The neuroprotective role of environmental enrichment against behavioral, morphological, neuroendocrine and molecular changes following chronic unpredictable mild stress: A systematic review

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Abstract

Environmental factors interact with biological and genetic factors influencing the development and well-being of an organism. The interest to better understand the role of environment on behavior and physiology led to the development of animal models of environmental manipulations. Environmental Enrichment (EE), an environmental condition that allows cognitive and sensory stimulation as well as social interaction, improves cognitive function, reduces anxiety and depressive-like behavior, and promotes neuroplasticity. In addition, it exerts protection against neurodegenerative disorders, cognitive aging and deficits aggravated by stressful experiences. Given the beneficial effects of EE on brain and behavior, preclinical studies focus on its protective role as an alternative, non-invasive manipulation, to help an organism to cope better with stress. A valid, reliable and effective animal model of chronic stress that enhances anxiety and depression-like behavior is the Chronic Unpredictable Mild Stress (CUMS). The variety of stressors and the unpredictability in the time and sequence of exposure to prevent habituation, render CUMS an ethologically relevant model. CUMS has been associated with dysregulation of the Hypothalamic-Pituitary-Adrenal axis, elevation in the basal levels of stress hormones, reduction in brain volume, dendritic atrophy and alterations in markers of synaptic plasticity. Although numerous studies have underlined the compensatory role of EE against the negative effects of various chronic stress regimens (e.g., restraint, social isolation), research concerning the interaction between EE and CUMS is sparse. The purpose of the current systematic review is to present up-to-date research findings regarding the protective role of EE against the negative effects of CUMS.

Figure 1. PRISMA flowchart of studies initially identified, included and excluded in this review

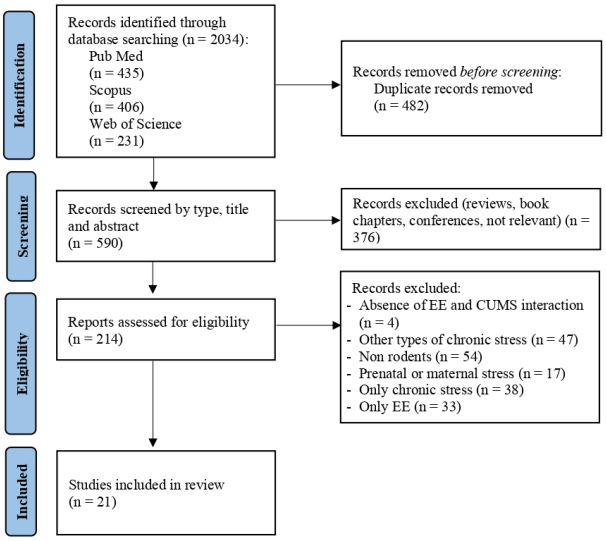

Table 1. CUMS and EE protocols included in the systematic review

	References	Strain/Species	Sex	CUMS		EE		Types of stressors	Behavioral tests
				Duration	*Age/Body weight*	*Duration*	*Age*		
1	*Dandi et al., 2022*	Wistar rats	Male + Female	4 wks	PND 66 - 95	10 wks	PND 23 - end	Overnight illumination (12h), light on/off (12h), tilt cage (15h or 5h), white noise (2h - 4h), food and water deprivation (14h or 15h), strobe light (3h - 7h), restraint (30min - 1h), rotation (30min - 1h), social isolation (15h)	EPM, OFT, FST, Barnes Maze
2	*Xu et al., 2022*	Sprague-Dawley rats	Male	5 wks	180-200gr	3 wks (after CUMS)	-	Food and water deprivation (24h), cage tilt (7h), wet bedding 24h), hot and cold swimming (5min), tail pinch (1min), overnight illumination, restraint (2h)	SPT, FST, OFT, MWM
3	*Costa et al., 2021*	Sprague-Dawley rats	Male	3 wks (3rd - 5th wk of EE)	11wks	7 wks (5days/wk., 2h/day)	8 wks	Immobilization (1h), restricted food access (2h), empty water bottle exposure (2h), overnight illumination, food and water deprivation (17h), wet bedding (17h), reversed dark/light cycle	SPT, FST, EPM, Y-Maze
4	*Gu et al., 2021*	Sprague-Dawley rats	Male	7 wks	180-200gr	final 3wks of CUMS	-	Electric foot shocks (10s), cold water immersion (4min), cage shaking (10min), tail clamp (1min), heat stress (5min), light/dark cycle reversal (24h), food and water deprivation (24h), restraint (2h), wet caging (24h)	SPT, FST, OFT
5	*Muthmainah et al., 2021*	Wistar rats	Male	21 days	6wks	21 days (concurrently with CUMS)	6 wks	Cold swim (4min), cage tilt (4h), cage darkened (3h), food and water deprivation (24h), predator noise (30min), damp sawdust (5h), cage shaking (10min), cage tilt (4h), tail pinch (2min), overnight illumination	EPM
6	*Nwachukwu et al., 2021*	Long-Evans rats	Male	4 wks	PND29	4 wks (concurrently with CUMS) **+icariin**	PND 29	Predator odor (10min), predator sound (10min), predator touch (5min)	FST
7	*Poggini et al., 2021*	C57BL/6 mice	Female	14 days	12-15wks	21 days (after stress) **+fluoxetine or minocycline**	17 wks	IntelliCage stressors	Saccharin preference (liking type anhedonia), progressive ratio reinforcement schedule (wanting type anhedonia)
8	*Ramírez-Rodríguez et al., 2021*	BALB/C mice	Female	6 wks	10wks	2nd week of stress-end (3hrs/day, dark phase) **+fluoxetine**	8 wks	Group housing (8h), food and water deprivation (18h), continuous light (24h), cold room (15min), strobe light (3h), constant motion (30min), white noise (12h), movement restriction (1h), dirty/wet cage (12h)	SPT
9	*Seo et al., 2021*	C57BL/6J mice	Male	4 wks	PND 56-83	5 wks	PND 21-55	Empty cage (24h), restraint (4h), tilt cage (4h), cold swim (5min), tail nip (1min), food and water deprivation (24h), wet cage (24h)	FST
10	*Shen et al., 2019*	Sprague-Dawley rats	Male	5 wks	180-200gr	3 wks (6hrs/day)/ after stress	-	Food and water deprivation (24h), restraint (2h), tail pinch (1min), hot water (5min), overnight illumination, soiled cage (24h), cage tilt (7h)	SPT, FST, OFT, MWM
11	*Seong et al., 2018*	Sprague-Dawley rats	Male	21 days	8wks	20 days **(**after stress)	11 wks	Restraint (6h), food and water deprivation (24h), cold swim (5min), tail pinch (5 min), isolation (24h), switch cage mate (24h), high density housing (24h)	FST, OFT
12	*Shtoots et al., 2018*	Sprague-Dawley rats	Male	3 days (1 stressor per day)	PND 27-29	30 days	PND 30-60	Forced swim (10min), elevated platform (30min x 3trials), restraint stress (2h)	EPM, OFT
13	*Smith et al., 2018*	Sprague-Dawley rats	Male + Female	PND 40-60	PND40-60	PND 33-60 (9:00-21:00)	PND 33-60	Hypoxia (30min), cold room (1h), orbital shaker (1h), restraint (30min), pedestal (5min), wet bedding (1h)	OFT, FST
14	*Alboni et al., 2017*	C57BL/6 mice	Male	14 days	15 wks	21 days (after stress) + **fluoxetine**	17 wks	IntelliCage stressors, social stress	LTA and WTA, cognitive bias activity (automatically administered by IntelliCage
15	*Gurfein et al., 2017*	BALB/C mice	Male	9 wks	6wks	9 wks	6 wks	Water deprivation (8h), food deprivation (overnight), lights on (03:00-08:00), cage vibration (3-6h), cage tilt (6h), strobe light (3h)	———
16	*Liu et al., 2017*	Sprague-Dawley rats	Male	6 wks	adult	3 wks (4th-6th week of stress) (12hrs/day) + **fluoxetine**	adult	Electric foot shocks (10sec), tail clamp (1min), light-dark cycle reversal (24h), heat stress (5min), food and water deprivation (24h), wet cage (24h), restraint (2h), white noise (1h)	Sucrose consumption, OFT
17	*Alboni et al., 2016*	C57BL/6 mice	Male	14 days	12-15wks	21 days (after stress) **+fluoxetine**	17 wks	Social stress and IntelliCage stressors	———
18	*Cordner et al., 2016*	CD-1 mice	Male	14 days	6 and 8 months	15 days (started 1 day before CUMS)	6 and 8 months	Restraint (30min), swim (10min), lights overnight, shaker (30min), white noise (6h), cold (30min), social stress (overnight),	OFT, NORT, Barnes Maze
19	*Vega-Rivera et al., 2016*	BalbC mice	Female	4 wks	7 months	45 days (6,5wks), before stress	6 months	Group housing (8h), food and water deprivation (18h), continuous light (18h), cold room (15min), strobe light (3h), constant motion (30min), white noise (12h), movement restriction (1h), dirty/wet cage (12h)	SPT, Porsolt's FST
20	*Zeeni et al., 2015*	Sprague-Dawley rats	Male	3 wks	8-10wks	5 wks (2wks before stress-end)	8-10 wks	Cage tilt (3h-4h), space reduction (4h-5h), restraint (1h-3h), cold swim (10min), warm water (10min), flashing light (3h-4h), neighbor cage (14h)	———
21	*Branchi et al., 2013*	C57BL/6 mice	Male	24 days	12 – 15wks	21 days + **fluoxetine** (after stress)	15-18 wks	Restraint stress (60min), social stress, FST (10min)	Saccharin preference
22	*Ilin & Richter-Levin, 2009*	Sprague Dawley	Male	3 days (1 stressor/day)	PND 27-29	30 days	PND 30-end	Forced swim (10min), elevated platform (30min x 3 sessions), restraint stress (2h)	OFT, EPM, Novel-setting exploration, TWS

Abbreviations: EPM, Elevated Plus Maze; FST, Forced Swimming Test; LTA, Liking Type Anhedonia; MWM, Morris Water Maze; NORT, Novel Object Recognition Test; OFT, Open Field Test; SPT, Sucrose Preference Test; TWS, Two-way shuttle avoidance task; WTA, Wanting Type Anhedonia.

Table 2. Behavioral findings in studies exploring the EE and CUMS interaction

Reference	Behavioral Tests	Outcomes of CUMS	Outcomes of CUMS + EE
Dandi et al., 2022	EPM, OFT, FST, Barnes Maze	**EPM**:↑ anxiety, **OFT**:↑ anxiety (males), **FST**:↑ immobility (females), **Barnes Maze**:↓ learning (males)	**EPM**:↓ anxiety, **OFT**↓ anxiety (males), **FST**: ↓ immobility (females), **Barnes Maze**:↑ learning (males)
Xu et al., 2022	SPT, FST, OFT, MWM	**SPT**:↓, **FST**:↑ immobility, **OFT**:↓ locomotor activity, rearing, grooming, **MWM**:↓ learning and memory	**SPT**:↑, **FST**:↓ immobility, **OFT**:↑ locomotor activity, rearing, grooming, **MWM**:↑ learning and memory
Costa et al., 2021	SPT, FST, EPM, Y-maze	**SPT**:↓, **FST**:↑ immobility,↓swimming, **EPM**:↑ locomotor activity, **Y-maze**:↓ memory	**FST**:↓ immobility,↑ swimming, **EPM**: ↑locomotor activity, **Y-maze**:↑ memory
Gu et al., 2021	SPT, FST, OFT	**SPT**:↓, **OFT**:↓ horizontal movement, **FST**:↑ immobility	**SPT**:↑, **OFT**:↑ horizontal movement, **FST**:↓ immobility
Muthmainah et al., 2021	EPM	↑ anxiety	↓ anxiety
Nwachukwu et al., 2021	FST	↓ dives and head shakes	↑ dives and head shakes
Poggini et al., 2021	Saccharin preference (LTA), progressive ratio reinforcement schedule (WTA)	**LTA**:↓ saccharine preference	**LTA**:↑ saccharine preference
Ramirez-Rodriguez et al., 2021	SPT	**SPT**:↓ sucrose preference	**SPT**:↑ sucrose preference
Seo et al., 2021	FST	**FST**:↑ immobility	**FST**:↓ immobility
Shen et al., 2019	SPT, FST, OFT, MWM	**SPT**:↓sucrose preference, **FST**:↑ immobility, **OFT**:↓ locomotor activity, rearing and grooming, **MWM**:↓ learning,↓ memory	**SPT**:↑sucrose preference, **FST**:↓immobility **OFT**:↑ locomotor activity, rearing and grooming, **MWM**:↑ learning,↑ memory
Seong et al., 2018	FST, OFT	**FST**:↑ immobility, **OFT**:↓ locomotor activity	**FST**:↓ immobility, **OFT**:↑ locomotor activity
Shtoots et al., 2018	EPM, OFT	**EPM**:↑anxiety, **OFT**:↑ anxiety	**FST**:↓ anxiety, **OFT**:↓anxiety
Smith et al., 2018	OFT, FST	**OFT**:↑ anxiety (females),↓ locomotor activity (males) **FST**:↑ immobility (females),↓ climbing (females)	-
Alboni et al., 2017	LTA and WTA, cognitive bias activity (CBA)	**LTA**:↑ anhedonia (FLX in stress condition), **WTA**:↑ anhedonia (FLX in stress condition)	**WTA**:↓anhedonia (FLX in EE condition), **CBA**:↑(FLX in EE condition)
Liu et al., 2017	Sucrose consumption, OFT	**Sucrose consumption**:↓, **OFT**:↓ horizontal movement	**Sucrose consumption**:↑, **OFT**:↑ horizontal movement
Cordner et al., 2016	OFT, NOR, Barnes Maze	**NOR**:↓(aged mice), **Barnes Maze**:↓ learning	**NOR**:↑, **Barnes Maze**:↑
Vega-Rivera et al., 2016	SPT, Porsolt test	**SPT**:↓sucrose preference, **Porsolt test**:↑immobility	**SPT**:↑ sucrose preference, **Porsolt test**:↓ immobility
Branchi et al., 2013	Saccharin Preference	↓	↑ +FLX in EE after stress,↓ +FLX in stress after EE
Ilin & Richter-Levin, 2009	OFT, EPM, Novel-setting exploration, TWS	**OFT**:↑anxiety, **EPM**:↑ anxiety,↓ locomotor activity, **Novel-setting exploration**:↓, **TWS**:↑ no escape responses	**OFT**:↓ anxiety, **EPM**:↓ anxiety,↑ locomotor activity, **Novel-setting exploration**:↑, **TWS**:↑ avoidance responses,↓ escape responses

Abbreviations: EPM, Elevated Plus Maze; FST, Forced Swimming Test; LTA, Liking Type Anhedonia; MWM, Morris Water Maze; NORT, Novel Object Recognition Test; OFT, Open Field Test; SPT, Sucrose Preference Test; TWS, Two-way shuttle avoidance task; WTA, Wanting Type Anhedonia.

The neuroprotective role of environmental enrichment against behavioral, morphological, neuroendocrine and molecular changes following chronic unpredictable mild stress: A systematic review

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Abstract

Environmental factors interact with biological and genetic factors influencing the development and well-being of an organism. The interest to better understand the role of environment on behavior and physiology led to the development of animal models of environmental manipulations. Environmental Enrichment (EE), an environmental condition that allows cognitive and sensory stimulation as well as social interaction, improves cognitive function, reduces anxiety and depressive-like behavior, and promotes neuroplasticity. In addition, it exerts protection against neurodegenerative disorders, cognitive aging and deficits aggravated by stressful experiences. Given the beneficial effects of EE on brain and behavior, preclinical studies focus on its protective role as an alternative, non-invasive manipulation, to help an organism to cope better with stress. A valid, reliable and effective animal model of chronic stress that enhances anxiety and depression-like behavior is the Chronic Unpredictable Mild Stress (CUMS). The variety of stressors and the unpredictability in the time and sequence of exposure to prevent habituation, render CUMS an ethologically relevant model. CUMS has been associated with dysregulation of the Hypothalamic-Pituitary-Adrenal axis, elevation in the basal levels of stress hormones, reduction in brain volume, dendritic atrophy and alterations in markers of synaptic plasticity. Although numerous studies have underlined the compensatory role of EE against the negative effects of various chronic stress regimens (e.g., restraint, social isolation), research concerning the interaction between EE and CUMS is sparse. The purpose of the current systematic review is to present up-to-date research findings regarding the protective role of EE against the negative effects of CUMS.

Keywords: Psychological stress, Enriched environment, Brain, Behavior, Stress hormones

Abbreviations: CMS; Chronic Mild Stress; CUMS: Chronic mild unpredictable stress; EE: Environmental Enrichment; FST: Forced Swimming Test; GCs: Glucocorticoids; HPA: Hypothalamic-Pituitary-Adrenal; OFT: Open Field Test.

1. Introduction

It is well known that environmental factors interact with biological and genetic factors to influence the development and health of an organism (Donkin & Barrès, 2018; Estrela et al., 2019). Stressful life events (e.g., abuse, significant loss, neglect), living in poverty, and absence of adequate cognitive, emotional and social stimuli disrupt normal development and are considered predictive risk factors associated with various health problems, including psychopathologies (e.g., anxiety, depression, PTSD), pathologies (e.g., cardiovascular or autoimmune diseases), as well as cognitive deficits (Juster et al., 2010; McEwen, 2017; Rokita et al., 2021; Singh et al., 2019). In contrast, living and growing in a socio-economic environment that provide higher educational opportunities, advanced health care system and chances of social interaction, increases life expectancy and reduces the risk of mental disorders, as well as the development of neurodegenerative diseases (Kotloski & Sutula, 2015).

Preclinical studies use environmental manipulation protocols to further explore the detrimental impact of stressful experiences on brain and behavior, as well as to test new therapeutic approaches. The Chronic Unpredictable Mild Stress (CUMS) protocol is an ethologically relevant and widely used model to cause anxiety and depressive-like symptoms, applied to study the effects of psychological stress (Antoniuk et al., 2019; D'Aquila et al., 1994; Hill et al., 2012; Willner, 2017; Zhu et al., 2019). On the contrary, Environmental Enrichment (EE), a well-established positive environmental manipulation paradigm, has proven to exert beneficial effects on brain and behavior. Thus, there has been a great interest in exploring its protective role as an alternative, non-invasive manipulation, to help organisms to cope better with stress and to restore impairments caused by previous exposure to stressful events (Smail et al., 2020).

Given that EE is the most used non-invasive environmental manipulation treatment and CUMS is considered a reliable and effective model to mimic humans' anxiety and depressive-like symptoms, it is surprising that only a limited number of studies have applied EE regimen in conjunction with CUMS. A plausible explanation may be the complexity and the demanding nature of CUMS in combination with EE manipulation. Indeed, the administration of two different stressors per day, requires not only a detailed and punctual experimental design, but also qualified research personnel and the use of appropriate lab equipment. In addition, the different CUMS

protocols may vary in duration and type of stressors being administered. Therefore, scientists prefer less demanding chronic stress protocols, usually consisting of one type of stressor (i.e., chronic restraint stress, chronic social isolation) and thus, data regarding the EE impact on the effects of CUMS is limited and sparse. The purpose of the present review is to summarize research conducted up to date, addressing the protective role of EE against the negative effects of CUMS using animal models.

1.1. Stress response

The term "stress" was first used by engineers in 17th century to describe materials' resistance (Cooper & Dewe, 2004) to later find application in the field of Physics, Biology and Psychology. Charles Darwin (19th century) underlined the importance of adaptation to environmental changes in order for an organism to survive (Rom & Reznick, 2015). As defined by Claude Bernard, the term adaptation refers to the ability of an organism to regulate and maintain stable the inner environment, regardless of the external environmental changes (Noble, 2008). Physiologist Walter Cannon was the first to give a psychological perspective to the term, by exploring the biological mechanisms and hormones related to stress (Cannon, 1914). He also expanded Bernard's theory, introducing the term homeostasis and "fight or flight response", a physiological reaction of an organism in response to a threat (Cooper, 2008). It was Hans Seyle (1907 – 1982), the father of modern stress research, who linked hypothalamic-pituitary-adrenal (HPA) axis with body coping stress mechanisms in response to acute and chronic stress (Tan & Yip, 2018). Since then, stress has been the subject of numerous clinical and pre-clinical studies, aiming to shed light on the physiological mechanisms that mediate stress response, as well as its impact on nervous system and behavior.

In everyday life, humans face various stressful situations threatening homeostasis. Physiological and behavioral responses to stressful conditions help to maintain homeostasis (Goldstein, 2019). Secretion of the catecholamines epinephrine and norepinephrine from adrenal medulla, and glucocorticoids from the adrenal cortex are both involved in stress response. Epinephrine acts immediately to prepare the body for the "fight and flight response", by increasing heart and respiratory rate and blood pressure, while glucocorticoids (GCs) are released in response to HPA axis activation (Nicolaides et al., 2014). In short, upon stress, neurons of the medial parvocellular paraventricular nucleus (PVN) in the hypothalamus secrete

corticoptropin-releasing hormone (CRH), which is transferred to the anterior pituitary gland via the hypophyseal portal system, stimulating the production and release of adrenocorticotropic hormone (ACTH). ACTH, in turn, enters the bloodstream causing the release of the primary glucocorticoid cortisol (in primates) or corticosterone (in rodents, reptiles, birds and other species) from the cortex (outer part) of the adrenal gland. Increased blood circulating cortisol levels exert negative feedback at several levels, including hippocampus, hypothalamus and pituitary gland, by acting on glucocorticoid receptors. GCs-mediated *negative feedback* is necessary for the termination of the HPA axis response to stress and restoration of GCs to basal levels (Herman, 2022).

Initial exposure to stress results in a range of physiological and behavioral adaptive responses such as increased blood pressure, heart respiratory rate, gluconeogenesis and lipolysis, as well as enhanced arousal and cognition (Charmandari et al., 2005). Although physiological alterations may be adaptive in the short-term, prolonged exposure to stressful events leads to HPA axis dysregulation, as indicated by the reduced efficacy of HPA axis negative feedback and the resulting long-term exposure to GCs (Vitousek et al., 2019). In addition, chronic exposure to stress has been linked to cognitive deficits, psychopathology (e.g., anxiety, depression, PTSD) (Deppermann et al., 2014; Heim & Binder, 2012; Marin et al., 2011; Myers et al., 2014), as well as pathology including cardiovascular diseases, immune system dysregulation (Gao et al., 2018; Saeedi & Rashidy-Pour, 2021), even to cancer development (Cui et al., 2021; Muthusami et al., 2020). The interest to unveil the underlying mechanisms behind human psychopathology related to chronic stress and the need to apply new therapeutic approaches, as well as the limitations in human studies, led to the development of animal models of chronic stress.

1.2. Chronic Stress protocol

In animal research, it was Katz and colleagues in the early 1980s who first introduced a chronic stress protocol as an experimental model of depression. They applied a variety of severe stressors (i.e., foot shock, cold water immersion and 48h food and water deprivation) on animals and observed a reduction in sucrose consumption and in open field activity, reversed by antidepressants (Katz, 1982; Katz et al., 1981). Later, Paul Willner developed a new chronic stress model, the Chronic Mild Stress (CMS) protocol (Willner et al., 1987). The severe stressors, initially

proposed by Katz and colleagues, were replaced for ethical reasons by milder ones, such as reversal of the light-dark cycle, social isolation, white noise, restraint stress and tilted cages. The unpredictability in the time and sequence of exposure to them to prevent habituation renders this paradigm an ethologically relevant model to study the effects of psychological stress (Antoniuk et al., 2019; D'Aquila et al., 1994; Hill et al., 2012; Willner, 2017; Zhu et al., 2019). Since its introduction, CMS has been widely used to study the impact of stress on health, behavior and emotion. It is important to note that CMS, in different variations, is also mentioned as Chronic Unpredictable Stress (CUS), Chronic Varied or Variable Stress (CVS) or Chronic Unpredictable Mild Stress (CUMS). In the present manuscript, research findings to be reviewed originate from studies that employed chronic stress protocols including a variety of different mild stressors, characterized by unpredictability in the time and sequence of exposure to them, to which we will refer as CUMS.

The CUMS is a valid, reliable and effective animal model of stress to cause anxiety and depression-like behavior (Willner, 2017). Existing evidence supports that CUMS causes dysregulation of the HPA axis and subsequent elevation in the basal levels of stress hormones (Algamal et al., 2021; Raghav et al., 2019; Ventura-Silva et al., 2020). In addition, reduction in brain volume, dendritic atrophy and alterations in markers of synaptic plasticity and exacerbated methamphetamine-induced neurotoxicity have been recorded as a result of CUMS exposure (Aydin et al., 2021; Jia et al., 2021; Li et al., 2021; Picard et al., 2021; Tata & Yamamoto, 2008). These negative outcomes are also reflected on emotional behavior and cognitive function, since chronically stressed rats show increased depressive and anxiety related behavior, along with cognitive impairments (Mohamed et al., 2020; Shen et al., 2018).

Beside the need to better understand the negative outcome of chronic stress, scientists have been also interested in the development of therapeutic approaches, against the deleterious effects of stress in animal models. Pharmacotherapy with antidepressants for example, has proven to ameliorate depressive and anxiety-related symptoms (Rafało-Ulińska & Pałucha-Poniewiera, 2022). Manipulation of environmental housing conditions is considered a non-invasive approach used in animal research to study non-pharmacological interventions in a variety of disorders, such as depression, generalized anxiety disorder and post-traumatic stress disorder (Arabin et al., 2021; Odeon & Acosta, 2019).

1.3. Environmental Enrichment

A well-established positive environmental manipulation is the EE protocol. In an effort to understand the interaction between heredity and environment on development, Donald O. Hebb (mid-1940s) explored the role of EE on behavior in a series of experiments including rearing rats at home. Interestingly, he reported that rats reared as pets, thus having access to an environment rich in sensory and social stimuli, presented improved learning and problem-solving ability in adulthood (Hebb, 1947 as cited in Brown, 2006). Subsequent studies were conducted to test in a systematic way this paradigm. To this end, laboratory large cages equipped with toys, platforms, ladders and running wheels, and in which more than two of the same-sex animals are housed, are used to form complex environments which promote social interaction, exploration and motor activity (Simpson & Kelly, 2011).

Existing studies support that EE improves cognitive function, reduces anxiety and depressive-like behavior in corresponding behavioral tests (Simpson & Kelly, 2011; Zheng et al., 2020), increases the expression of neurotrophins and cortical weight and promotes neurogenesis and dendritic growth (Gualtieri et al., 2017; Rostami et al., 2021; van Praag et al., 2000). Additionally, EE has proven to be an effective treatment to a variety of pathologies and brain-related injuries occurring during lifespan or due to prenatal exposure to various harmful conditions (Dandi et al., 2018; Dorantes-Barrios et al., 2021; Joushi et al., 2021; McCreary & Metz, 2016; Yuan et al., 2021). Moreover, it exerts protection against neurodegenerative disorders, cognitive aging and other deficits aggravated by stressful experiences (Hutchinson et al., 2012; Wright & Conrad, 2008).

Up to date, the compensatory role of EE against the negative effects of various chronic stress regimens, such as restraint or social isolation stress, has been well documented (Bahi & Dreyer, 2020; Bhagya et al., 2017; Cordner et al., 2021; Hutchinson et al., 2012; Mesa-Gresa et al., 2016; Shilpa et al., 2017; Thamizhoviya & Vanisree, 2019; Veena et al., 2009; Wright & Conrad, 2008). In contrast, research concerning the interaction between EE and CUMS protocol is limited. Thus, the main goal of this review is to summarize the available data on EE exposure in CUMS rats.

2. Methods

2.1. Search strategy

The present review was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021) using the electronic databases PubMed, Scopus and Web of Science. The terms used in this literature search were the following: *environmental enrichment OR enriched environment AND chronic stress OR chronic unpredictable stress OR chronic variable stress OR chronic mild stress OR chronic unpredictable mild stress OR juvenile stress*. All studies published up to October 2022 were considered.

2.2. Eligibility

The following criteria should have been met for an article to be *included* in the present review: (a) experimental studies published in peer-reviewed journals; (b) conducted only in rodents; (c) articles studying interaction between EE and CUMS; (e) articles written in English language. *Exclusion* criteria included: (a) other types of chronic stress (i.e., restraint, social isolation); (b) prenatal chronic stress; (c) no investigation of interaction effect between EE and CUMS; (d) use of non-rodents; (e) gray literature (e.g., theses); (f) reviews, meta-analyses, book chapters, and conference abstracts. The eligibility of each retrieved record was verified by two reviewers (E.D, D.A.T).

3. Results

The literature search detected a total number of 1.212 records. Following removal of duplicates (n = 485), 727 articles were subjected to title and abstract screening, and 479 of them were excluded (reviews, book chapters, not relevant) leaving 248 articles for full text review. Following eligibility criteria, 22 studies were identified as eligible for inclusion in this review (see Table 1 for CUMS and EE protocols included). The PRISMA flow diagram details all information about identification, screening as well as eligibility of articles (see Figure 1).

3.1. Effects of EE and CUMS on emotional behavior and cognitive function

CUMS protocols vary in respect to stressors administered, animals' strain and sex, as well as duration and age at the time of exposure. Another important difference among laboratories is whether CUMS precedes, follows or coexists with EE manipulation. In adult male rats, long-term (5, 6 or 7 weeks) CUMS exposure resulted in cognitive impairment, as well as depressive-like behavior, such as decreased sucrose consumption, increased immobility in the Forced Swim Test (FST) and

reduced locomotor activity in the Open Field Test (OFT). EE housing for 3 weeks (6hrs or 12hrs/day) starting either during the last days of CUMS (Gu et al., 2021; Liu et al., 2017) or following stress termination (Shen et al., 2019; Xu et al., 2022) restored cognitive deficits and counterbalanced the emotion-related behaviors. Housing adult male rodents in EE for 7 weeks (5 days/week, 2hrs/day), before, during and after exposure to stress, prevented depressive-like behaviors and memory impairments caused by CUMS (Costa et al., 2021). Cordner and Tamashiro reported differences between young and older male mice exposed to stress. Specifically, while older male mice exposed to CUMS presented cognitive impairments in both Barnes Maze and Novel Recognition Task, younger adult mice exhibited moderate impairments only in Barnes Maze, while concurrent EE housing prevented the negative effect of CUMS in both groups (Cordner & Tamashiro, 2016). Although most studies support the beneficial effects of EE against the negative outcome of CUMS on emotional behavior and cognitive function, Gurfein and colleagues found no main effect or interaction of EE and CUMS on anxiety behavior as estimated by the Elevated plus maze. This contradictory result may be partially attributed to strain differences, as BALB/C mice demonstrate less exploratory behavior compared to other strains (Gurfein et al., 2017).

It is important to note that CUMS and EE affect adult and adolescent animals to a different extent. Adolescence is a critical developmental period, sensitive to environmental manipulations, and is characterized by increased neural plasticity (Eiland & Romeo, 2013). Brain areas involved in stress response, such as prefrontal cortex and limbic structures, undergo significant maturation processes, thus juvenile exposure to stress has more detrimental and long-lasting effects than adult exposure (Chaby et al., 2015; Drzewiecki & Juraska, 2020; Hollis et al., 2013). Short-term variable stress in juvenile male rats caused cognitive deficits, as well as anxiety and depressive-like behavior, but subsequent exposure to EE housing, until adulthood decreased anxiety (Shtoots et al., 2018), increased motivation and improved learning abilities for stressed rats even to a greater extent compared to non-stressed rats (Ilin & Richter-Levin, 2009). In addition, adolescent EE prevented depressive-like behavior induced by subsequent CUMS in adult male mice (Seo et al., 2021). Similarly, Smith and colleagues found that exposure of adolescent male and female rats (PND33-60) to CUMS (4 weeks) resulted in passive coping behavior in FST and decreased exploration in OFT in adulthood, in females only, while housing in EE, initiated in

adolescence and prior to CUMS, attenuated passive coping behavior, but did not affect exploration (Smith et al., 2018). Likewise, our group recently reported that adult CUS induced depression-like behavior, as indicated by increased immobility time in FST, only in females, an effect that was prevented by EE housing (Dandi et al., 2022). Concerning the sex-related effect of CUMS, it has been previously reported that females are especially susceptible to the long-term negative effects of CUMS on emotional behavior. More specifically, while adolescent females tested in FST, immediately after CUMS, exhibited no differences compared to non-stressed ones, they displayed an increase in immobility time as adults (Wulsin et al., 2016).

Sex-related differences in psychiatric disorders have been well documented with women being more vulnerable and characterized by higher prevalence of stress-related mental disorders than men (Dalla et al., 2010; Pawluski et al., 2020). However, only few pre-clinical studies have included both sexes for direct comparison of males and females. According to existing evidence, females tend to be more susceptible to emotion-related behavioral effects of chronic stress than males, while they are more resilient to stress-associated cognitive impairments (Bowman, 2005; Dalla et al., 2005; Luine et al., 2017; McFadden et al., 2011; Peay et al., 2020; Vieira et al., 2018). In a recent study investigating the interaction between EE initiated in adolescence and adult CUMS, stress-related spatial learning impairments were limited to male rats. Interestingly, living in an enriched environment protected against these deficits (Dandi et al., 2022).

The impact of EE seems to depend on factors such as the duration of EE exposure and the initiation time in relation to the stress protocol (i.e., prior, following or concurrently with CUMS). More specifically, in certain protocols, EE is terminated prior to stress experimental manipulations, while in others it is extended during a stress protocol or follows it. According to an interaction model presented by Macartney and colleagues, EE exerts the most beneficial effects when administered post stress (Macartney et al., 2022). It is worth mentioning that termination of EE has been reported to induce depressive-like behaviors and HPA axis dysregulation in adult male rats (Smith et al., 2017), while it had no effect on female adolescent or adult rats (Smith et al., 2018; Vega-Rivera et al., 2016). Similarly, EE housing had long-lasting protective antidepressant effect on adult female mice against subsequent exposure to a 4-week CUMS protocol, even after its cessation (Vega-Rivera et al., 2016).

Environmental factors seem to influence the effectiveness of certain pharmacological agents or natural compounds. Our literature search retrieved seven (7) studies exploring the drug-by-environment interaction, employing CUMS and EE alongside with antidepressant treatments. All studies have concluded that environment plays an important role in the efficacy of antidepressant drugs. Specifically, adult male and female animals that underwent CUMS and then treated with fluoxetine while living in EE, presented reduced depression-like symptomatology. On the contrary, depression-like behavior was worsened in animals treated with fluoxetine while being exposed to CUMS instead of EE (Alboni et al., 2017; Branchi et al., 2013; Liu et al., 2017; Poggini et al., 2021). Similarly, the antibiotic minocycline, a drug with neuroprotective and anti-inflammatory properties, had the same antidepressant effects in previously stressed adult female mice either being administered under EE or CUMS conditions (Poggini et al., 2021). Even a shorter duration exposure to EE enhanced fluoxetine action against depressive-like phenotype caused by previous CUMS exposure in adult female mice (Ramírez-Rodríguez et al., 2021). Two additional studies compared separately the effect of EE and fluoxetine in CUMS. More specifically, Seong and colleagues reported decreased helplessness in FST and anxiety behavior in OFT in adult CUMS rats that were exposed to both EE and fluoxetine administration (Seong et al., 2018). Interestingly, 3 weeks of EE housing of male rats, concurrently with CUMS, elicited greater anxiolytic effect when tested in Elevated plus maze than fluoxetine administration (Muthmainah et al., 2021). In addition to pharmacological agents, administration of natural compounds (i.e., icariin) in adolescent CUMS male rats exerted the strongest positive effects on emotional resilience when combined with EE housing (Nwachukwu et al., 2021) (see Table 2 for behavioral results).

3.2. Effects of EE and CUMS on brain and neuroendocrine function alterations

Based on existing evidence, EE housing attenuates cognitive deficits, as well as depressive and anxiety-related behaviors due to stress exposure, by enhancing brain plasticity, promoting neurogenesis and dendritic growth, increasing the expression of neurotrophins and restoring HPA axis dysregulation (Bhagya et al., 2017; Thamizhoviya & Vanisree, 2019; Wu & Mitra, 2021). Concerning the interaction of EE and CUMS, it has been found that 5-week exposure to EE (2 weeks prior and 3 weeks during CUMS) attenuated the increase in serum corticosterone and ACTH

levels in adult male rats (Zeeni et al., 2015). Additionally, Costa and colleagues have recently reported that EE housing prior, during and after CUMS reduced epinephrine levels in both stressed and non-stressed male animals and attenuated the secretion of corticosterone and norepinephrine induced by CUMS (Costa et al., 2021). Furthermore, EE attenuated stress-associated increases in hypothalamic angiotensin II (peptide hormone that regulates neurophysiology of certain brain regions) (Costa et al., 2021). Interestingly, Muthmainah and colleagues found no difference in plasma corticosterone levels in male rats exposed concurrently to CUMS and EE for 3 weeks, despite increased anxiety in stressed animals (Muthmainah et al., 2021). However, EE alone or in combination with the natural compound icariin resulted in lower corticosterone levels in CUMS adolescent animals compared to standard-housed CUMS animals (Nwachukwu et al., 2021).

Exposure to CUMS during adulthood decreased the expression levels of synaptic plasticity-associated proteins in certain hippocampal regions and dentate gyrus (DG) in male rats, but subsequent EE attenuated this effect (Liu et al., 2017; Shen et al., 2019). Other studies have shown reduced LTP in males (Alboni et al., 2017), but not in females (Poggini et al., 2021) after CUMS. In adult female rats, EE has proven to exert long-term protective effects even after 4 weeks of cessation, against reduced hippocampal neurogenesis caused by CUMS. Specifically, the EE-associated increase in newborn cells (BrdU), mature neuronal phenotypes, as well as doublecortin-positive cells, was not affected by subsequent exposure to CUMS (Vega-Rivera et al., 2016). Daily EE of shorter duration administered during the last 4 weeks of a 6-week CUMS period did not reverse the reduction in hippocampal neurogenesis caused by CUMS in adult female mice (Ramírez-Rodríguez et al., 2021), while restored markers of synaptic plasticity, such as reduced synaptophysin hippocampal levels in adult males (Liu et al., 2017). It is worth mentioning that in the aforementioned studies, EE alone or in combination with fluoxetine decreased depressive symptomatology induced by CUMS, indicating that different neuroplastic mechanisms may mediate the beneficial effects of EE when combined with fluoxetine (Ramírez-Rodríguez et al., 2021). In addition, administration of fluoxetine under EE condition reduced the CUMS-associated elevations of corticosterone in adult males (Alboni et al., 2017; Branchi et al., 2013). Interestingly, while exposure to EE alone or in combination with fluoxetine subsequent to CUMS increased the levels of hippocampal neurotrophic factors (i.e., BDNF and VEGF) (Branchi et al., 2013;

Seong et al., 2018), it had no effect on neurogenesis. However, fluoxetine decreased the number of proliferating cells and caused reduction of CA1 volume when administered in adult male mice in a stressful condition (Alboni et al., 2017).

Chronic unpredictable stress can also cause the release of pro-inflammatory cytokines, thus there has been an interest in investigating the role of inflammation on behavior, specifically in CUMS-induced depression. In a recent study, Gu and collaborators found that EE housing during the last 3 weeks of a total 7-week CUMS protocol blocked the pro-inflammatory activation of microglia by inhibiting the pro-inflammatory genes and promoting the anti-inflammatory genes (Gu et al., 2021). It has been recently found that 3 weeks of EE following CUMS (5 weeks) also produced anti-inflammatory and protective effects through the induction of autophagy in the hippocampus (Xu et al., 2022). Interestingly, EE housing did not restore the alterations in blood concentration of monocytes and peritoneal macrophage caused by juvenile variable stress, but increased IL-10 activation ratio in stressed animals, indicating an indirect modulatory action of EE against the negative effects of juvenile stress (Shtoots et al., 2018). Existing evidence suggests that Selective Serotonin Reuptake Inhibitors (SSRIs), such as fluoxetine, regulate emotion through changes in immune function, mainly exerting its effects through anti-inflammatory action (Caiaffo et al., 2016). However, there are studies suggesting a pro-inflammatory action of antidepressants. Alboni and colleagues reported that the effect of fluoxetine treatment on inflammatory markers depends on the environment. More specifically, while fluoxetine increases inflammatory markers in male mice when administered in environmentally enriched conditions, it decreases their expression in stressful environments (Alboni et al., 2016). In another study, fluoxetine had no effect on levels of inflammatory markers in adult female mice previously exposed to CUMS (Poggini et al., 2021).

Studies investigating the role of EE in adolescence also suggest its restorative action against the detrimental effects of subsequent exposure to adolescent or adult CUMS. Specifically, EE in adolescence decreased basal levels of circulating corticosterone in adult male rats that had been previously exposed to juvenile CUMS and caused greater increases in the expression of the L1 Cell Adhesion Molecule, an important molecule for neuroplasticity and memory formation, in dorsal cornu ammonis area 1 (dCA1), compared to those observed in stressed or non-stressed standard-housed rats (Ilin & Richter-Levin, 2009). Seo and colleagues reported that

epigenetic modification due to adolescent EE can help the organism to cope better with adulthood stress and prevent CUMS-induced increases in corticosterone levels (Seo et al., 2021). Living in EE during early or late adulthood protected male mice against CUMS-associated impaired cognition, reduced Bace1 expression as well as promoter methylation (Cordner & Tamashiro, 2016). CUMS in late adolescence also decreased adrenal responsiveness to ACTH following subsequent acute stress, while EE increased basal corticosterone concentration in female but not in male rats. In addition, EE concurrently with CUMS condition reversed the decreases in peak adrenocortical responsiveness caused by adolescent CUMS in adult females (Smith et al., 2018). Interestingly, stress-induced corticosterone elevations in response to an acute stressor have been detected only in male rats previously exposed to CUMS and EE-initiated in adolescence-increased corticosterone levels in non-stressed males and CUMS females (Dandi et al., 2022) (see Table 3).

4. Conclusions – Implications for future studies

The present review was an attempt to summarize available research evidence regarding the EE as an intervention manipulation against the negative outcome of CUMS. Interestingly, while there are many studies investigating the effects of CUMS and EE separately, the vast majority of research studying the interaction between chronic stress and EE has employed other chronic stress regimens, with chronic restraint stress being the most widely used. The difficulty to administer concurrently two different and rather complex protocols, as well as to manage all the variables involved, have resulted in a limited number of studies applying the EE regimen under a CUMS condition. Specifically, our literature search retrieved 21 articles investigating the protective role of EE against related behavioral, morphological and molecular changes caused by CUMS exposure.

Most of these studies agree that EE housing initiated before, during or post CUMS restores cognitive deficits and counterbalances depressive and anxiety-related behavior caused by stress exposure (Cordner & Tamashiro, 2016; Dandi et al., 2022; Gu et al., 2021; Ilin & Richter-Levin, 2009; Liu et al., 2017; Seo et al., 2021; Shen et al., 2019; Shtoots et al., 2018; Xu et al., 2022). Additionally, it attenuates CUMS-associated increases in basal levels of stress hormones and compensates the reduced expression of synaptic plasticity markers, as well as the decreased neurogenesis and dendritic atrophy (Branchi et al., 2013; Dandi et al., 2022; Liu et al., 2017; Seong et

al., 2018; Smith et al., 2018). In addition, there are studies investigating the role of inflammatory factors in the brain of stressed animals, since inflammatory changes have been linked to depression (Beurel et al., 2020). Concerning the role of EE on the release of pro-inflammatory cytokines caused by CUMS, it has been found that EE blocks the pro-inflammatory activation of microglia and produces anti-inflammatory and protective effects through the induction of autophagy (Alboni et al., 2017; Gu et al., 2021; Xu et al., 2022). Our literature search also retrieved studies exploring the drug-by-environment interaction, employing CUMS and EE alongside with antidepressant treatments. Interestingly, the effectiveness of certain pharmacological agents (i.e., fluoxetine) or natural compounds (i.e., icariin) as treatment approaches in CUMS animals, is more profound when administered in an EE, while their effectiveness is reduced under stressful conditions (Alboni et al., 2017; Branchi et al., 2013; Liu et al., 2017; Nwachukwu et al., 2021; Poggini et al., 2021). These findings, in agreement with previous studies, employing other chronic stress regiments, support the beneficial role of EE against the detrimental effects of CUMS.

It should be noted, however, that there are various factors mediating the effects of EE on the impact of CUMS exposure. Differences in duration and time of CUMS in relation to EE (i.e., whether EE precedes, follows or coexists with CUMS) as well as the sex of animals may explain the inconsistent results. More specifically, it is proposed that EE exerts the most beneficial effects when administered post stress (Macartney et al., 2022), while its cessation before the end of all experimental procedures is considered a stressful situation causing depressive-like symptomatology in male rats (Smith et al., 2017). In contrast, in female rats EE exerts long-lasting protective effects, even if it is terminated prior to CUMS initiation (Vega-Rivera et al., 2016). Interestingly, while housing in EE for a short period restores markers of synaptic plasticity in adult male rats (Liu et al., 2017), it does not reverse the reduced hippocampal neurogenesis caused by CUMS in adult female mice (Ramírez-Rodríguez et al., 2021), a finding indicating that a longer period of EE housing may be needed to exert its beneficial effect in females.

Despite the fundamental behavioral and hormonal differences observed between genders and the risk of mental disorders to be more prevalent in women than men (Altemus et al., 2014; Balta et al., 2019), relatively few pre-clinical studies have included both sexes for direct comparison. In fact, according to our literature search both sexes have been included only in two studies and their results indicate sex-

related differences. Specifically, stress-related spatial learning impairments were limited to males (Dandi et al., 2022), while CUMS induced depression and anxiety-like behavior only in female rats (Smith et al., 2018). The behavioral and neurobiological sex-related differences observed in models of anxiety and depression, raise the question whether the outcome of many studies which employed only males, could be replicated in females (Kokras & Dalla, 2014). More importantly, the inclusion of both sexes in preclinical and clinical studies will promote a better understanding of sex-dependent differences in psychiatric disorders and lead to more efficacious sex-orientated treatments (Pavlidi et al., 2022).

Through the presentation of research evidence regarding behavioral, cognitive, neuroendocrinological and brain morphological alterations, the current paper aimed to provide an up-to-date review regarding the EE and CUMS interaction. Although the effects of both EE and CUMS have been well documented, data on their interaction has been sparse. Thus, more research needs to be conducted to clarify the underlying mechanisms of CUMS-associated behavioral changes and explore the effectiveness of EE and other non-pharmacological interventions against stress-related disorders.

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Declarations of Interest

None

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Table 3: Findings regarding brain and neuroendocrine function alterations in studies exploring the EE and CUMS interaction

References	Marker	Area of Interest	CUMS	CUMS/EE
Synaptic plasticity and development				
Poggini eta al., 2021	LTP	CA1	-	LTP (+minocycline)↑
Shen et al., 2019	SYN, PSD (thickness), PSD95, SIRT1, miR-134	CA1	PSD↓, SIRT1 (in vivo)↓ miR-134 (in vivo)↑, SYN and PSD95 (in vivo)↓	PSD ↑, SIRT1 (in vivo)↑, miR-134 (in vivo)↓, SYN and PSD95 (in vivo)↑
Alboni et al., 2017	GluR1, GluR2, Ser845-GluR1, Ser831-GluR1, Ser880-GluR2, NMDA (GluN2A, GluNR1, GluN2B), LTP	HPC, CA1 (LTP)	Ser845-GluR1↓, GluN2A↑, GluNR1↑, LTP↓(+FLX)	Ser845-GluR1 ↑, GluN2B ↓ (+FLX)
Liu et al., 2017	SYN	CA1, CA2, CA3, DG	CA1↓, CA3↓, DG↓	-
Ilin & Richter-Levin, 2009	L1-CAM	PFC, BLA, CA, dCA1, TL	BLA ↑, TL↑	dCA1↑, TL↓
Neurogenesis and neuronal growth				
Ramírez-Rodríguez et al., 2021	Ki67, BrdU, DCX, CR, BrdU+/Neun-, calretinin cells	DG (ventral, dorsal)	Ki67↓, DCX↓, CR↓, BrdU+/Neun-↓, calretinin cells↓	Ki67↑,CR ↑, BrdU ↑(higher in dorsal), DCX ↑, CR↑, BrdU+/Neun-↑, calretinin cells↑ (+FLX)
Shen et al., 2019	BDNF, number of branches and spines	CA1	number of branches and spines↓ BDNF (in vivo) ↓	number of branches and spines↑ BDNF (in vivo) ↑
Seong et al., 2018	BDNF, TrkB, VEGF-positive cells	HPC, SLM	BDNF↓, TrkB↓, VEGF (in SLM)↓	BDNF↑, TrkB ↑, VEGF (in SLM) ↑
Alboni et al., 2017	DCX, Ki67, BDNF, CREB, phospho-ERK1/2 levels, CA1 volume	HPC, MPFC	**HPC**: Ki67↓, CA1 volume↓, CREB↓, phospho-ERK1/2 levels↓ **MPFC**: ERK1/2 levels↓, CREB↓ BDNF ↓, (+FLX)	**HPC**: BDNF↑ **MPFC**: ERK1/2 levels ↑, (+FLX)
Cornder et al., 2016	Bace1, Gsk3b, BDNF, Fkbp5, App	HPC, PFC, amygdala	**HPC**: Bace1↑, Gsk3b ↑, BDNF (aged mice)↓ **PFC**: Bace1 (aged mice)↑, BDNF (aged mice)↓ **Amygdala**: Bace1 (aged mice)↑	**HPC:** Bace1↓
Vega-Rivera et al., 2016	Ki67, BrdU, BrdU/S100β, BrdU/NeuN, DCX	DG	Ki67↓(40%), BrdU/NeuN↓, DCX ↓	Ki67↓(66%), BrdU/NeuN↑, DCX ↑
Branchi et al., 2013	BDNF	HL, HPC	↓	HL, HPC (+FLX in EE after stress exposure) ↑, HL, HPC (+FLX in stress after EE)↓
Depression				
Seong et al., 2018	TPH-positive cells	DRN	↓	↑
Stress response				
Dandi et al., 2022	CORT, adrenal glands	Peripheral	CORT↑♂, adrenal glands↑♂	CORT ↑♀
Costa et al., 2021	CORT, CAT (NOR, ADR), RAS (Ang I, II, IV)	HL (RAS)	CORT↑, NOR↑, ADR↑, Ang II↑ Ang I, IV ↑	CORT↓,NOR↓, ADR↓, Ang II↓ Ang I, IV↑
Muthmainah et al., 2021	CORT	Peripheral (retro-orbital plexus)	-	-
Nwachukwu et al., 2021	CORT, DHEA, T	Peripheral (fecal)	CORT ↑, DHEA↑, T↑	CORT ↓, DHEA↑
Poggini et al., 2021	CORT	Peripheral	↑	-
Seo et al., 2021	CORT	Peripheral	↑	↓

Smith et al., 2018	ACTH, CORT (after FST), adrenal glands	Peripheral	peak responsivity ↓♀	peak responsivity ↑♀
Alboni et al., 2017	CORT, GR	Peripheral, HPC	GR ↓, (+FLX)	CORT ↓, (+FLX)
Gurfein et al., 2017	1.CORT, 2.GR	1.Peripheral (fecal), 2.splenic cells ($CD19^+$B, $CD4^+$T, $CD8^+$T, monocytes, NK, neutrophils)	1.FCM ↑, 2.neutrophils ↑(28%), T lympocytes ↑	2. neutrophils ↑(33%), $CD19^+$B ↓
Zeeni et al. 2015	ACTH, CORT	Peripheral (inferior vena cava)	ACTH ↑, CORT ↑	ACTH ↓, CORT ↓
Cordner et al., 2016	CORT, adrenal glands	Peripheral	CORT ↑, adrenal glands ↑	-
Vega-Rivera et al., 2016	CORT	Peripheral	↑	↑
Branchi et al., 2013	CORT	Peripheral (before and after CUMS and EE)	-	-
Ilin & Richter-Levin, 2009	CORT	Peripheral (Basal levels)	↑	↓
Immune regulation				
Xu et al., 2022	Iba-1, CD68, NLRP3, caspase1 P20, ASC, autophagy	CA1	Iba-1 ↑, CD68 ↑, NLRP3 ↑, caspase1 P20 ↑, ASC ↑, autophagy ↓	Iba-1 ↓, CD68 ↓, NLRP3 ↓, caspase1 P20 ↓, ASC ↓, autophagy ↑
Gu et al., 2021	Microglia (IBA-1, M1, M2), cytokines (IL-1β, IL-6, TNF-α, IL-10), NF-kB p65	HPC, peripheral serum	IBA-1 ↑, M1 ↑, (IL-1β ↑, IL-6 ↑, TNF-α ↑, IL-10 ↓ in serum), (IL-1β ↑, IL-6 ↑, TNF-α ↑, IL-10 ↓ in HPC), NF-kB p65 ↑	IBA-1 ↓, M2 ↑, (IL-1β ↓, IL-6 ↓, TNF-α ↓ in serum and HPC), IL-10 ↑ in HPC, NF-kB p65 ↓
Poggini et al., 2021	cytokines	HPC	-	-
Shtoots et al., 2018	Peritoneal macrophage, blood monocyte, CCL2, CCR2, CCL3, CCL4, CCL5, IL-10 (activation ratio)	Peripheral, peritoneum	Peritoneal macrophage ↓, blood monocyte ↑ , CCL2 ↑, CCR2 ↓, IL-10 ↓	Blood monocyte ↑, peritoneal macrophage ↓, CCL2 ↑, CCR2 ↓ IL-10 ↑
Gurfein et al., 2017	Splenic immune cells ($CD19^+$B, Thy 1.2^+T $CD4^+$T, $CD8^+$T, $CD11b^+$, $CD49^+$b NK, Ly-$6g^+$)	Spleen	B cells ↓, T cells ↓, spleen cellularity ↑ (27%),	$CD19^+$ B lymphocyte ↓, spleen cellularity ↓(32%), B:T ratios ↓
Alboni et al., 2016	IL-6, TNF-α, IL-1β, IFN-γ, IL-10, IL-4, TGF-β, CD14, TLR4, caspase1	HPC	TNF-α ↓, IFN-γ ↓(+FLX)/ TLR4 ↑, caspase1 ↑ (no FLX)	IL-1β ↑, CD14 ↑ , TLR4 ↑ (+FLX) / TLR4 ↓, caspase1 ↓ (no FLX)
Epigenetics				
Seo et al., 2021	p11 mRNA, AcH3, HDAC5, H3K4me3, H3K27me3	HPC	p11 mRNA ↓, AcH3 ↓, HDAC5 ↑ H3K4me3 ↓, H3K27me3 ↑	p11 mRNA ↑, AcH3 ↑, HDAC5 ↓ H3K4me3 ↑, H3K27me3 ↓
Alboni et al., 2017	p11 mRNA	HPC		↑(+FLX)

Abbreviations: BLA, Basolateral Amygdala; CA, Dorsal Cornu Ammonis / dCA1, area1; DG, Dentate Gyrus; DRN, Dorsal Raphe Nucleus; FLX, Fluoxetine; HL, Hypothalamus; HPC, Hippocampus; PFC, Prefrontal Cortex; SLM, Stratum Lacunosum Moleculare; TL, Thalamus.