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Animal Models in Neuroscience: A Focus on Stress

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1 Introduction

The first decades of the twenty-first century have been associated with a rise in stresses experienced at home, in the workplace, and in public areas as a result of technological advances, an escalation of social violence, more frequent and intense natural disasters, and the outbreak of warfare and genocide around the globe. Thus, the incidence of stress-related disorders such as anxiety, depression, post-traumatic stress disorder, conduct disorders, and drug use/abuse has dramatically increased, and the World Health Organization (WHO) has dubbed stress the health epidemic of the twenty-first century (Meyers, 2018). It is therefore incumbent on neuroscientists and clinicians to better understand and more effectively treat these problems.

An important issue for studying stress in humans is that prospective research on chronic stress cannot be done because of its deleterious effects, but retrospective studies have examined stress effects in Holocaust survivors (Yehuda et al., 2007), victims of childhood abuse (Chapman et al., 2004), and war veterans (Aschbacher et al., 2018), for example. Effects of mild, acute stresses can be studied in humans (Wolf et al., 2016). However, the heterogeneity of human populations, due to both nature and nurture, complicates such studies, and it is difficult to determine the biological bases of the effects. Thus,

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animal models have been developed in order to assess physiological effects of stress and to understand its biological underpinnings. Animal models consist primarily of rodents, rats or mice, or small primates like tree shrews that are subjected to stressful experiences and then analyzed during and after the stressful experience. This chapter provides a review of previously and currently applied animal models for analyzing stress and how they may be utilized to develop novel and, hopefully, more effective therapies for disorders that are precipitated by or related to stress.

2 The Stress Response

2.1 Temporal Aspects of Stress

Stress, depending on its intensity and duration, results in adaptive or maladaptive physiological changes in systems ranging from neural cells in culture to laboratory rodents and subhuman primates to humans, a concept put forward by Selye (1976). For example, short periods of mild stress are associated with enhanced availability of energy and oxygen, inhibition of digestion, growth and immune function, and enhanced cognitive processing and analgesia in CNS¹ responses which help to cope with the stressor. However, when the duration of the same stress is increased, these responses become maladaptive to the organism and result in fatigue, myopathy, gastro-duodenal ulcers, impaired immune function, and, ultimately, death (for further discussion, see Nelson, 2000; also see Chap. 3 of the present book). More recently, McEwen and colleagues introduced the concept of allostasis/allostatic load to describe the stress response. Allostasis refers to adaptive processes by which the body responds to stressors in order to maintain homeostasis, which is defined as maintenance of equilibrium in physiological processes. When stress is chronic or repeated, allostatic load (“wear and tear on the body”) accumulates, and the individual is no longer able to maintain homeostasis (McEwen & Stellar, 1993). As long as allostatic processes maintain homeostasis, effective coping occurs, but when stress imparts too large an allostatic load, stress becomes debilitating to both peripheral and central nervous systems (see McEwen, 2016 for further information).

Recent studies show that some brain functions, including learning and memory, are also subject to the same adaptive/maladaptive pattern (Luine & Gomez, 2015). This outcome has major implications for maintaining the

¹ CNS: central nervous system.

safety and effectiveness of workplaces, especially those involving teams piloting complex technical systems such as aircraft, space shuttles, or nuclear reactors, and for dealing with the aftermath of disasters and war (Fauquet-Alekhine & Rouillac, 2016). Thus, it is important to develop animal models for understanding and coping with job-related stresses.

2.2 Types of Stressors

In experimental animals, two major categories of stressors are acknowledged: systemic and processive stressors (Anisman et al., 2001). Systemic stressors, which constitute an immediate physiological threat to homeostasis, include immunologic challenge, hemorrhage, excess alcohol, and hypoglycemia. These stresses are relayed directly to the paraventricular nucleus (PVN) via brain stem monoaminergic projections to influence hormone release (Herman & Cullinan, 1997). These stressors are not common in relation to stresses that humans usually experience, and, thus, their use as animal models to study stress has waned. Processive stressors, on the other hand, require integration and interpretation at higher brain centers before relay to the PVN through limbic forebrain circuits in the prefrontal cortex, hippocampus, and amygdala (Herman & Cullinan, 1997) and are experienced by humans. There are two types of processive stressors, psychogenic and neurogenic. Psychogenic stressors are mainly psychological such as exposure to a predator or a novel open field for animals, and anxiety over an upcoming event or exam for humans, while neurogenic stressors are mainly physical such as foot shock, pedestal, cold swim, immobilization, social defeat, and isolation for animals. For humans, neurogenic stressors include overcrowding in subways or buses, exposure to natural disasters like hurricanes, earthquakes, and so on, and traffic accidents. Some processive stressors used in animal research have both psychogenic and neurogenic elements such as overcrowding, restraint stress (subject is placed in a plastic tube or wire mesh container where they have limited movement), and resident intruder or social defeat stress. Restraint and overcrowding have psychogenic elements in that subjects cannot escape and they are not physically painful, but unlike a purely psychogenic stressor, they involve physical components that limit the response/defensive style of the subject (McIntyre, et al., 1999). Thus, a variety of stress paradigms are available for analyses which to some degree model the human experience.

It needs to be acknowledged that animal models do not replicate human stress situations as accurately as, for example, models for breast cancer or diabetes. However, it should be noted that, as described here, various kinds of

Table 4.1 Types of stresses utilized in rodent studies

Stress method	Description	Comments	References
Restraint	Commercially available plastic tubes keep rats or mice unable to escape but not immobilized	Acute or chronic use; mesh restrainers are not allowed by some regulatory agencies	Watanabe et al., 1992; Conrad et al., 2003; Bowman et al., 2009
Resident intruder	Aggressive mouse introduced to home cage	Not usable for females or young animals	Nasca et al., 2019
Social isolation	Removed from cagemates daily	None	Carnevali et al., 2012; Rowson et al., 2019
Social instability/ isolation	Change cagemates or groups daily 2x/week Removed from cagemates each day	None	Rowson et al., 2019
Pedestal	30-inch-high platform, 30 min -1 h	Some protocols put the platform in water pool	Zanca et al., 2018; Avital et al., 2005
Forced swim	Put in water pool for various periods of time	Monitoring health is important	Avital & Richter-Levin, 2005
Tail or foot shock	Brief shocks given intermittently	Need to carefully monitor health	Wood et al., 2001
Chronic variable or unpredictable stress (CVS or UCS)	Different stressors applied daily for days or weeks	Various investigators combine different types of stress	Hodes et al., 2015

stressors lead to the same final, common outcome: elevations in stress hormones, cortisol (humans), or corticosterone (animals). Further, animal models provide the advantage of being able to examine internal organs, circulating hormones and other factors, and the brain of subjects before, during, and after stress (see Table 4.1 for a list and short description of commonly used stressors in rodent research and studies that have utilized these stressors).

The hallmark of stress is enhanced synthesis and release of cortisol or corticosterone which occurs within minutes following the initiation of stress and is sustained for hours. It is notable that major differences in amount of release between various stresses are not apparent. However, it is difficult to compare the intensity of the stimulus between stressor types. Rivier (1999) assessed the effects of the neurogenic stressor, foot shock, and the systemic stressor, alcohol, and found similar levels of circulating corticosterone following their application. Likewise, Anisman et al. (2001) compared corticosterone levels in mice exposed to the psychogenic stressors—a rat or fox odor—and the

neurogenic stressors—restraint, foot shock, cold swim, acoustic startle, or open field—and found more similarities than differences in circulating corticosterone. Thus, a variety of stressors are effective in causing release of generally high and similar levels of circulating corticosterone.

2.3 Sex as a Biological Variable

While there is general agreement on the debilitating effects of chronic stress, it is remarkable to note that the vast majority of studies were conducted in only males of these species. Thus, it is only quite recently that the response of females to stress has been investigated. Surprising to some researchers, but not to most neuro-endocrinologists, responses in females can be different than in males (Luine et al., 2017b, 2018). The importance of determining possible sex differences in stress responses is firstly that a model derived solely in a male may not pertain to a female and vice versa, and thus the model does not provide translational value. Furthermore, determining the extent and nature of sex differences in responses may provide information for understanding why some stress-related diseases have different incidence rates between the sexes. For example, women have a higher incidence of anxiety disorders, post-traumatic stress disorder, and major depression than men, while they have a lower incidence of alcohol and drug abuse and behavior disorders than men (Bangasser & Valentino, 2014). A better understanding of sex differences in stress responding and their bases may inform the development of novel and more effective therapies for these disorders, which are often precipitated by or related to stress and which pose an enormous burden on society. Examples of sex differences in stress responses are detailed below.

3 Stress Alters Anxiety, Depression, and Cognitive Function

3.1 Effects in Adult Rodents

The assessment of complex interactions between stress, gender, hormones, mood, and neural plasticity requires study in a vertebrate where physiological and behavioral indices can be assessed. Thus, rodents are ideal for study since they display mood characteristics, and cognition can also be measured. Changes in mood and behavior have been measured following numerous stress paradigms, and tissue sizes are sufficient such that multiple analyses can

be conducted from a single subject, thus reducing the total number of animals needed. In addition, there is a large database of literature on rats and mice on which to base studies. Finally, the relatively low cost of large numbers of rodents needed for stress studies makes rats or mice the species of choice.

In the last 20 years, more research has focused on higher-order neural functions, and results show a more complex picture than with other physiological systems, with several factors influencing outcomes including the sex of the subject as well as the specific domain assessed. The vast majority of experiments have been conducted in males, but recent studies have included females and bring fresh insight into the nature of stress effects. In male rodents, acute stress enhances some forms of learning including classical conditioning (Wood et al., 2001), and generally enhances consolidation of memory but impairs retrieval and working memory (Green & McCormick, 2013). In contrast, chronic stress, one–three weeks of stress elicited by daily restraint or different daily stressors (unpredictable chronic stress, UCS), results in impaired learning and memory in spatial tasks such as radial arm maze (RAM), Morris water maze, Y-Maze, and recognition memory tasks such as object and place recognition (Luine et al., 2017b, 2018; Conrad et al., 2003; Ortiz & Conrad, 2018). Remarkably, female rodent cognitive responses to stress are often different: acute stress impairs classical conditioning (Wood et al., 2001) and does not affect object placement (Luine et al., 2017a), while chronic stress enhances performance of spatial memory tasks (Bowman et al., 2001; Conrad et al., 2003), but does not alter object recognition (Gomez & Luine, 2014). Our lab was the first to show that chronic stress impairs RAM performance in males (Luine et al., 1994) and enhances it in females (Bowman et al., 2001), and we continue to consider sex as a variable in our studies.

While less investigated than cognition, anxiety increases following chronic stress in both male and female rats as assessed by either the elevated plus maze or the open field. It appears that males show stress-dependent increase in anxiety before females (seven days; Bowman et al., 2009) but by 21 days females are also affected (Noschang et al., 2009). However, few studies include females, and further study is required. Depression in rodents is assessed by preference for sucrose-containing vs plain water wherein less consumption of sucrose indicates anhedonia (loss of pleasure) and therefore depression. In males, exposure to a variety of stressors (one–four weeks) including predator stress, social defeat coupled with chronic isolation, and variable stressors increases male depression in the sucrose preference test (Dalla et al., 2010; Carnevali et al., 2012; Gronli et al., 2005; Burgado et al., 2014). Few studies have utilized females but we found that both sexes exhibited decreased sucrose preference at seven, 14, and 21 days of restraint stress (Buenaventure et al.,

unpublished) and seven days of unpredictable stress decreased preference in female but not male mice (Hodes et al., 2015). Thus, stress appears to alter anxiety and depression, but the parameters may be somewhat different between the sexes and require further investigation.

Thus, current studies have shown that stress deleteriously impacts the higher-order neural functions of cognition and mood in rodents. Importantly, if stress is not severe or maintained for extended periods, the changes are generally reversible (Ortiz & Conrad, 2018). For example, we found that if RAM training and testing begins immediately after chronic stress, males are impaired, but if training and testing begins 14 days following stress cessation, impairments are not present (Luine et al., 1994). This pattern follows anatomical results which show retraction of hippocampal pyramidal neuron dendrites following chronic stress and re-growth of the tree by 10 days following cessation of stress (McEwen, 2001) (see Sect. 4 for further discussion of neural changes).

3.2 Stress during Developmental Periods Causes Long Lasting Behavioral and Cognitive Changes

As indicated above, when stress is experienced during adulthood, effects are generally reversible and transitory in nature, but during the pre- and post-natal stage, chronic stress can result in long-lasting effects (reviewed in Weinstock, 2016). The long-lasting effects of environmental agents, food restriction, or toxic chemicals at this time period are also well documented and long known. In regard to stress in rats, we restrained pregnant dams and found that at adulthood, anxiety was increased in female but not male offspring, and RAM performance was impaired in males and enhanced in females (Bowman et al., 2004). Other research shows similar stress effects in males, but females are not often assessed (Weinstock, 2016). Stress during the post-natal period (up to weaning at 21 days) is also associated with permanent alterations in neural and behavioral function (Hodes & Epperson, 2019).

In recent years, the developmental period for the exerting of long-lasting effects has been lengthened to include periadolescent/adolescent and pubertal effects of drugs, environmental factors, and hormones at adulthood (Green & McCormick, 2013). Adult performance on mazes is impaired in rats given stress at 28 days of age (Isgor et al., 2004). Adolescent stress increases anxiety in both sexes at adulthood (Holder & Blaustein, 2014). Adult males show depressive-like behavior and cognitive impairment following adolescent stress but females do not (Gomes & Grace, 2017; Klinger et al., 2019). Further

research in females is clearly warranted in order to determine the extent of sex differences at adulthood following adolescent stresses.

Thus, acute and chronic stress given across the rodent lifespan is associated with alterations in higher-order brain functions like cognition and mood. Therefore, animal models of stress can provide important translational information for developing new treatments for stress-related diseases.

4 Neural Effects of Stress

The main advantage of conducting stress experiments in animals, as in most pre-clinical research, is that post-mortem examinations can be made to determine which neural systems are affected and how. Such results can inform the design of clinical studies and treatment paradigms. Chronic stress is associated with sex-specific alterations in areas important for learning and memory like the prefrontal cortex and hippocampus and for mood and anxiety in areas like the amygdala and prefrontal cortex. However, the vast majority of neural studies, like the behavioral studies, fail to include females. A brief synopsis of important changes follows, and the pattern of results suggests that stress often downregulates neural activity or expression in males but either does not alter activity or causes different changes in females.

Chronic stress differentially affects neuronal survival; in males short-term survival of new dentate gyrus neurons is decreased whereas survival is increased in females (Westenbroek et al., 2004). Retraction/pruning of dendrites in CA3 pyramidal neurons of the hippocampus following stress has been well described in male subjects (Watanabe et al., 1992; Ortiz & Conrad, 2018). As shown in Fig. 4.1, three weeks of daily restraint stress causes loss of tertiary dendrites (most distal dendrites) from the apical dendritic tree, but basal dendrites remain unaffected. The density of dendritic spines (small protrusions from the dendrites which are the site of synapses) is not changed following stress. Galea et al. (1997) confirmed retraction of CA3 apical dendrites following 21 days of restraint stress in males and found that females do not show changes in these dendrites but that basal CA3 dendrites retract. McLaughlin et al. (2009) also showed that estradiol treatment of female rats without circulating estradiol because of ovariectomy prevents CA3 dendritic retractions and increases CA1 spine density (number of spines/segment of dendrite). These results provide some morphological evidence for the idea that the female hormone, estradiol, may confer cognitive resilience to stress in females.

Chronic stress leads to retraction or loss of tertiary apical dendrites (most distal dendrites) in hippocampal CA3 pyramidal neurons in male rats. The

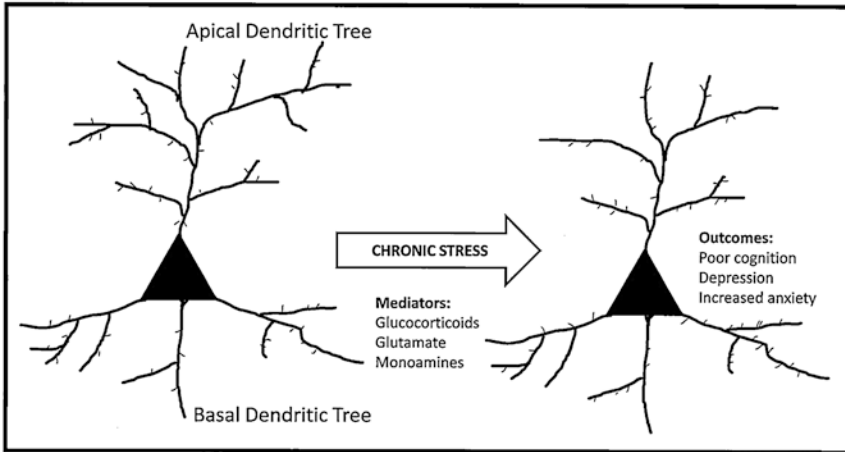


Fig. 4.1 Schematic of hippocampal CA3 pyramidal neuron pre and post chronic stress

basal dendrites are not affected, but other sub-regions are impacted when chronic stress is sufficiently robust or long-lasting. Density of dendritic spines is not altered in CA3 apical or basal spines. Retraction of apical dendrites underlies impaired hippocampal function and contributes to decrements in learning and memory and alterations in mood. Mediators of dendritic retraction are prolonged exposure to the stress hormones glucocorticoids, increases in glutamatergic activity, and changes in monoaminergic activity (adapted from the studies of Watanabe et al., 1992; Galea et al., 1997; and Ortiz & Conrad, 2018).

Several neurotransmitters show sexually dimorphic responses to stress. Glutamate activity is critical in learning and memory, and 21 days of restraint stress decreased glutamatergic neurotransmission and surface expression of glutamate receptors in the PFC² of male, but not female, rats (Wei et al., 2014). Chronic unpredictable mild stress for 21 days caused a 50% decrease in the endocannabinoid receptor, CB1, in the dorsal hippocampus of male rats, whereas an approximately 150% increase was found in females (Reich et al., 2009).

Monoaminergic systems are among rapid and important stress responders/mediators, and following 21 days of restraint decreased levels of norepinephrine, dopamine, and serotonin (5-hydroxytryptamine, or 5-HT) levels are found in the hippocampus of males (Beck & Luine, 1999; Sunanda et al., 2000), but opposite changes in these monoamines are found in females (Beck & Luine, 2002). We examined effects of restraint for a shorter period,

²PFC: prefrontal cortex.

Table 4.2 Sex differences in chronic stress effects on monoamine metabolites in brain areas of male and female rats

AREA	MHPG		HVA		5-HIAA	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
CA1	↑ 153%	↑ 122%	↓ 63%	↑ 18%	↓ 45%	↑ 30%
CA3	↓ 41%	↑ 105%	↑ 46%	↓ 11%	↓ 22%	↑ 24%
MP-CORTEX	↓ 18%	↑ 21%	=	=	=	=
BL-AMYGDALA	↓ 18%	↑ 21%	↓ 24%	↓ 4%	↓ 25%	=

Entries show significant increases (↑) or decreases (↓) in metabolites for norepinehrine (MHPG, 3-methoxy-4-hydroxyphenolglycol), dopamine (HVA, homovanillic acid), and serotonin (5-HIAA, 5-hydroxyindole acetic acid). An arrow of the same color but a lighter hue indicates a significant difference. Significant differences range from $p < 0.05$ to 0.001 . Adult male and female rats served as controls or received seven days of 6 h/day restraint stress. Data from Bowman et al. (2009)

one week, and like the behavioral responses, monoaminergic systems showed robust sex differences (Bowman et al., 2009, Table 2). Changes in metabolites of the major amines, 3-methoxy-4-hydroxyphenolglycol (MHPG) for norepinephrine, homovanillic acid (HVA) for dopamine, and 5-hydroxyindole acetic acid (5-HIAA) for serotonin, are shown by arrows in CA1, CA3, medial prefrontal cortex, and baso-lateral amygdala in Table 4.2. Metabolites provide a measure of activity, but other assessments like *in vivo* release of transmitters need to be made. In the majority of cases, metabolites decreased in males and increased in females following stress.

The cortex showed the fewest changes, with only MHPG affected: an 18% decrease in males and a 21% increase in females. The largest changes were found in CA1 where both sexes showed an increase in the NE metabolite, MHPG, but males showed a significantly larger increase, 153%, than females, 122%, and both HVA and 5-HIAA decreased 45–63% in males and increased 18–30% in females. However, the largest difference between the sexes occurred in CA3 where MHPG decreased 41% in males and increased 105% in females. These remarkably different patterns in monoaminergic activity in the sexes following stress may be critically important in mediating memory and mood changes. However, further experiments are necessary to link the changes in activity to behavior and to determine their translatability.

5 Ethical Considerations

Stress procedures, like all research in animals, must be approved by a committee which oversees animal care and protection at the institution where the research is carried out. Researchers must insure welfare of the animals and

demonstrate that procedures do not cause undue harm or morbidity/mortality. Examples include daily weighing of subjects to insure against excessive weight loss because chronic stress causes weight loss. All researchers need to be trained in proper implementation of the stressors and to monitor untoward effects, in which case the stress must be stopped. Such symptoms can include porphyrin discharge from the eyes and excessive weight loss. Stress experiments have been conducted for many years, and thus if researchers follow standard protocols and are attentive to animal well-being, problems should not arise. In the United States, researchers adhere to guidelines from the National Institutes of Health (*Guide for the Care and Use of Laboratory Animals*, Eighth Edition, n.d.). Many European researchers also follow these guidelines, but many countries issue their own. Researchers should check with their home institution's research office for the guidelines which they should follow.

6 Conclusions

An array of acute and chronic stress models is available in rodents to model stress in humans. Proper implementation can lead to critical information for understanding the mechanisms underlying stress effects and for developing new treatments. Since stress is a growing issue for our society, obtaining such information is ever more critical.

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