CHAPTER 12

Early-Life Stress: Rodent Models, Lessons and Challenges

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Lactating rat in nest nursing her litter (left), and licking/grooming her pups (right)

Prototypical maternal care behaviours in rats. The arched back nursing posture (left) is an active nursing posture characterized by a tented (arched) back. Active sensory input from the mother is crucial for pup neurodevelopment and is provided by the dam in the form of licking and grooming (right). See Companion Website for animation www.wiley.com/go/russell/stress.

12.1 Why study early-life stress?

The early postnatal brain is far from mature and hence the perinatal period represents a critical stage of neural development. During this period, the brain is highly vulnerable to both organizing and disorganizing influences from the environment, including environmental stress. What is perhaps most striking about the influences of early-life stress (ELS) on brain development is how permanent and progressive the deleterious effects can be. Indeed, a relatively brief period of stress occurring during just the first few days of life often has life-long consequences for brain structure and function, ultimately impacting on behaviour and vulnerability to subsequent stress. Understanding the mechanisms for the enduring consequences of ELS on brain function has been

an active area of neuroscience research, as this knowledge is critical for identifying clinically plausible therapeutic strategies.

Throughout the literature, the term ‘early-life’ has been used to describe different developmental windows. Thus, it is best to define the term in relation to its most common usage and its specific meaning in the context of this chapter. Here, we will use the term ‘early-life’ or ‘early-life period’ to refer to early postnatal life, including the day of birth through the time of weaning from the mother (in most rodent species, weaning occurs around postnatal day 21). The effects of stress during other critical periods can also impact on brain development, but will not be the focus of this chapter (see Maccari and Morley-Fletcher, 2007, and McCormick and Green, 2012, for comprehensive reviews on prenatal and adolescent stress respectively).

12.1.1 Early-life adversity is a major risk factor for psychopathology

Epidemiological data indicate that various forms of ELS in humans can have lifelong impacts on cognitive and emotional function. Adverse early-life conditions, including poverty, loss of parent, substance abuse by the mother or maternal depression, are consistently associated with vulnerability to develop various psychopathologies later in life. Stress-related disorders, including depression, anxiety and post-traumatic stress disorders, appear to be especially sensitive to the effects of ELS, but cognitive and executive functions are also impaired following childhood adversity. Among the most influential studies of these effects are those of institutionally reared children, where chronic impoverished care was shown to be associated with cognitive and emotional deficits; the associated consequences were partially reversed by fostering, therefore highlighting the importance of early life care per se.

The societal impact of ELS is substantial, as the majority of children worldwide grow up under some form of chronic stress. In the United States alone, there are as many as 1.2 million verified cases of child abuse each year, and, of course, many more cases remain unreported. The situation seems to be even worse in countries stricken by poverty, famine and war (UNICEF, 2005). Given the scope of the underlying problem, complete prevention of ELS is unlikely, and research into interventions with translational and clinical potential is therefore greatly needed. Moreover, many of the symptoms resulting from ELS may not emerge until later in life, making it difficult to identify affected or vulnerable individuals until well past the critical developmental window. Thus, any therapeutic interventions will need to be effective when applied post hoc, after the stress has occurred.

12.1.2 The need for animal models of chronic early-life stress

Although the epidemiological studies described above suggest that ELS influences later pathology, the correlational nature of these studies precludes direct causal inferences. Animal models therefore provide an important tool for asking mechanistic questions regarding ELS, distinguishing between the roles of genetic and environmental factors. In addition, parameters of interest can be manipulated and subsequent experiences can be controlled throughout the entire

period of investigation. Finally, direct access to specific brain regions, coupled with neuroanatomical, biochemical and genetic approaches can tease out the regions, circuits, mediators and signalling cascades that might contribute to the profound effects of early-life maternal interaction on adult outcome.

Over the past 60 years, researchers have developed primate and rodent models to manipulate early-life experiences. Given the importance of mother–infant interactions, most of these models focus on manipulating the quantity and/or quality of maternal care in various ways. From this research, it has become clear that different forms of ELS can result in a spectrum of positive and negative consequences on brain structure and function. The ultimate outcome appears to depend on the 'stressful' nature of the experience: its quality, severity and duration. For example, several models of 'enhanced' early-life experience involve brief separation of the pups from the dam (mother rat – pregnant or lactating) and increased maternal care upon reunion of the litter. This manipulation could be considered a 'mild' form of ELS and, interestingly, these studies generally report improved brain function and reduced stress sensitivity. Although it remains unclear whether these animal models have any relation to the human condition, or whether similar experiences would truly be beneficial in different contexts, they have been proposed as a model for the development of resilience to stress and psychopathology. On the opposite end of the spectrum, models of severe early-life stress, designed to provoke chronic rather than intermittent stress, find impairments in cognitive and emotional function; these models of severe or chronic stress are the focus of the current chapter.

ELS models using non-human primates, whose brains and sociality most closely represent the human condition, have provided powerful insights into the development of complex psychiatric disorders. The seminal work of Harlow and colleagues (Seay et al., 1962), using maternally isolated rhesus monkeys as a model were the first to demonstrate that maternal–infant interactions are indeed required for normal cognitive and emotional brain development. Although primate models of ELS continue to provide important insights, the many practical and ethical concerns associated with the use of primate species precludes their widespread use. As an alternative, simpler animal model, rodent species provide tractable ways to test the consequences of ELS on basic developmental processes and have therefore become widely adopted.

12.2 Vulnerability of the developing brain

As indicated above, the developing brain is particularly vulnerable to the deleterious effects of environmental perturbations and hence the impact of ELS has been an area of intense research. This vulnerability is due in large part to the developmental processes that are still taking place during the early-life period. For example, in rodents, the organization of the hippocampal formation, a brain region implicated in cognitive and emotional function, takes place largely after birth, continuing through the first few weeks of life. Brain development in humans is characterized by a particularly prolonged trajectory, as the refinement of cortical synapses and connectivity patterns continues for years into the adolescent or pubertal period.

12.2.1 Maturation of the stress system

The stress-response network consists of several overlapping circuits (Figure 12.1), all of which are still developing during early postnatal life. The neuroendocrine component, comprising the hypothalamic– pituitary–adrenal (HPA) axis, is activated by stress and is initiated by the release of corticotropin releasing hormone (CRH) from neurons in the paraventricular nucleus (PVN), resulting in the eventual release of glucocorticoids from the adrenal gland. In rats, CRH mRNA expression is robust at gestation day 18 (day 0: vaginal semen plug found), decreases perinatally (gestation days 20 and 21) and finally increases to reach adult levels by the end of the first postnatal week. In the mouse, hypothalamic PVN CRH expression is first detected a little earlier, on gestation day 13.5, but also decreases around the time of birth and then climbs to adult levels.

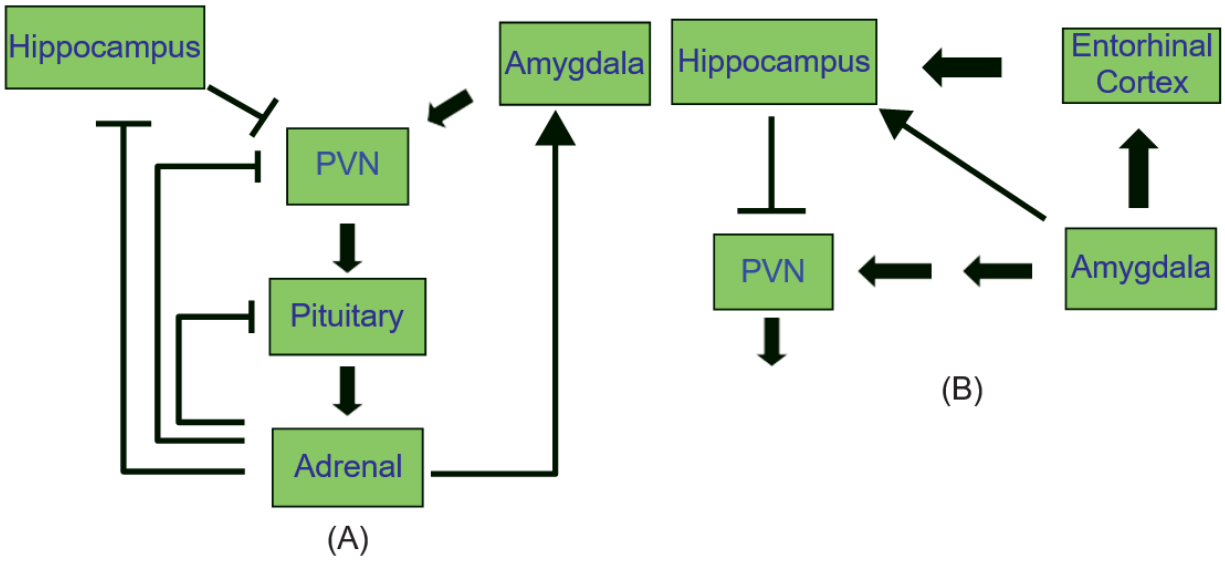

Figure 12.1 Central stress processing pathways. Stress-activated pathways include

(A) the neuroendocrine hypothalamic–pituitary–adrenal axis (HPA) and (B) the central, limbic stress-loop. (A) 'Physiological' stress signals reach the hypothalamus, causing secretion of corticotropin-releasing hormone (CRH) from neurons of the paraventricular nucleus (PVN). CRH induces release of adrenocorticotropic hormone (ACTH) from the pituitary and ACTH elicits secretion of glucocorticoids (GCs) from the adrenal gland. (B) Stress involving higher-order sensory processing activates limbic pathways constituting the 'central' stress circuit. Stressful stimuli reach the key processor, the central nucleus of the amygdala (ACe), activating the numerous CRH-producing neurons in this region. Locally released CRH acts on cognate receptors on projection neurons of the amygdala, which convey stress-related information (directly or indirectly via the entorhinal cortex) to the hippocampal formation. Arrows indicate facilitatory projections but do not imply monosynaptic connections. Blunt-ended lines denote inhibitory feedback loops. Adapted from Avishai-Eliner et al., 2002, and reproduced by permission of Elsevier Limited.

Interestingly, in contrast to the well-known negative feedback action that glucocorticoids have on hypothalamic CRH levels during adulthood, CRH expression is not regulated by glucocorticoids during fetal life. This lack of regulation is not due to the absence of glucocorticoid receptors: glucocorticoid receptor mRNA has been shown in the hypothalamic PVN as early as the 16th gestation day. The onset of glucocorticoid negative feedback on CRH expression does not appear until the end of the first postnatal week, and this lack of inhibition may, in part, explain the particularly damaging effects stress has on the brain when it occurs during the first week of life.

12.2.2 The stress hyporesponsive period

Initial work on the ontogeny of the neuroendocrine stress response system indicated that in the first two weeks of life in rodents (postnatal days 4–14 in rats; 1–12 in mice; day of birth is usually denoted day 0), the HPA system is relatively unresponsive to stress (termed the stress hyporesponsive period or SHRP). This characterization is based on low basal corticosterone levels, reduced sensitivity to CRH and the apparent lack of a stress response to a variety of 'typical' stressors during this age. The SHRP probably reflects the still ongoing maturation of the HPA axis and this reduction in HPA tone has been hypothesized to protect against the deleterious effects of glucocorticoids on brain development. Importantly, the initial concept of a SHRP has been proven to not fully represent the stress status of immature brains. In humans, stress responses to pain exist throughout the neonatal period. In rodents, immature pups have 300–400% increases in plasma corticosterone levels in response to age-appropriate stressors, i.e. maternal separation or hypothermia. These hormonal responses are mediated by stress-induced activation of CRH release and associated with stress-induced enhancement of CRH expression in hypothalamic PVN neurons. Thus, rather than being unresponsive, the developing stress system seems to be tuned specifically to the types of stress that may be relevant to the early-life period.

12.3 The mother is key: ELS models and manipulation of maternal input

In humans, chronic early-life stress has both physical and emotional com- ponents, and the emotional aspects are dominant. In large part, many forms of ELS in humans derive from abnormal patterns of maternal care, varying from neglect to inconsistency and lack of sensitivity. Several rodent models have attempted to recapitulate these stressful conditions by manipulating interactions of pups with the dam.

As is the case in humans, maternal care plays a critical role in rodent development. Beyond simply providing nutrition and safety in the nest, the dam is critical for providing important sensory stimulation and relaying environmental cues to the pups. Maternal care has been well

characterized in rodents and consists of several stereotyped behaviours, including licking and grooming (LG) of the pups and nursing pups in an active, arched back nursing (ABN) posture, collectively referred to as LG-ABN. These maternal behaviors are critical for normal development of the pups. are critical for normal development of the pups. It is therefore logical to focus on dam–pup interactions as a potent way to manipulate the early-life environment and provoke stress. Simply removing the dam for extended periods of time would lead to hypothermia and starvation, so many models use intermittent maternal deprivation, resulting in intermittent stress. An alternative approach has been to provoke chronic, persistent changes in maternal care, when the dam remains present. We will present here examples of both types of models.

12.3.1 Maternal separation

Models of ELS based on separating pups from the dam are by far the most common. Separating pups from the nest (maternal separation, abbreviated as MS) is an easy, efficient method of disrupting dam–pup interactions. As such, various MS models have been developed, which vary according to both the length of the separation (1–24 hours) and the number of days that the separations occur (1–14 days during the first 2 postnatal weeks). Importantly, MS procedures elicit a stress response in neonatal rats that is dose-dependent, with longer durations of MS associated with the highest serum corticosterone levels. As a general rule, MS procedures with shorter separations usually employ repeated episodes over several consecutive days, whereas the longer (24-hour) separation procedures are employed just once during early life (most commonly around postnatal days 3–5).

In addition to the duration and frequency of the separation, there are

several critical variables to consider across different MS procedures. The first issue is related to the details of how the pups are separated; specifically, whether each pup is separated in total isolation or whether the entire litter is kept together during the separation procedure. The former method is a more severe stress, as it has the additional component of complete social isolation and as a general rule the effects of MS are more robust when pups are also separated from their siblings. Additional environmental factors, such as temperature and even the light–dark phase during which the separation procedure occurs, can also have a significant impact on the pattern of results. Most MS models experimentally control the temperature of the isolation chamber, maintaining euthermic conditions at around 30–33 ∘C throughout isolation.

In a way, MS models take a relatively broad approach to manipulating the early environment: separating the pups from the dam removes all maternal input for that period of time. MS therefore reduces the available time for maternal care and, presumably, the total amount of maternal care received by the pups during early life is reduced. Detailed studies by Levine and colleagues (Levine, 1957) have demonstrated that the MS-induced stress response in neonatal rats is mediated by the reduction in both feeding/nursing and the loss of tactile stimulation associated with anogenital licking and grooming, whereas some of the molecular changes in the HPA axis are mediated specifically by the tactile sensory signals from the mother. Importantly,

the MS procedure itself may provoke compensatory changes in maternal care, and in fact a recent examination of maternal behaviour in the MS paradigm found alterations in both absolute levels and diurnal distribution of active maternal care following MS. Interestingly, if foster pups are provided to the dam during the MS procedure, therefore reducing effects directly on the dam, pups are protected from the alterations in the HPA axis resulting from MS, suggesting that the long-term effects of MS may result in large part from alterations in the dam's behaviour, perhaps through altering the quality of maternal care.

12.3.2 Manipulation of maternal care of a dam that remains with the pups

Although MS models have provided a vast amount of data on the effects of reducing (or at least altering) maternal input on pup development, data from human studies of chronic childhood stress, including war, famine and neglect/abuse, suggest that the mother is typically present, but her behaviour is abnormal. Thus, alternative models, which examine the effects of differential care of a dam that remains with the pups, may provide additional insights into the processes by which early-life experience, including stress, influences lifelong resilience and vulnerability to neuropsychiatric disorders.

12.3.2.1 Natural variations in maternal care

To some degree, the variable patterns of maternal care provoked by models of MS are reproduced in nature. In rats, the amount of time the dams spend licking and grooming (LG) pups and displaying the arched-back nursing posture (ABN) typically follows a normal distribution; the extremes of this curve (defined as one standard deviation above and below the mean) can be used to identify dams that display particularly high LG-ABN or low LG-ABN behaviour. This variation was first described by the work of Myers and colleagues, and later expanded by several other groups (see Champagne et al., 2003), to study how natural variations in maternal care regulate brain and behaviour development. Studies using this model have demonstrated that relatively subtle differences in maternal care are associated with the later emergence of several phenotypic differences. Interestingly, the maternal care phenotype appears stable across multiple generations, suggesting a strong genetic or epigenetic component.

12.3.2.2 Direct manipulation of maternal care quality

Beyond looking at the effects of natural variation in maternal care, more recent paradigms have been developed to experimentally produce extreme forms of fragmented or erratic maternal care, in an effort to mimic the quality of care that often characterizes depressed, severely stressed or drug abusing mothers. Baram and colleagues have developed a paradigm of 'simulated poverty' that provokes alterations in maternal care. In this model, on postnatal day 2, ELS litters and dams are transferred to cages with limited nesting material. Specifically, the bedding in the home cage is removed, cages are fitted with plastic-coated mesh bottoms and a single paper towel is

provided for rudimentary nesting maternal. The impoverished cage environment prevents the dam from constructing a satisfactory nest, which, in turn, results in chronic stress in the dam herself. This chronic stress for the dam alters the pattern of maternal care she displays, resulting in fragmented and erratic nurturing behaviours. Specifically, detailed analysis of maternal care in this model demonstrates a pattern characterized by shortened bouts of each nurturing behaviour and frequent shifts between different behaviours (Figure 12.2). There is also an apparent reduction in the drive or the ability of the dam to keep the pups in the nest area, as the dam spends more time away from the pups and the nest is more frequently dispersed. The disrupted maternal care leads to chronic stress in the pups, as evidenced by plasma glucocorticoid levels and by the presence of hypertrophied adrenal glands at the end of this one week stress period (postnatal day 9). The consequences of this ELS model are profound, including progressive loss of cognitive functions as the rats mature. Importantly, the limited nesting paradigm has been used to provoke chronic stress in mice and has been successfully adopted and modified by several other groups.

12.4 Validity and reliability of ELS models

Ideally, an animal model for ELS should be both reliable and valid.

Validity is a theoretical concept, referring to the accuracy with which the model reflects the human condition. When evaluating ELS models for human psychopathology, researchers often consider several subtypes of validity, including: predictive validity (the ability of the model to predict the outcome in the human condition, i.e. predict successful treatments); face validity (how similar the symptoms in the model match the symptoms in the human disease); construct validity (whether the theoretical rationale for the model matches the human condition); and etiological validity (whether the model's disease etiology, or underlying cause, matches the suspected cause of the disease in humans). Given the difficulty of modelling complex neuropsychiatric disorders in non-primate species, many rodent models of ELS will not meet all aspects of validity; however, these concepts provide useful tools to compare different models and design new and improved models over time.

Reliability refers to the consistency and stability of the variables in the model and is of obvious importance for replicating and expanding results over time and across laboratories. Although some models show generally consistent effects, there can be a striking lack of reliability. This variability may in part relate to methodological differences. Detailed descriptions of ELS protocols are thus extremely important to reproduce findings, as seemingly minor variations can impact the results. For example, in Sprague–Dawley rats, 6-hour daily separations on postnatal days 2–10 has no effect on activity in the open field, whereas 4.5 hour separations across the first 3 weeks of life reduces open field activity.

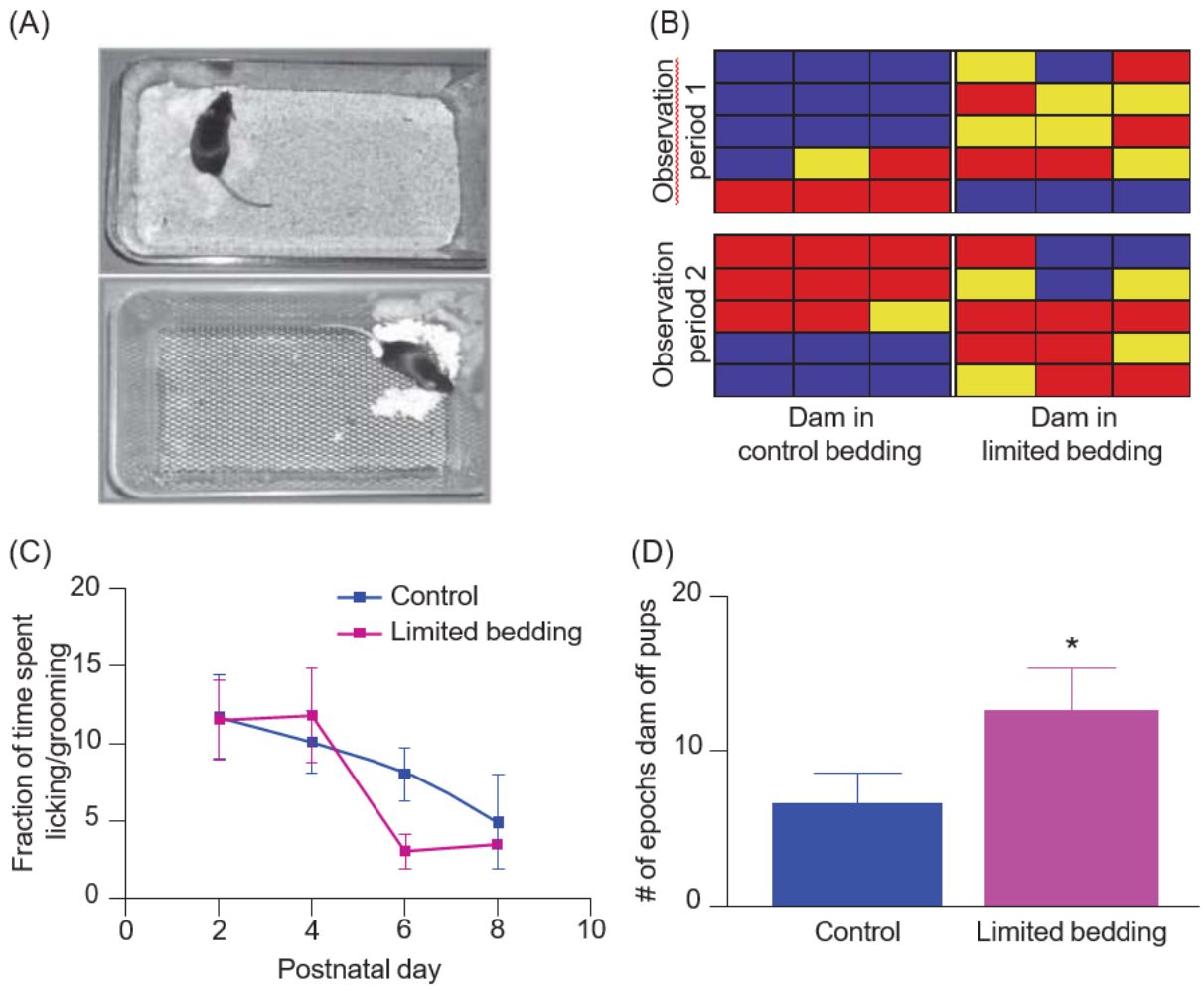

Figure 12.2 Limited nesting model. (A) Photographs demonstrating the setup of a control cage in comparison with limited nesting material cages in a mouse system: top, control dam in a cage with the standard amount of bedding and one square of felt-like nesting material; bottom, limited nesting cage with minimal bedding and limited nesting material (one half square). Note the elevated mesh platform that permits dropping elimination. (B) Representative activity grids of control and stressed dams during two matched observation periods. Each grid depicts one dam's activity during 15 1-min epochs. Individual panes are colour-coded to represent the dam's location/activity during that epoch: blue, dam in nest in contact with pups for entire epoch; red, dam outside nest area for entire epoch; yellow, a mixed activity epoch. The consistency of control dam behaviour is contrasted with the fragmented pattern in the limited nesting dam. (C) Interestingly, the fragmentation resulted in little change in total care: the total licking/grooming duration among dams in the limited nesting cages was comparable to that of dams in the control condition. (D) The limited nesting environment also increases the number of epochs the dam spends away from the nest, an additional measure of fragmentation of care. Adapted from Baram et al., 2012, and reproduced by permission of the American Psychiatric Association.

Other sources of variability may relate to the background strain of the rodent (see below) or whether animals were bred in-house or shipped from commercial vendors. If animals are purchased from a commercial vendor, the time at which the animals are shipped may have an impact on the study, as the shipment process itself (housing, transport and handling) may serve as a potent stress for the subjects.

Beyond these technical considerations, variability may also reflect fundamental underlying biological mechanisms, such as sex differences or pre-existing genetic factors. Given the range of variables to consider, we have attempted to identify those that are considered critical for comparing and interpreting results across studies.

12.4.1 The timing of stress manipulation

It was initially assumed that the neonatal period represented more or less stable, unidirectional processes of brain development, such that the effects of stress across this period were consistent and cumulative. However, this assumption has turned out to be largely inaccurate, as even within the early postnatal period defined here, the direction and magnitude of stress-induced changes can vary dramatically according to precisely when the stress occurs. This has been directly tested by studies using a single 24-hour MS procedure; for example, a 24-hour MS during postnatal days 3–4 leads to a hyper-responsive HPA axis later in life, whereas the same MS procedure just days later (postnatal days 7–8 or 11–12) results in a hypo-responsive stress system. Similar differences have been extended to learning and memory functions: MS on postnatal day 3 impairs active avoidance and conditioned freezing, whereas MS on postnatal day 9 improves performance in these tasks. These data suggest that the early-life period consists of complex and overlapping developmental processes and that these may drastically influence the consequences of any environmental perturbations.

12.4.2 Genetics: species, strain and sex differences

12.4.2.1 Species and strain

Although rarely directly compared within the same study, differences in the direction and magnitude of the effects of ELS have been noted across different rodent species and strains, suggesting an interaction between stress and genetic factors. Thus, results from rats and mice, or even from different strains within the same species, should be compared with caution. For example, repeated MS during early life has divergent effects on anxiety and stress responses in male Wistar rats bred for either high or low anxiety-related behaviour. Significant strain differences in the consequences of MS models have also been reported in mice (for a comprehensive review, see Millstein and Holmes, 2007). In fact, a recent study suggests that C57BL/6 mice may even be resilient to MS, whereas Balb C mice might be sensitive to even 15 minutes of MS. Although these differences make broad interpretations difficult, they highlight the potential influence of pre-existing genetic factors in the development of cognitive and emotional functions, and their vulnerability to environmental factors.

12.4.2.2 Sex

Until relatively recently, most of the research on ELS has focused exclusively on males. This bias has led to some overgeneralizations on the effects of ELS; those studies that directly compare males and females often report sex-dependent effects. As a general rule, males appear more vulnerable to the deleterious effects of ELS, although it often depends on the type of behavioural test used. For example, the MS-induced increases in anxiety observed in male rats are often attenuated in female rats or may even be absent. More recent research has found that the effects of MS on cognitive function are also dependent on sex, even prior to the onset of puberty: MS impairs spatial and non-spatial memory performance in pre-pubertal Long Evans male rats, but has little effect (and in some cases enhances) performance in age-matched females.

The recognition that sex modulates stress processing, and in particular the vulnerability to ELS, has significant relevance to the human condition; compared to males, females are approximately twice as likely to develop stress-related emotional disorders, such as depression and anxiety. Although the underlying cause(s) for the sex-dependent effects of stress on brain function are still under investigation, they are likely to involve the interaction of the stress system with gonadal hormone receptors through- out the brain, including the hippocampus.

12.4.3 The nature of the comparison group

The choice of the comparison group has been identified as an additional important factor. Initial studies typically compared MS animals to completely undisturbed or ‘non-handled’ animals (NH). However, this completely undisturbed condition is different from standard housing conditions in animal facilities, which involve regular cage changes, and thus regular handling and environmental stimulation. Thus, ‘animal facility reared’ (AFR) animals, which are maintained with regular husbandry conditions, perhaps reflect a more ‘normal’ control group.

Interestingly, the nature of the effects of ELS often differs according to which control group (NH or AFR) is used for comparison. For example, when compared to the AFR condition, MS generally leads to increased anxiety, but these effects are not found if MS animals are compared to completely NH animals. Measures of the stress response also yield inconsistent results; some studies report comparable corticosterone levels between MS and NH animals, whereas others report higher stress-induced corticosterone levels in NH compared to MS animals. Furthermore, although MS increases CRH mRNA expression throughout the brain compared to briefly handled rats, they are not distinguishable from NH controls.

12.5 Consequences of chronic early-life stress

Although a complete review of the consequences of ELS in rodent models is beyond the scope of this chapter, this section attempts to summarize the most consistent findings associated with two prominent models: maternal separation and limited nesting environment.

12.5.1 Maternal separation

12.5.1.1 Effects on the HPA axis

By far the most consistent results from MS models are related to alterations in the development and function of the stress system. As mentioned above, despite the relatively hyporesponsive HPA axis in early life, MS results in a marked activation of the stress system in both rat and mouse pups. Beyond this acute activation, studies by Levine and colleagues (Levine, 1957) suggest that MS may accelerate the development of the HPA axis in rats, resulting in an earlier termination of the SHRP: following a single 24-hour MS procedure on postnatal day 11, 12- or 16-day-old rat pups have a potentiated hormonal response to novelty stress, responding more or less identically to adults.

The effects of MS on the HPA system appear to be permanent. As adults, rats exposed to MS have increased baseline circulating ACTH and corticosterone levels, as well as increased CRH-immunoreactivity within the PVN. MS permanently reduces expression of both glucocorticoid and mineralocorticoid receptors within the hippocampus, suggesting an impairment in the negative feedback regulation of the HPA axis. Similarly, adult rats exposed to repeated maternal separations early in life have exaggerated hormonal and neural responses to various acute stressors. Taken together, these data are consistent with a higher 'setting' (i.e. the level at which an automatic control is set to operate) of the HPA axis.

12.5.1.2 Effects on emotional function

Although less consistent than the effects on the HPA axis, MS is generally associated with changes in emotional function, including increases in anxiety and/or depressive-like behaviours. Following repeated or prolonged MS, adult rats often have heightened measures of anxiety and an increased arousal or fear in response to novelty. MS has also been reported to decrease sucrose consumption and increase passive swimming in a forced swim task, both measures of a depressive-like state. Importantly, negative results (i.e. no effects) on both anxiety and depressive-like behaviour have been reported and seem particularly inconsistent when MS rats are compared to completely non-handled controls. For depressive-like behaviour, the most dramatic outcomes are observed only when MS adults are exposed to additional chronic stress, suggesting that early-life stress increases the vulnerability to a 'second hit' later in life. As such, one of the major consequences of early-life stress may be to alter the subsequent effects of additional stressors experienced later in life, somehow lowering the threshold for a negative impact of the stressors. Interestingly, the increased anxiety following MS is attenuated if MS rats are housed with control rats after weaning, indicating that the quality of subsequent social experience may modulate the effects of early-life stress.

12.5.1.3 Other effects and considerations

Beyond stress and emotional function, MS seems to have a broad impact on the development of several other systems. For example, both spatial and non-spatial learning and memory impairments have been found following MS, often associated with morphological changes in hippocampal neurons. MS has also been found to alter the pattern of male reproductive behaviour, ethanol consumption and even the dopamine reward pathway and responses to drugs of abuse. Although these and other consequences of MS are still under active investigation, they are often associated with changes in activity in various neurotransmitter and neuropeptide systems, as well as alterations in levels of several neurotrophic and growth factors. It should be noted that the majority of MS models have employed rats.

As mentioned above, there is evidence that certain mouse strains differ significantly on measures of emotional and stress-related behaviours and have differential sensitivity to the effects of MS. Although there have been relatively fewer studies on the long-term effects of MS in mice, several report MS-induced increases in anxiety-like behaviour and stress reactivity in adulthood in C57BL/6J mice. The importance of strain (i.e. genetic) differences cannot be ignored (Millstein and Holmes, 2007).

12.5.2 Limited nesting environment

12.5.2.1 Effects on cognitive function and the hippocampus

Initial studies using the limited nesting model focused on cognitive functions, and specifically on learning and memory. Because deficits in hippocampus-dependent learning and memory were observed, attention focused on development of the hippocampus. It has been well established that the hippocampus is particularly vulnerable to the effects of stress and when that stress occurs early in life, the deleterious effects are often permanent. It is important to note that when rats exposed to the limited nesting model described above reached adulthood, the neuroendocrine parameters of their stress system returned to baseline and were in fact indistinguishable from those of conventionally reared rats. However, despite the lack of permanent alterations in the stress response system, this form of ELS leads to enduring and profound changes in learning and memory, probably as a result of altered developmental growth and establishment of synaptic connectivity, leading to abnormal structure and function of hippocampal neurons.

During young adulthood, rats that were exposed to the limited nesting environment performed quite well in the Morris water maze (MWM) test of spatial learning and memory. Long-term potentiation (LTP) in response to high-frequency stimulation is also normal in both areas CA1 and CA3, although subtle changes in the properties of CA3 pyramidal cells are evident at this age. However, by 7–10 months of age, rats exposed to the early-life stress have significantly impaired performance in the MWM, as well as in the relatively stress-free novel object recognition test.

12.5.2.2 Mechanisms of hippocampal dysfunction

In an effort to identify the possible mechanisms for the observed impairments in learning and memory function following ELS, atrophy of hippocampal CA1 and CA3 apical dendrites was discovered, with commensurate loss of dendritic spines. Because dendritic spines carry excitatory synapses in the hippocampus, these findings have important implications for information processing and memory. Indeed, accompanying these structural changes, the early-life stress also attenuated LTP in both CA3 and CA1 neurons. Thus, a single week of chronic stress, induced simply by limiting the nesting material of the home cage for the first week of life, provokes enduring and potentially progressive disturbances in synaptic plasticity and in memory processes, at least in part via loss of dendrites, dendritic spines and excitatory synapses (Figure 12.3).

Of the possible molecular signals acting within the hippocampus, CRH has been identified as a critical mediator of the consequences of ELS. Interneurons expressing CRH are abundant in the hippocampus and these cells release the peptide into the intercellular space during stress. Moreover, the primary receptor for CRH, CRH receptor type 1 (CRHR1), resides on dendrites of CA1 cells, the same neurons sustaining dendritic atrophy after early-life stress. Strikingly, administration of a pharmaco- logical antagonist of CRHR1 immediately following the ELS period has been shown to rescue cognitive function, dendritic structure and synaptic plasticity of early-stressed rats. Consistent with these rat studies, mice lacking CRHR1 in the forebrain are resistant to the deleterious effects on hippocampal structure and function following chronic early-life stress.

12.5.2.3 Other effects and considerations

It is important to note that although the initial studies using this paradigm focused on learning and memory function, the limited nesting model also leads to profound changes in emotional behaviour as well, including depressive-like behaviour following subsequent stress. Sullivan and colleagues (see Moriceau et al., 2009) have adapted this model to study the development of early olfactory learning and found that rearing pups in a limited nesting environment disturbs the development of pup attachment behaviour via an amygdala-locus coeruleus-olfactory bulb network perturbation. Although still relatively new, the continued adoption of this model may provide a valuable tool for identifying the long-term consequences of ELS on brain development and vulnerability to disease, and the underlying mechanisms.

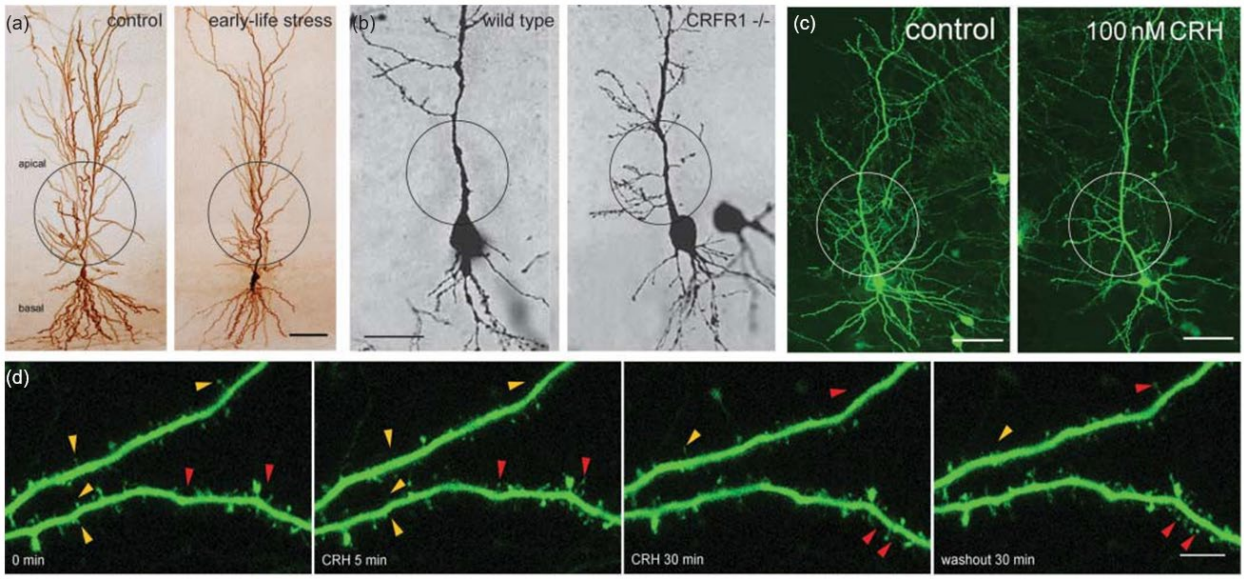

Figure 12.3 Early life stress (ELS) and the hippocampus. ELS shapes the hippocampal dendritic structure, probably through a CRH signalling mechanism. (a) Dendritic impoverishment in pyramidal cells of adult rats that have experienced ELS via the limited nesting model. Photomicrographs of biocytin-labelled CA1 pyramidal cells illustrate the reductions in total dendritic length and dendritic arborization in the early-stress group (right) compared to controls (left). Scale bar, 80 μm. (b) In the absence of CRHR1, the dendritic trees of CA1 pyramidal neurons are exuberant. Photomicrographs of Golgi-impregnated CA1 pyramidal cells of postnatal day 6–7 mice show increased dendritic length and branching in CRHR1 knock-out mice (right) compared to wild-type mice (left) (note that these images are from standard-facility reared mice; ELS was not used in this study). Scale bar, 40 μm. (c) CRH application on to hippocampal organotypic slice cultures reduces dendritic complexity. Cultures were prepared from postnatal day 1 yellow fluorescent protein (YFP)-expressing mice and grown either in control media (left) or in the presence of CRH (100 nM; right) for 2 weeks. Scale bar, 70 μm. The circles in (a) to (c) illustrate the similar distribution of dendritic changes induced by stress and altered CRH signalling. (d) A potential mechanism by which CRH may attenuate dendritic length and arborization is through an initial loss of dendritic spines: infusion of CRH (100 nM) on to hippocampal organotypic slice cultures leads to a rapid and reversible loss of spines. High-magnification imaging reveals accelerated spine disappearance that is apparent already 5 min after the onset of CRH exposure; CRH-induced spine elimination is partially reversed by a 30 min washout. Red arrowheads denote newly formed spines; yellow arrowheads show eliminated spines. Scale bar, 6.6 μm. Adapted from Maras and Baram,2012, and reproduced by permission of Elsevier Limited.

12.6 Perspectives

Animal models of ELS provide useful tools to study normal adaptation, as well as the basis of resilience and vulnerability to stress-related disorders. Whereas much has been accomplished through the use of these models, several challenges remain. These include:

- Identifying interactions between ELS and stresses experienced later in life. Individuals exposed to ELS are likely to experience stress throughout their life span. How do early stressful experiences impact the consequences of stress experienced later in life? Are there other sensitive windows during which the brain is particularly vulnerable to stress (e.g. adolescence)?

- Determining the importance of genetic predispositions on the impact of ELS. Although early-life adversity is a major risk factor for later pathology, there is substantial variability in the outcome of ELS. What genetic (or epigenetic) factors impart vulnerability or resilience to the effects of ELS?

- Elucidating the nature and underlying biology of sex-specific consequences of ELS. Human epidemiological data indicate that stress-related psychopathologies are more prevalent in females, yet there is a paucity of rodent models studying how sex modulates vulnerability to ELS. How do genetic and hormonal sex differences regulate the consequences of ELS?

- Addressing the daunting issue of the generalization and translation of rodent studies to the human condition. Rodent models have proven extremely useful in describing the consequences of ELS on brain development and identifying mechanisms of psychopathology. However, it is absolutely critical to continually aim to apply these findings and theories back to the human condition.

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Appendix. Protocol for limited nesting model of ELS

Note. This model has been validated in both rats and mice, but may be adapted to any altricial rodent species in which the dam provides significant levels of maternal care. All animals should be housed in temperature-controlled, quiet, uncrowded conditions on a 12-hour light, 12-hour dark schedule, with free access to food and water.

Set up pregnant females (dams)

- House dams in an all-female room, preferably used exclusively for breeding purposes.
- Order time-pregnant females from your animal supplier or arrange to breed animals in-house (see below).
    - To limit the effects of previous experience on the dam's maternal behaviour and response to stress, always use virgin naïve females.
    - Time pregnancies so that at least two dams will give birth within the same 24-hour period.
    - This is important because pups will be mixed across litters on the day of manipulation (see below). Given the viability of your conditions, this may require ordering or breeding spare females.
    - If breeding litters in-house:
        - Pair a single male with a single female in a standard cage for up to 10–12 days to allow sufficient time to mate. During that period, check the female's vagina and cage floor twice daily for the presence of a sperm plug, a hardened or gelatinous secretion indicating the male has successfully deposited sperm. Remove the male from the breeding cage as soon as a sperm plug is confirmed (pregnancy/gestation day 0) or, if none is observed, at the end of the 10–12 day period. Leave the female undisturbed, except for checking for a swollen abdomen (indication of pregnancy) at 14 days after finding a plug or daily between 2 and 14 days after removing the male.
- Once dams are pregnant, limit disturbing them.

    - Keep the housing room as quiet as possible and limit the number of times cages or racks are moved. Depending on husbandry regulations, this should involve temporarily stopping cage changes from gestation day 15 through the termination of the stress exposure (note that the experimenter will change cages on postnatal days 2 and 9).
- If breeding in-house, check for births at least twice daily on the days sur- rounding expected parturition; make note of any pups and the time they were observed. Check for births twice daily beginning at 21 days after initial pairing with the male through 21 days after removing the male. Note the first presence of any pups and the time they were observed; day of birth is commonly noted as postnatal day 0 (PND0).

Prepare limited nesting cages

- Start with clean, empty standard housing cages.
- Position a fine-gauge aluminum mesh platform (mesh dimensions 0.4 cm × 0.9 cm, catalogue no. 57398; McNichols Co., Tampa, Florida) to sit approximately 2.5 cm above the cage floor.
    - Fold edges of the mesh along the length approximately 3 cm so that platform sits above the bottom of the cage, permitting droppings to fall below the platform without trapping the pups. To reduce odours, cover the cage floor with a small amount of standard bedding material (do not add enough for dam or pups to contact bedding material).
- Provide a limited amount of nesting material on top of the mesh platform.
    - We have found that at least some nesting material is required to reduce pup mortality. For rats, add one-half of a single paper towel to the cage. For mice, add one-half of a single NESTLET square (Ancare, Bellmore, New York).

Limited nesting manipulation

Note. This procedure should be done in the morning of postnatal day 2. To reduce the influence of genetic differences across litters, any pups born within the same 10–12 hour window can be mixed together. It is important that the separation time is not more than 15–20 minutes.

- For each litter, quickly and gently remove all pups from the home cage; identify the sex of each pup (using anogenital distance) and place males and females into separate, euthermic holding cages. Repeat for each litter, keeping separate holding cages for male and female pups.
- Once all litters are removed and sorted, randomly assign dams to the control or limited nesting conditions.
    - Control cages. Place the dam into a fresh, clean standard cage (with normal amounts of bedding and nesting material). Randomly transfer pups from the male and female holding cages to the control cage with the dam.
    - Limited nesting cages. Place dam into the limited nesting cage (with mesh platform and limited bedding and nesting material). Randomly transfer pups from the male and female holding cages to the experimental cage with the dam.

Note. Litter size should be held as constant as possible (4–6 per mouse litter; 10–12 perrat litter) and the final sex ratio should be approximately 1:1. Counterbalance the order inwhich you replace pups with the dams between control and ELS conditions to limitdifferences in the total duration pups are separated.

- Leave control and limited nesting cages undisturbed (unchanged) until postnatal day 9.
    - Observed maternal behaviour during that period, as described in Ivy et al. (2008).
- On the evening of postnatal day 9, change all cages (control and ELS) to fresh, standard cages with normal bedding.
    - Return cages to standard husbandry and changing schedules.
- On postnatal day 21, wean animals from the dam. House same-sex littermates in the same cage.