, Kessels HW, Hu H. 2014. The mouse that roared: neural mechanisms of social hierarchy. *Trends in Neurosciences* **37**:674–682. DOI: https://doi.org/10.1016/j.tins.2014.07.005, PMID: 25160682
Wetherall K, Robb KA, O'Connor RC. 2019. Social rank theory of depression: a systematic review of self-perceptions of social rank and their relationship with depressive symptoms and suicide risk. *Journal of Affective Disorders* **246**:300–319. DOI: https://doi.org/10.1016/j.jad.2018.12.045, PMID: 30594043
Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, et al. 2019. Welcome to the tidyverse. *Journal of Open Source Software* **4**:1686. DOI: https://doi.org/10.21105/joss.01686
Wilke CO. 2019. cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2". 1.0.0. R package. https://rdrr.io/cran/cowplot/
Williamson CM, Lee W, Curley JP. 2016. Temporal dynamics of social hierarchy formation and maintenance in male mice. *Animal Behaviour* **115**:259–272. DOI: https://doi.org/10.1016/j.anbehav.2016.03.004
Williamson CM, Lee W, DeCasien AR, Lanham A, Romeo RD, Curley JP. 2019. Social hierarchy position in female mice is associated with plasma corticosterone levels and hypothalamic gene expression. *Scientific Reports* **9**:7324. DOI: https://doi.org/10.1038/s41598-019-43747-w, PMID: 31086272
World Health Organization. 2017. *Depression and Other Common Mental Disorders: Global Health Estimates* Geneva: World Health Organization. https://apps.who.int/iris/handle/10665/254610.
Young LJ, Pfaff DW. 2014. Sex differences in neurological and psychiatric disorders. *Frontiers in Neuroendocrinology* **35**:253–254. DOI: https://doi.org/10.1016/j.yfrne.2014.05.005, PMID: 24882637

List of Figures

List of Tables

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Declaration of Contributions

Mice handling

CMS paradigm	Elena Brivio, Rainer Stoffel
Behavioural tests	Elena Brivio
Blood collection and processing	Elena Brivio, Juan Pablo Lopez
CORT measurement	Rainer Stoffel
Ovariectomy	Albin Varga
Estrus monitoring	Elena Brivio
Organs collection and dissection	Elena Brivio, Juan Pablo Lopez, Aron Kos, Bianca Schmid, Daniela Harbich, Andrea Ressle

scRNA-seq

PVN dissection	Elena Brivio
Single cell suspension	Elena Brivio
10x Genomics Chromium Controller loading	Juan Pablo Lopez
3’ single cell library preparation	Elena Brivio
Sequencing	Sequencing Core Facility, *Max Planck Institute for Molecular Genetics - Berlin*
Data pre-processing	Gil Sterlzer, *Weizmann Institute of Science*
Data QC, clustering, label transfer, and differential expression, pseudotime, CCInx	Elena Brivio
Pathway and RRHO analyses	Elena Brivio

Validation

Tissue fixation, preparation, and immunostaining	Elena Brivio

Bioinformatics

Statistical analysis	Elena Brivio
Graphing and figures preparation	Elena Brivio
Web App	Elena Brivio, Stoyo Karamihalev

Affidavit

Affidavit

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I hereby declare, that the submitted thesis entitled:

"Modulation of Acute Stress Transcriptional Signatures by Sex and History of Stress at Single-Cell Resolution"

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Rehovot, 03.05.2022

place, date

Elena Brivio

Signature doctoral candidate

Confirmation of Congruency

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN | Promotionsbüro Medizinische Fakultät | MMRS

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I hereby declare, that the submitted thesis entitled:

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is congruent with the printed version both in content and format.

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List of Peer-Reviewed Publications

	Publications
Review Articles	**Brivio E**, Lopez JP, Chen A. – "Sex differences: Transcriptional signatures of stress exposure in male and female brains", (2020) Genes, Brain Behav.
First-author Research Articles	**Brivio E**, Kos A, Karamihalev S, Ressle A, Stoffel R, Stelzer G, Schmidt MV, Lopez JP*, Chen A*. – "Sex shapes cell-type-specific transcriptional signatures of stress in the mouse hypothalamic paraventricular nucleus", (2022) *in revision* Karamihalev S*, **Brivio E***, Eren N, Flachskamm C, Kos A, Bordes J, Lopez JP, Schmidt MV, Chen A. – "Spontaneous behavioral synchrony in mouse dyads", (2022) *in preparation* **Brivio E**, Lopez JP, Ressle A, Stelzer G, Albin V, Schmidt MV, Chen A. – "Female-specific GABAergic Response is Estrogen-Dependent", (2022) *in preparation* Karamihalev S*, **Brivio E***, Flachskamm C, Stoffel R, Schmidt MV, Chen A. – "Social dominance mediates behavioral adaptation to chronic stress in a sex-specific manner", (2020) eLife
Contributions to Research Articles	Karamihalev S, Eren N, **Brivio E**, Flachskamm C, Huettl RE, Touma C, Kuperman Y, Shemesh Y, Chen A. – "Deep automated phenotyping links social context to individual behavior and physiology", (2022) *in preparation* Dournes C*, Dine J*, Lopez JP, **Brivio E**, Anderzhanova E, Roeh S, Kuehne C, Holzapfel M, Huettl R, Stoffel R, Tietze L, Eggert C, Schieven M, Jakovcevski M, Deussing JM, Chen A. – "Hypothalamic glucocorticoid receptor in CRF neurons is essential for HPA axis habituation to repeated stressor", (2020) *under revision and BioRxiv* Sen P, Sotillos L, **Brivio E**, Lopez JP, Ortiz O, Chen A, Wurst W, Deussing JM. – "A bipolar disorder-associated polymorphism alters protein function and mouse behavior", (2022) *in preparation* Kos A, Lopez JP, Bordes J, De Donno C, Dine J, **Brivio E**, Karamihalev S, Luecken MD, Almeida S, Dick A, Miranda L, Büttner M, Stoffel R, Flashskamm C, Theis FJ, Schmidt M, Chen A. – "Early life stress alters cell-type specific transcriptomic patterning in the ventral hippocampus leading to disturbed excitation and inhibition transmission", (2022) *in preparation* De Donno C*, Lopez JP*, Luecken MD*, Kos A, **Brivio E**, Yang H, Deussing JM, Schmidt MV, Theis FJ, Chen A. – "Single-cell atlas of stress response in the adult murine hippocampus" (2022) *in preparation*

	Lopez JP, Lücken MD, **Brivio E**, Karamihalev S, Kos A, De Donno C, Benjamin A, Yang H, Stoffel R, Ressle A, Flachskamm C, Roeh S, Huettl RE, Parl A, Eggert C, Holzapfel M, Hauger B, Di Giaimo R, Schmidt MV, Deussing JM, Eder M, Dine J, Theis FJ, Chen A. – "Ketamine exerts antidepressant effects via cell-type specific regulation of Kcnq2", (2022) Neuron
	Lopez JP, **Brivio E**, Santambrogio A, De Donno C, Kos A, Peters M, Rost N, Czamara D, Brückl TM, Roeh S, Pöhlmann ML, Engelhardt C, Ressle A, Stoffel R, Tontsch A, Villamizar JM, Reincke M, Riester A, Sbiera S, Fassnacht M, Mayberg HS, Craighead WE, Dunlop B, Nemeroff CB, Schmidt MV, Binder EB, Theis FJ, Beuschlein F, Andoniadou CL, Chen A. – "Single-cell molecular profiling of all three components of the HPA axis reveals adrenal ABCB1 as a regulator of stress adaptation", (2021) Sci. Adv.
	Häusl AS, Brix LM, Hartmann J, Pöhlmann ML, Lopez JP, Menegaz D, **Brivio E**, Engelhardt C, Roeh S, Bajaj T, Rudolph L, Stoffel R, Hafner K, Goss HM, Reul JMHM, Deussing JM, Eder M, Ressler KJ, Gassen NC, Chen A, Schmidt MV. – "The co-chaperone Fkbp5 shapes the acute stress response in the paraventricular nucleus of the hypothalamus.", (2020) Mol. Psych.
	Gandaglia A, **Brivio E**, Carli S, Palmieri M, Bedogni F, Stefanelli G, Bergo A, Leva B, Cattaneo C, Pizzamiglio L, Cicerone M, Bianchi V, Kilstrup-Nielsen C, D'Annessa I, Di Marino D, D'Adamo P, Antonucci F, Frasca A, Landsberger N. – "A Novel Mecp2^{Y120D} Knock-in Model Displays Similar Behavioral Traits but Distinct Molecular Features Compared to the Mecp2-Null Mouse Implying Precision Medicine for the Treatment of Rett Syndrome.", (2018) Mol. Neurobiol.
	D'Annessa I, Gandaglia A, **Brivio E**, Stefanelli G, Frasca A, Landsberger N, Di Marino D. – "Tyr120Asp mutation alters domain flexibility and dynamics of MeCP2 DNA binding domain leading to impaired DNA interaction: Atomistic characterization of a Rett syndrome causing mutation", (2018) Biochim. Biophys. Acta – Gen. Subj.
Science Communication Articles	Loganathan S*, Pölchen D*, **Brivio E***, Comes AL, Haas SS, Kalman JL, Krontira AC, Stamp F, Hoch E, Wotjak CT. – "Be Careful What You Feed Your Brain: Cannabis and Mental Health", (2020) – Front. Young Minds

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“Non sono una futurologa. Posso solo vedere quello che capita oggi.
Il passato lo conosco.
Il futuro… speriamo”.

Rita Levi-Montalcini

“I’m not a futurologist. I can only see what happens today.
I know the past.
The future… you have to hold hope for it”.

Rita Levi-Montalcini