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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

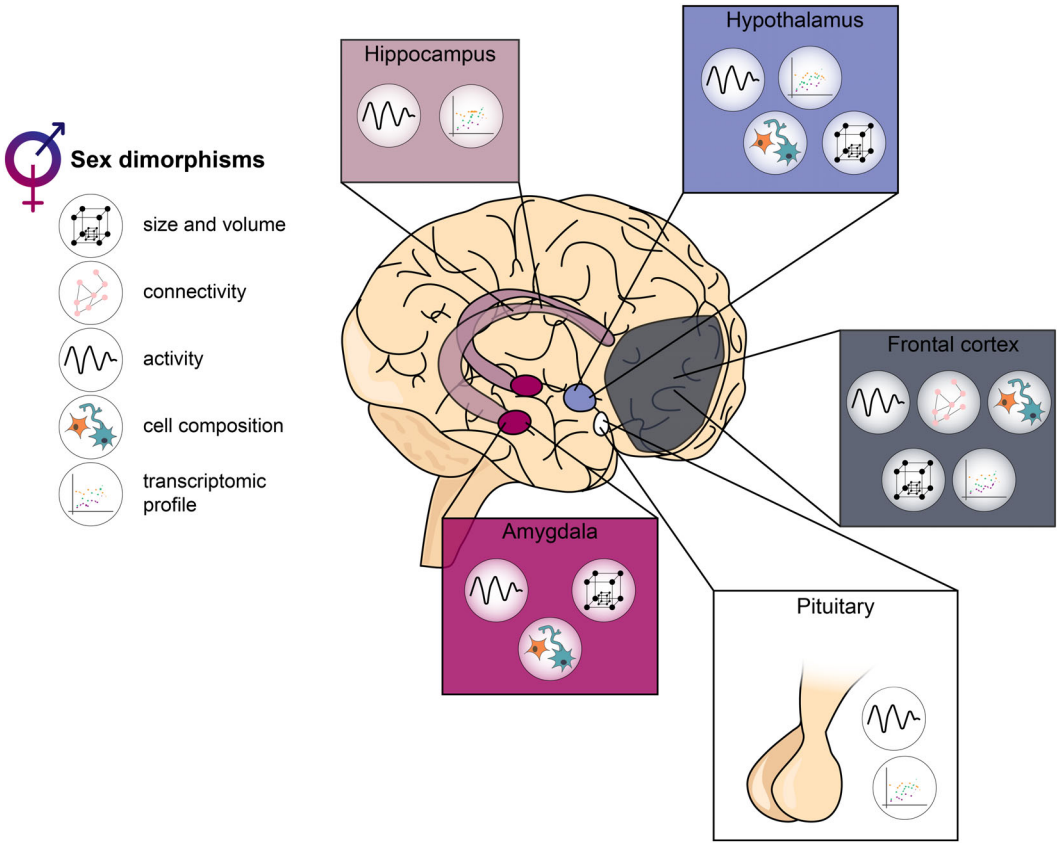

FIGURE 1 Sex dimorphism in the human brain stress system. Schematic representation of the main brain regions of the stress system that have been shown to be sexually dimorphic in adulthood. Dimorphism in size or volume has been found in the amygdala,[18-23,26] frontal cortex[18-23,26] and hypothalamus.[26] 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

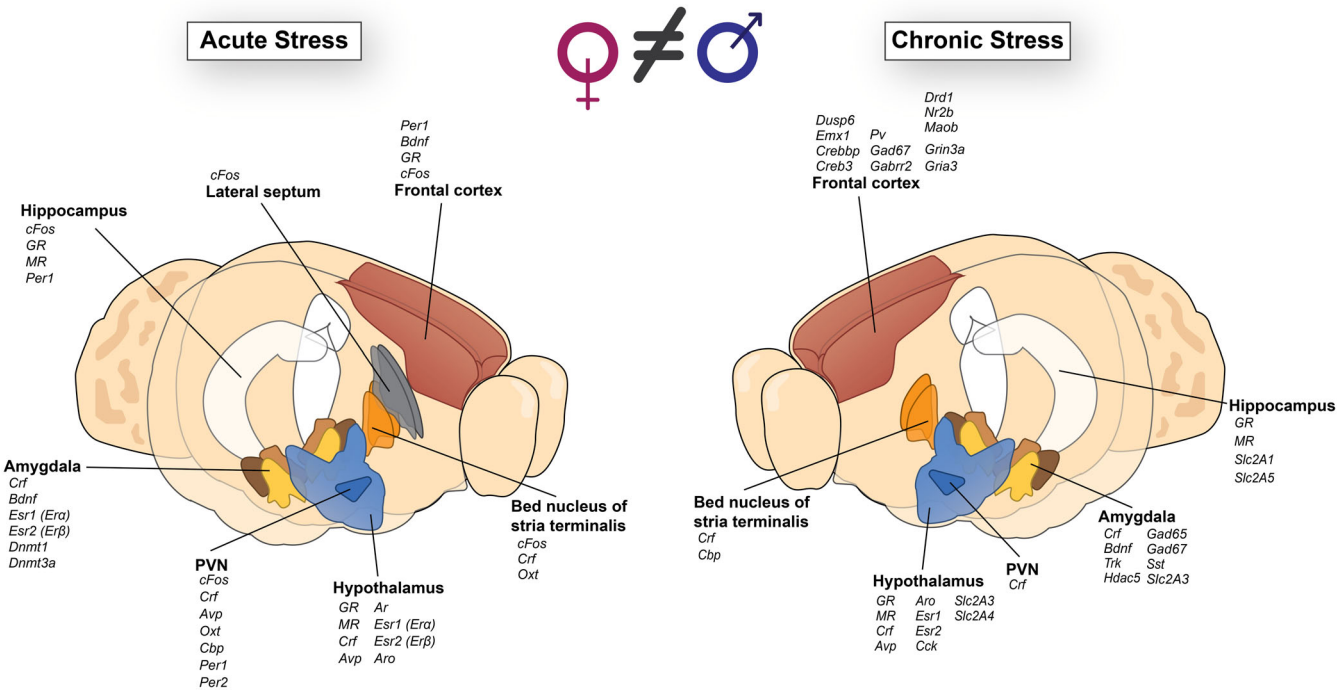

FIGURE 2 Genes differently affected by acute and chronic stress in male and female rodent brain regions. Schematic representation of genes affected by either acute (left panel) or chronic (right panel) stress in the rodent brain in a sex-specific fashion. Several stress-related genes such as *Nr3c1*, *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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