An assessment of the validity of laboratory animal behavioural research as translatable models of early childhood adversity

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Summary

In mammals, early-life experiences, from post-partum to adolescence, shape the response to chronic stress and its related disorders, later, during their adult lives. Laboratory animals (predominantly rodents) are used in preclinical research models of early life adversity, to determine the pathogenesis of stress-related psychiatric disorders, such as depression, Post-Traumatic Stress Disorder (PTSD) and anxiety disorders. By tracking the behaviour of rodents and obtaining neurochemical data, the impact of early-life traumatic events can provide insights into stress-induced risk of psychiatric disorders in humans. The value of using rodents in models of childhood adversity is dependent on an integrative approach of finding common endophenotypes between the species.

This review finds that there are deficiencies in dealing with conceptual issues, including genetic influences and inter-individual differences in vulnerability or resilience to negative consequences of stress. The need for standardisation and quality control, incorporating better experimental design and an understanding of the strengths and limitations of the models used, is prominent in current literature. This review outlines the frameworks and hypotheses that form the basis for the use of rodent models of early childhood adversity.

The outcome of this review is that there is justification for the use of laboratory animal models to investigate individual symptoms, or markers, of early childhood adversity and associated psychiatric disorders. The frequently used rodent paradigms of early years adversity, provide a significant wealth of information of the impact of early-life stress on the functioning and architecture of the relevant brain regions and associated behavioural and physiological changes in mammals. Future research should focus on developing high quality rodent models of early childhood stress with the benefit of input from specialists designing human clinical research. This, combined with reproducible, carefully designed experiments, incorporating translatable rodent models, will help achieve increased validity of preclinical research of early childhood adversity.

Introduction

Context

The author is the laboratory animal facility Manager and oversees the research and the provision of access to laboratory animal services to the Life Sciences, and *in vivo* research groups in University College Cork (UCC), Ireland. The diagram below, outlines the daily duties that form a context for interaction with laboratory animal behavioural research, that is linked to the research hypotheses of this thesis.

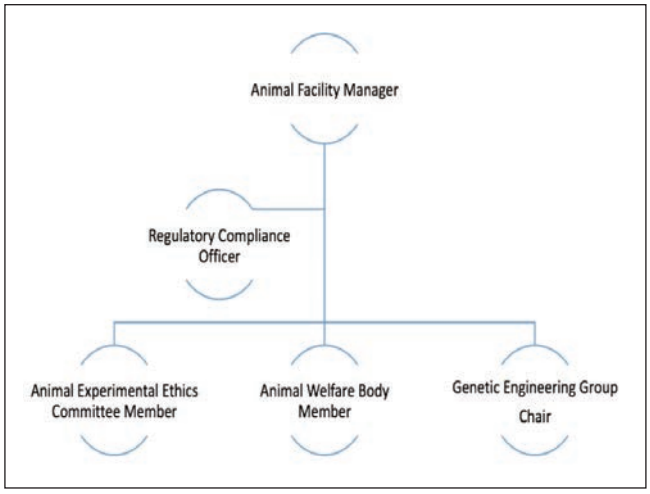


Figure 1. Relevance of career pathway to this FIAT Thesis.

Background

A laboratory animal model is an experimental paradigm specifically designed to study a known phenomenon present in humans. The current animal models, incorporating rodents, are not complete models of human early-life stress but are designed to provide information on specific elements of the disorder being assessed. All rodent models of early childhood adversity, provide information on the biological basis of anxiety and depressive disorders in humans but due to the complex nature of these disorders cannot be compared directly to the human disorders.[1] Figure 2 provides an overview of the principle areas researched in this thesis.

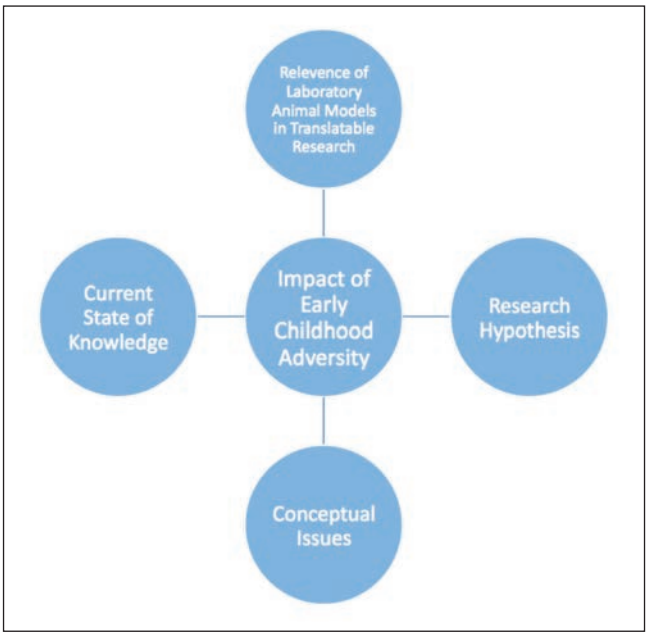


Figure 2. Areas of research for FIAT thesis.

Adverse early life experiences are believed to have a lifelong impact on the mental health and emotional quality of life experienced thereafter. One such early life adversity is a deficiency in the "normal" mother-offspring relationship in early life.[2] Maternal Separation/Maternal Deprivation (MS/MD), which is the most widely used animal model for early life (intermittent) stress, involves removing rodent pups from their mother for protracted periods of time from postnatal day (PND) 2.[3] More recent research has resulted in the development of models that include chronic, rather than intermittent stress, in which the mother rat is present or in close proximity, to the developing pups but in environments that adversely affect and interrupt the normal nurturing of the offspring.[2,4]

For a model to be translatable, there is a requirement to identify common endophenotypes (elemental components of a given disorder that have been shown to have the same effect in both species) which can be studied under a synchronised experimental regime.[5]

To achieve this refinement and standardisation of experiments input from specialists in the areas of psychiatry, scientific research, scientific ethical review, genetics, psychology, behavioural sciences and neurosciences will be required.[6]

Methodology

Introduction

In researching the validity of laboratory animal models for early childhood adversity, a detailed review of the literature relating to both animal models of early childhood adversity and the impact of early childhood adversity on subsequent mental health was undertaken. University College Cork library was the primary source.

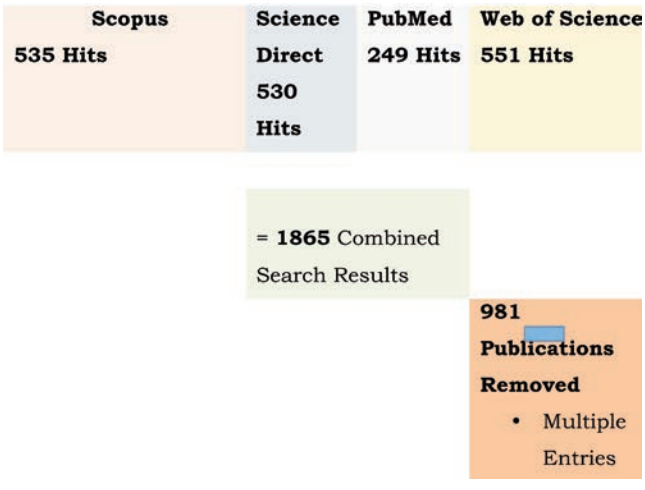
Using 'nested' searches, the keywords used were (rodent OR model) AND (early life OR juvenile adversity) AND (maternal OR separation) AND (anxiety OR depression) AND Validity). These search words were entered in the following databases: PubMed, Scopus, Web of Science and Science Direct (Figure 3).

All final selection publication references were saved to the Mendeley referencing system library.[10] Mendeley's function of alerts for relevant journals and articles of interest, produced a significant number of the additional publications used in this research.

The author is a reviewer of behavioural research projects (Figure 1) involving models of early life adversity in both rats and mice and therefore also, had access to relevant journals through the references provided during these reviews.

The search was refined to 71 peer reviewed journals published 2007-2019 (Figure 3). The journals returned and saved to Mendeley library, were sorted into categories in accordance with the objectives of the report (Table 1). The Vancouver referencing system is used throughout this thesis.

Data-Bases Search Results



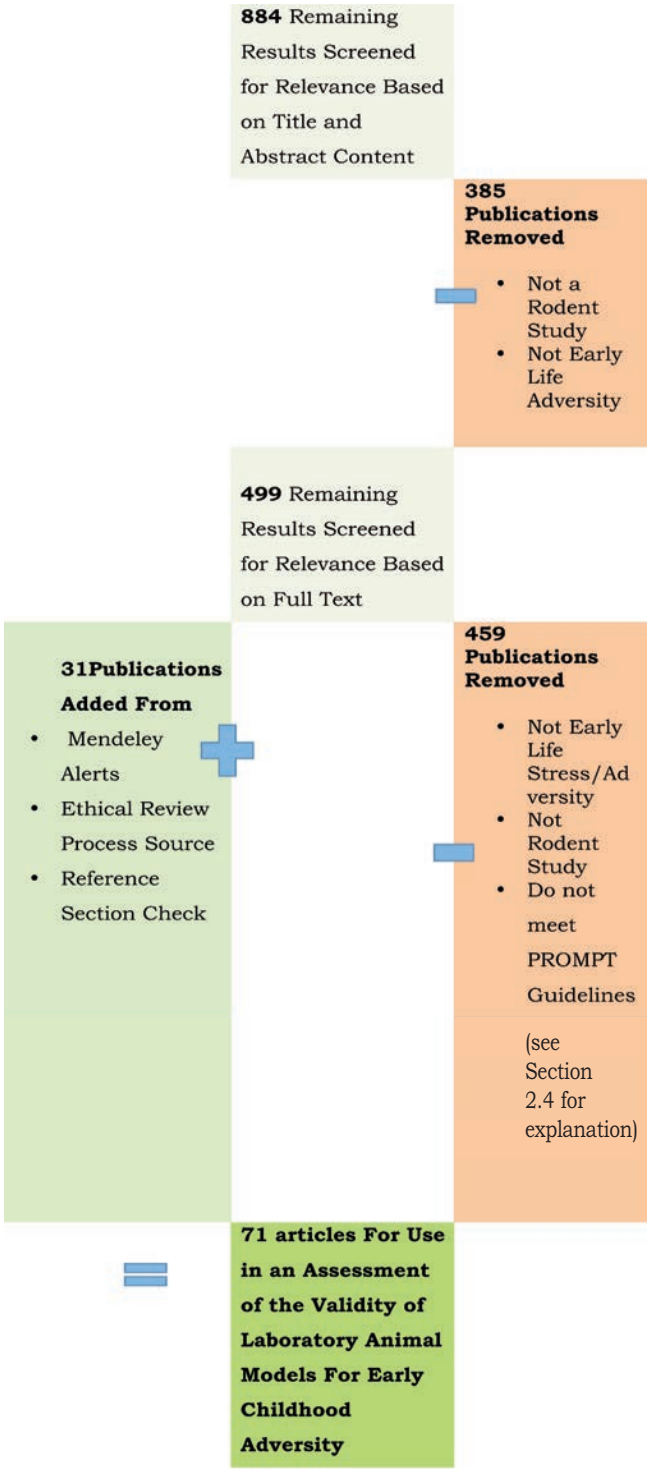


Figure 3. Selection process for articles for systemic review of the validity of Laboratory Animal Models of Early Childhood Adversity. (Adapted from [11])

Categories of Articles in Final Selection

The majority of the journals selected were original research articles. In order to develop chapters by themes, the articles were categorised and saved as per Table 1. Not all articles listed are cited in the references, as some were retained for supplemental reading/understanding of the research into early life adversity and the complexity of the animal models developed to investigate them.

Article Subject Matter	Number	Publication Year (Range)
Detailed rodent experimental models of early life adversity	25	2011-2019
Behavioural and psychological impact of early life adversity/stress in both humans and rodents as evidenced by published research	29	2010-2019
Conceptual issues relating to the use of rodents as models for early life adversity	10	2011-2017
Quality control and public interest	7	2011-2018

Table 1. Categories of journal articles in the final review.

Data Analysis

The information was evaluated using Presentation, Relevance, Objectivity, Methodology, Provenance, Timeliness (PROMPT) guidelines.[12] This included an evaluation of whether the information was **presented** in a clearly laid out structure. The **relevance** of the material to the objectives of the project was also evaluated. The **objectivity** of the author of each article was assessed for bias or vested interests. The **methodology** was assessed for being appropriate and trustworthy. The **provenance** of the material was assessed against the supporting institutions and publisher. The **relevance** of the information was checked during the filtering stages and old/obsolete material discarded. The **timeliness** of the article's publication in relation to the research cited within was also assessed.

A meta-analysis of the data collected was not possible in this project as quantitative data provided in the 25 animal model studies could not be analysed against the quantitative data published from the 9 human clinical studies. The variety of the information gathered in the total 71 studies was deemed to provide insufficient information for an effective meta-analysis.

The impact of early childhood adversity

Background

The postnatal period of development in both rodents and humans has been identified as a significantly sensitive and important window of brain development. Indeed, growth and connectivity in the brain is at its highest during the early developmental years.[13]

The mother-child relationship during this time is seen as a critical influence on the development of the offspring. Adversity during this period such as trauma or parental neglect are identified as a major risk factor for psychiatric disorders such as Post Traumatic Stress Disorder (PTSD) and depression, later in life.

Causal Factors of Early Life Adversity

Children and adolescents experience traumatic incidences in their lifetime, with 15 to 20% experiencing a relatively severe encounter.[14] Stress can be defined as a common, frequently occurring experience which incorporates a variety of physical, psychological and social factors in mammals, including humans.[15] The stress response is an important and necessary mechanism to adapt to environmental changes and challenges. However severe, traumatic and chronic stress can constitute a serious threat to the physical and mental health of the individual experiencing the adversity. There are many factors that cause early childhood adversity, for example, lack of adequate nutrition, poverty and parental neglect. This research reviews the use of animal models of early childhood adversity with a significant focus on the influence and the impact of the mother and her presence/absence on the offspring.

Early life adversity experienced from, or in the presence of an attachment figure, most normally the mother, has been shown to produce more detrimental effects, both immediately and in the long term, than adversity experienced in situations where the mother is absent and the juvenile is in isolation.[16] Moreover, studies on children in orphanages indicate that the environmental impact of inadequate maternal and social stimulation and support drastically increases the risk of subsequent psychiatric disorders.[13] In addition, early life adversity results in an inability of the developing child to regulate emotions in a normal, healthy manner and these children have limited adaptive skills in comparison to children reared in a non-aversive environment.[17]

Vulnerability

Vulnerability, in the context of early life adversity relates to sensitive periods when environmental cues exert a maximum impact on the developing organism.[18] Vulnerable periods differ from critical periods, in that critical periods require an 'all or nothing' response. The sensitive period of vulnerability is thought to be an evolutionary development in order to condition the organism to adapt to the changing environment. Vulnerable individuals have preconceived expectations of the future, based on the immediate environmental information gained but are at risk from adversity that differs from the expected adversity.[19]

A three hit concept of vulnerability to the negative effects of adversity and stress proposes that genetic predisposition, coupled with early life environment plus later life environment, result in cumulative stress, increasing vulnerability and a difficulty in stress coping resulting from early life adversity.[20] This is supported by an assumption that the vulnerability genes and plasticity genes are one in the same. This suggests that early life adversity in combination with genetic predisposition can lead to programming effects in the brain which can manifest to unpredictable outcomes in later life, once challenged with adult adversity (Figure 8). The nature and severity of the adult adversity are also mitigating factors in the psychiatric outcome.[21]

Resilience

Not all children that experience early life adversity and trauma develop a psychiatric disorder in later life. Although the basis for resilience is unclear, there is evidence that some children are resilient, while others are susceptible, when exposed to similar adversity in early life.[22] This suggests that the brains of the resilient individuals might be pre-prepared through gene-environment preprogramming, to be resilient, unlike the brains of the vulnerable individuals.[23] On the other hand, research also indicates that early childhood adversity, artificially induced in rodents, protected against or slowed down the occurrence of pain hypersensitivity.[24] This suggests that these rodents were capable of developing resilience as a result of being able to adapt to later challenges, following early life stress exposure.

Adaptation

The post-natal period of brain development is characterised by higher rates of remodelling, synaptic re-organisation and neuronal plasticity. The view that most early life stress is maladaptive is widely held but is also disputed in some publications, for example several hypotheses, detailed in this thesis, suggest that a certain level of childhood adversity may confer some beneficial adaptation properties on the developing organism.[25] Andrew Sih (2011) proposes a behavioural ecologist's view, that moderate stress, experienced at the 'right' time prepares the developing mammal by inoculating and developing a stress response model for the future.[26] This research also suggests that maternal influence and emitted cues may be focussed on preparation of the offspring for independent living, by providing a representative level of care and attention during early development. The impact of genetic predisposition is also a factor in the impact of early childhood adversity in humans.[5]

In order to research adaptation, the effects of MS/MD model in two strains of rats, the stress normo-sensitive Wistar strain and the more stress susceptible Wistar-Kyoto (WKY) which have an innate heightened vulnerability to stress were assessed in behavioural paradigms. The strains that underwent MS/MD showed a marked difference in their response to anxiety-

provoking anxiety behavioural tests. Adult WKY rats that underwent MS showed increased coping behaviour in the behavioural tests in comparison to the stress normosensitive Wistar strain which showed increased anxiety-like behaviours. This indicates that the genetic predisposition to stress-like phenotype prepared the WKY rats for future stress later in adult life. This supports the theory of an adaptive capability in mammals (in this case, a genetic predisposition) to deal with early life adversity and proposes that early stress exposure might actually confer protective properties on the developing mammal that will be beneficial in later adulthood.[27]

Not all of the effects of early life adversity are irreversible. Behavioural plasticity facilitates adaptation in order to arm mammals for unanticipated adverse events.[21] The effects of moderate early life adversity could potentially be beneficial in all mammals, even those without a genetic predisposition to anxiety and depression.[28] Previous research identified that development of the fear instinct in normally reared rats occurred at post-natal day (PND)10,[2] however rats reared under the Chronic Early Life Stress (CES) model displayed an earlier emergence of fear instinct at, PND 7, due to adaptation of the amygdala, the threat recognition region in the brain.[29]

Pathological Consequences

The central nervous system (CNS) is designed to cope with the stress threat to evaluate the threat and make a decision to fight, flight or freeze. Under certain conditions, for example, early development stage, the stress adaptation capability of the CNS fails and this results in long term cognitive, emotional and mood disorders. This suggests that early childhood adversity is a major causal factor in the pathogenesis of many disorders, such as anxiety and depression, in later life (See Figure 4).[30] The impact of early childhood stress and trauma at specific developmental stages doubles the risk of psychopathology, primarily in the forms of anxiety, depression and behavioural problems.[14] Environmental adversity induces remodelling and can imprint morphological, neurological and behavioural changes on the developing brain with chronic effects.[21] In the pathogenic state, the amygdala becomes hyperesponsive and exhibits neuronal hypertrophy. The brain becomes damaged by neuroinflammation and the neurotoxic effects of cortisol, the hormone released during stress on brain function and structure, which in turn leads to reduced hippocampal neuronal plasticity. Cortisol is released in humans and corticosterone is released in rodents.[31] The volume of the hippocampus is reduced as a result of neuronal atrophy. The medial Prefrontal Cortex (mPFC) also exhibits atrophy. This pathology may explain some of the symptoms of PTSD and depression such as cognitive impairments (due to disruption of the HIPP and mPFC functioning) and heightened anxiety (See Figure 4).

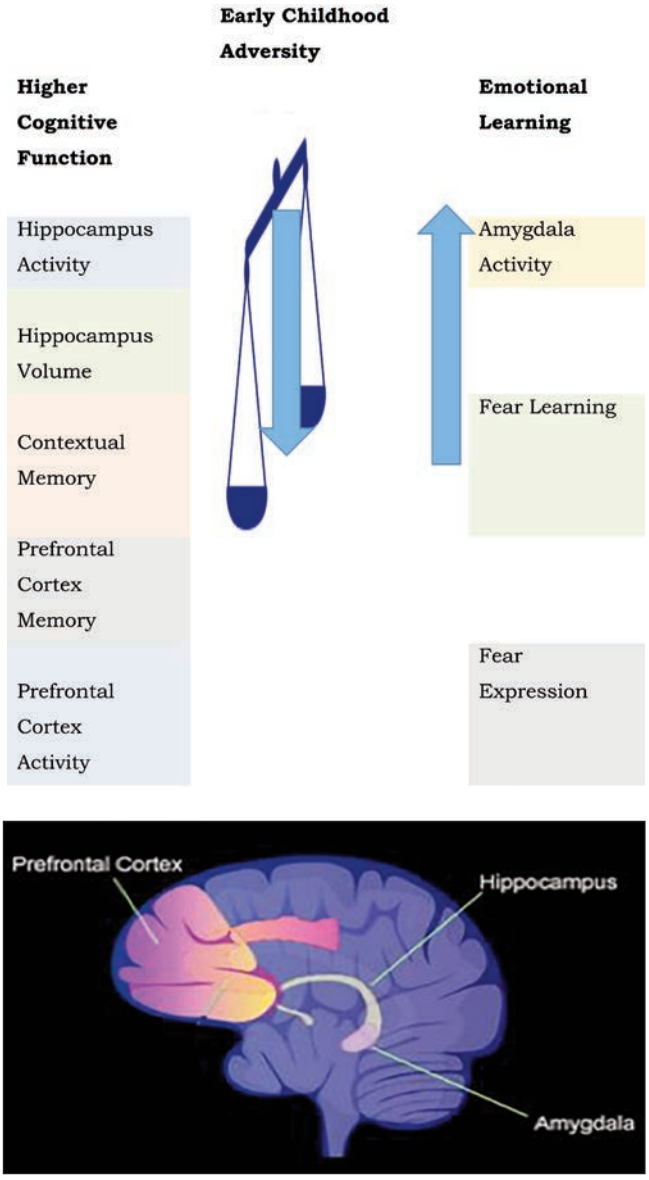


Figure 4. Impact of early childhood stress on higher cognitive function and emotional learning. (Adapted from Krugers, H.J. *et al.* 2017[13]), image courtesy of Google Images) Illustration of the brain regions associated with early childhood stress: Prefrontal Cortex, Hippocampus and Amygdala.

(Photo courtesy of www.psypost.org)

Laboratory animal models of early childhood adversity

Background

The use of laboratory animal models has been developed to examine the biological basis and cellular and molecular pathways of human diseases. Humans and certain mammals have similar genetic and epigenetic (the biological mechanism for controlling gene expression without altering the genetic code) markers as well as other peripheral characteristics in common. This forms the basis for the use of laboratory animals, predominantly rodents, in researching human

diseases such as stress related psychiatric disorders. Animal models of such related psychiatric disorders are used because there are limitations in the study of the neurobiological mechanisms of anxiety and depression in humans. The use of blood and faecal matter, from samples taken in human clinical studies, for *in vitro* research has limited value in terms of gaining behavioural or psychological data. Human post-mortem brain tissue, when available, may have been compromised by drug treatment and comorbid diseases and *in vivo* neuroimaging techniques do not have resolution at a cellular level, thus precluding the study of the human brain at cellular and molecular level.[5] Therefore models of laboratory rodents have been developed, over decades, to deliver a mechanism for studying the biological imprint factors and behavioural consequences of early life adversity.[4] The European Scientific Animal Protection (SAP) legislation ensures that the animals used in such studies are necessary, the numbers of animals required is justified and that all pain and distress is kept to an absolute minimum.[32] Here, 3 different laboratory animal models of early life adversity will be presented.

Maternal Separation/Maternal Deprivation (MS/MD)

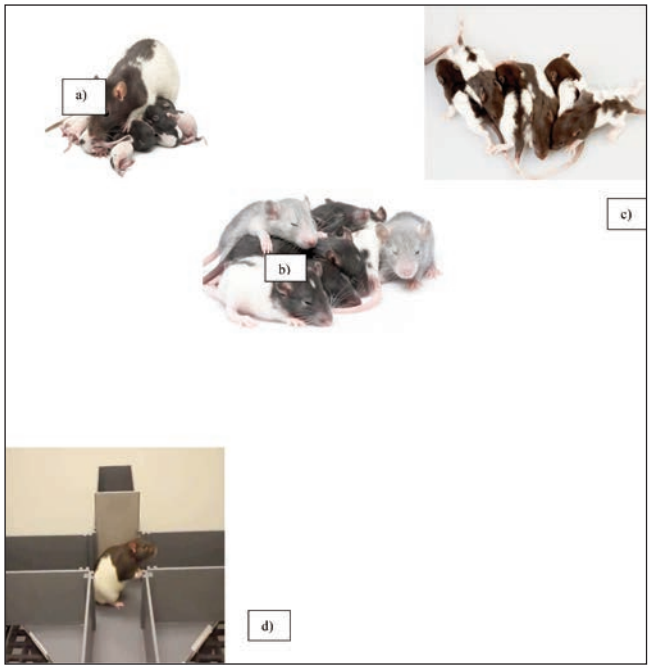


Figure 5. Maternal Separation/Deprivation Model (adapted from Tan, S. *et al.* 2017[33]).

Images courtesy of Google Images and Colourbox.

a) Mother rat with litter.
b) The mother rat is removed for 3 hours at a time from PND 2 to 14.
c) Rats are weaned and allowed to grow to adulthood under normal housing conditions.
d) Adult offspring undergo behavioural tests to determine the effects of the Maternal Separation/Deprivation.

The rat MS/MD models are referenced throughout this project as they are the most frequently used animal models of early life adversity researched in this review. The impact of childhood adversity on brain and behaviour cannot be achieved in a human clinical environment and therefore models have been refined in a laboratory environment using rodents. Figure 5 illustrates the experimental paradigm. The dam is removed from the litter daily from PND 2 for a period in excess of 3 hours and then returned for a short duration, primarily to allow lactation and essential grooming. This process normally occurs daily until PND 14, though some continue to PND 21, until the rodents are weaned (Table 2). There is a control group whereby the mother and litter have constant access to each other. The rodents are weaned and grouped into their two respective experimental groups (MS/MD, control) and allowed to grow on to adulthood. Both the MS/MD groups and the control groups are assessed, at adulthood, for behaviour and physiological changes and the results assessed for differences.[33] Often blood samples are taken both during the experiment for neurochemical and stress hormone evaluation and at post mortem stage and post mortem tissue samples, primarily the brain, are taken for histological, cellular and molecular analysis.

Rodent Experience	⇒ Normal	3 Hours MS/MD	No MS/MD	Weaning	Normal Group Housing	Behavioural Tests
Post Natal Days	⇒ 0 ⇔ 2	2 ⇒ 14	14 ⇒ 21	21 ⇒ 24	24 ⇒ 60	60 ⇒ 90

Table 2. Prototypical Schedule for Maternal Separation/Maternal Deprivation (MS/MD) models.

Chronic Early-Life Stress (CES) Model

This model was developed by the Baram Laboratory as a CES model of early life adversity in the presence of the mother and commences at PND 2 in rats (Table 3).[2] The mother and pups are placed on a perforated floor base in a modified home cage setting from PND 2 to PND 9 (Figure 6). This contrasts with the normal solid home cage base of the control group which typically holds 3cm of wood chip and nesting material in the form of commercially available, purpose designed, shredded paper or wool. The CES model home cage has a sparse scattering of wood chip added, in addition to a single sheet of nesting substrate. The result is a barren cage space experimentally designed to simulate a (human) impoverished living environment. The mother rat is forced to alter her nurturing behaviour, to accommodate a dispersed caring system for her pups. The lack of adequate nesting material in the CES home cage (available to the control mother rat) results in maternal stress which in turn creates erratic early life care for the rat pups, therefore experimentally generating chronic stress in that environment.

During the CES (PND 2 to 9) the mother rat demonstrated rough manipulations of the pups and shortened bouts of feeding/caring for her offspring when compared to the behaviour of the control dam. Some rat mothers showed adaptability by hoarding both faecal pellets and chunks of uneaten food to compensate for insufficient nesting substrate. It is significant that once returned to normal caging on PND 9, the mother rat invariably reverts to normal caring and handling of the pups, as the stress of the impoverished living conditions is lifted. The rats are allowed to develop to adulthood in a normal manner as in the MS/MD model detailed in Section 4.2. The test (CES) and control offspring are assessed at adulthood for behavioural/neurological and physiological differences.[2] Samples for analysis are taken as in the MS/MD paradigm description in *Maternal Separation/Maternal Deprivation* Section.

Figure 6. Chronic early-life stress (CES) model of early life adversity (Image courtesy of the Baram Laboratory).[2] The mother rat and litter are maintained on a gridded floor with a minimal amount of bedding/ nesting substrate.

Rodent Experience	⇨	Normal	Grid Floor Housing	Normal Cage	Weaning	Normal Group Housing	Behavioural Tests
Post Natal Days	⇨	0 ⇳ 2	2 ⇨ 9	9 ⇨ 21	21 ⇨ 24	24 ⇨ 60	60 ⇨ 90

Table 3. Schedule for chronic early-life stress (CES) model in rats.

Early Life Trauma Witness Model

The Early Life Trauma Witness Model seeks to deliver a rodent model of chronic early childhood adversity by emulating chronic early life adversity caused by post-natal witnessing of violence, abuse and/or dominance against the mother.[4] The mother rat is removed from the home cage and placed in the home cage of an aggressive (Long Evans) male rat. The resident male rat, attacks the female rat, as a mark of aggression towards the perceived intruder. The resident male forces the intruding mother rat into submission and maintains an aggressive stance for the allowed period (3 intervals of 10 minutes with 5-minute rest period between) daily from PND 21 to PND 24. The offspring of the mother are housed individually in arena type transparent Perspex caging around the perimeter of the aggressive male home cage thus exposing them as unavoidable witnesses to repeated aggressive social defeat of their mother on a daily basis (Figure 7). 4 days following the last exposure to stress, (at PND 28), the pups are weaned and separated according to sex and returned to normal husbandry until adulthood (Table 4). Upon reaching adulthood, the offspring then undergo a series of behavioural assessments to determine the impact of the early Life Trauma Witness Model. Trauma witness animals are compared to control animals, which undergo the same protocol above with the exception that the male, is known to, and socially acceptable to, the female and therefore is not aggressive towards her.[4] Samples for analysis are taken as described previously in Maternal Separation/ Maternal Deprivation Section.

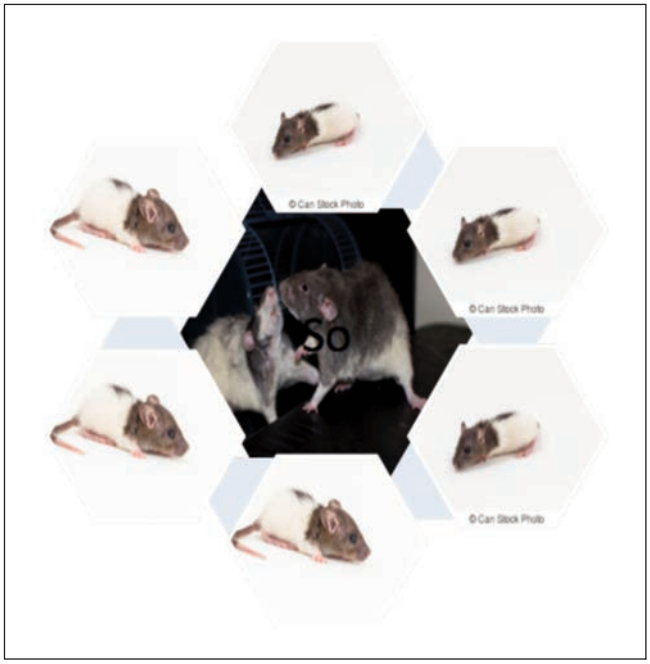


Figure 7. Witnessed aggression model of early life adversity (Adapted from, images courtesy of Brattleboro Rat/Can Stock Photo).[4] The 6 offspring have clear sight of their mother being attacked into submission by the larger male aggressor.

Rodent Experience	⇨	Normal Cage Housing	Witnessed Aggression	Weaning /Normal Group Housing	Behavioural Tests
Post Natal Days	⇨	0 ⇨ 21	21 ⇨ 24	28 ⇨ 60	60 ⇨ 90

Table 4. Schedule for early life trauma witness model.

Relevance of laboratory animal behavioural research as translatable models of early childhood adversity

Background

The ability to reproduce all the symptoms of a human psychiatric disorder is rarely achieved in experimental animal models, due mainly to the complexity of brain and behavioural differences between humans and animals.[34] The use of laboratory animal models for evaluating early childhood adversity has been a source of controversy and scepticism with the main argument relating to the difficulty in replicating the higher cognitive and emotional ability in humans. Behavioural researchers seek to achieve, in the study of early life adversity, the generation of a state of anxiety or depression in rodents that simulates humans experiencing these disorders. The models of stress in rodents, detailed in this report (see Laboratory Animal Models of Early Childhood Adversity) were originally developed for drug screening and have been refined to study specific elements of symptoms that closely resemble early childhood adversity.[35] The use of laboratory animals in the development of new treatments and therapies for mental health is acknowledged as a necessary and ethical use of animals but will not be discussed in further detail here as it is beyond the scope of this thesis.

The 3Rs

As part of the legislative authorisation process, every researcher carrying out an experiment involving the use of live laboratory animals must carry out a harm-benefit analysis. The stress and disruption (the harm) being inflicted on, for example, the mother and offspring rats must be shown to be necessary in order to achieve benefits to human (and/or animal) welfare (the benefit). All applications for authorisation for proposed models must demonstrate that they have considered Replacement Reduction and Refinement (the 3Rs) in their approach to the experimental design. The 3Rs were developed > 50 years ago and have evolved to become a guiding framework for the humane use of animals in biomedical research.[36] The impacts are seen at laboratory animal cage level in terms of improved experimental design but also at regulatory levels as they guide policy and regulatory change in animal welfare matters. The 3Rs have had a significant impact on the uptake of new, improved approaches to designing animal experiments and several of the current journals reference its influence, for example.[37]

The evaluation process at University College Cork (UCC) incorporates the principles of the 3Rs in the Animal Welfare Body (AWB) and Animal Experimental Ethics Committee (AEEC) review processes and a representation of how this applies is outlined further (Research Ethics, University College Cork, 2018). The 3Rs evaluation process has been validated by the regulatory authority, The Health Products Regulatory Authority (HPRA) as an appropriate model of institutional assessment and is fully compliant with the Irish and European legislation (S.I. 543 of 2012 and Directive 2010/63 EU).

In terms of **replacement** of a rodent model of early childhood adversity: the main objectives of a rodent model of early life adversity, incorporating the mother-child relationship are to investigate the development of complex behaviours as a result of being deprived of the normal mother/offspring relationship. The impact is measured by assessing the behaviour of the whole organism over time. *In vitro* (cellular) or simulation methods cannot replicate the behavioural and physiological processes involved in the models detailed in Chapter 4.[35] The specific timing of critical interventions is not feasible in humans and thus live animals need to be used. The following websites are regularly accessed, in UCC, for information on where to look for possible alternatives to using the animal models detailed in Laboratory Animal Models of Early Childhood Adversity.

https://www.niehs.nih.gov/health/topics/science/sya-iccvam/
http://animalresearch.thehastingscenter.org/facts-sheets/alternatives-to-animals/
https://www.nc3rs.org.uk/the-3rs

In terms of **reduction**, all laboratory animal experiments undergo a rigorous evaluation of the numbers required to complete the experimental model. A typical experiment involves a detailed examination of relevant published literature to determine likely effect sizes for behavioural readouts because of early life adversity exposure in rodents. Comprehensive power analysis (typically = 0.05, power = 80%) are performed to determine the minimum number of mothers with litter of pups required, for each of the models, in order to achieve statistical significance. The aim is to achieve, statistically sound, relevant results with a potential to benefit future clinical experimental design. Qualified biostatisticians assess the models and associated research projects including the sample sizes and overall animal numbers to be used before the research achieves authorisation.

In terms of **refinement**, the applicants must indicate that they have considered refinements under the following headings: most appropriate choice of species, the most refined choice of procedures, the appropriate policies in place to minimise suffering, the details of welfare monitoring, the details of caging, husbandry and care and the details of environmental enrichment used. Each individual procedure involved in the models detailed in Laboratory Animal Models of

Early Childhood Adversity, has undergone refinements to achieve models that consider the above during the experimental design stages. For example, MS/MD models of early childhood stress are required to detail how the welfare of the pups is monitored while the mother is removed and any interventions required should the pups become too distressed or at risk of unnecessary pain.

Criteria for Selection an Animal Model of Early Life Adversity

Four strategies form the basis for the development of rodent models of early childhood adversity: (A) Genetic predisposition of the species being investigated, for example, the use of WKY rats with an innate susceptibility to anxiousness. (B) Selective breeding for the characteristics required to carry out the experiments, for example, the use of the Long Evans rat as an aggressor in the witness model of early life adversity (see Laboratory Animal Models of Early Childhood Adversity). (C) Physical and environmental manipulations required to achieve the model selected, for example, the housing of the mother rat with offspring on a perforated cage floor, as opposed to normal cage substrate. (D) Tightly controlled adherence to protocol, in order to ensure reproducibility and repeatability between laboratories.[5]

Validation Criteria for Laboratory Animal Models

Establishing the credibility of a laboratory animal research model is based on meeting certain validity. The three main validity criteria assessed are Face Validity, Predictive Validity and Construct Validity.[5]

Face Validity refers to the clinical symptoms of the human disease that must be observed in the animal model.[3] Face Validity is difficult to achieve in entirety in psychiatric disorders but symptoms of human stress can be identified in early life rodent models, for example, agitation, and sleep disruption and food/water intake variations.[38] This supports the current research that suggests researching specific symptoms or endophenotypes rather than the entire disorder results in better translational value.[5] The difficulty in achieving DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, fifth edition) criteria of psychiatric disorders supports the suggestion that face validity is the most difficult of the validities to achieve in rodent models of early childhood stress.[38]

In Construct Validity, the cause, or mechanism of the human disease must be represented in the experimental design for the animal study. The rodent models of early childhood adversity detailed in Laboratory Animal Models of Early Childhood Adversity attempt to simulate the cause of early childhood adversity by altering the mother/offspring normative relationship in an adverse manner. By inclusion of, for example, WKY rats, predisposed to vulnerability in the MS/MD paradigm,[27] the researchers have achieved Construct Validity in that they incorporated the gene-environment interaction, that has been identified as a vulnerability or resilience factor in human early life adversity.

In Predictive Validity, the response to the therapy/treatment of the animals is expected to be also seen in the human patients.[39] Predictive Validity has been challenged in terms of effectiveness in relation to psychiatric disorders. For example, it is unclear whether Predictive Validity refers solely to the predictive effect of the treatment or to the ability to predict some of the markers specific to the disease.[40] Treatment and therapy are not discussed in this report but many of the adult rodent behavioural tests referred to in Laboratory Animal Models of Early Childhood Adversity, incorporate treatment during the experimental behavioural assessment.

A proposal for a modified set of criteria to include Pathogenic Validity, Homological Validity and Mechanistic Validity while retaining but modifying the meaning of face validity and predictive validity have been proposed.[40-41] Pathogenic Validity refers to the similarity of the process that leads to the pathogenesis of the disease disorder. Homological Validity refers to the assessment of both the species and the strain of the species in terms of their relevance and suitability at both behavioural and biological levels. Mechanistic Validity refers to our interpretation of what we know is working in the chosen animal species versus what we assume to be working in the human pathogenic condition.

This amended framework of assessing animal model validity involves prioritising in accordance with the objectives of the given experiment. A scoring system is proposed to assess the validated rating of each procedure. The proposer acknowledges that the categories of validities may not all apply to models of early childhood adversity, with pathogenic validity cited as an example of irrelevance.[40]

In recent years behavioural research has developed into modular experiments which can be used in isolation or combined to study specific aspects of the disease.[3] This has increased the validity values of the current laboratory animal models of early childhood adversity, due to identifying common endophenotypes. The recent availability of feedback information from human clinical trials of depression has led to the development of more accurate and specific design of the independent variables. This is achieved by breaking down the illness into individual behaviours common to and measurable in, both humans and animals (endophenotypes). Endophenotypes of the models are

measured using behavioural tests. This results in rodent models that better represent the childhood adversity outcome in terms of relevance to human psychiatric disorders.[39] Endophenotypes allow direct comparison across species, provided that they have the elemental components of the disorder.[19]

Reproducibility and Translational Value

The use of laboratory animals as models of early life adversity in research is highly regulated and governed by strict legislation as detailed in the 3Rs section. The harm-benefit analysis, assesses the objectives and the benefits of the research against the potential distress and physical harm inflicted on the laboratory animal(s). Recent guidelines such as Animal Research: Reporting *in vivo* Experiments (ARRIVE) have further encouraged researchers to focus on the 3Rs by requiring reporting of details on the laboratory animal experimental design, relevance to human biology and diseases, statistical analysis, and the health status of the animal at the commencement of the experiment.[42] These guidelines are taking some time to have effect with some publication review bodies not enforcing the need to meet the ARRIVE criteria.[6] However, the Journal of British Pharmacology (BPS) require authors to use the ARRIVE guidelines which is encouraging.

The Planning Research and Procedures on Experimental Animals: Recommendations for Excellence (PREPARE) guidelines complement the ARRIVE guidelines. They were developed on the basis that better reporting in retrospect will not improve the quality of an experiment already completed but will provide valuable information for future experiments. ARRIVE are a set of guidelines which include the formulation of the study, the communication process between the researcher and the animal facility staff and details of the quality control measures that will be implemented for each aspect of the study. PREPARE focusses on a wide variety of aspects which are seldom reported in publications but if followed can have a significant influence on both the validity and reproducibility of the research. They act as a useful checklist for researchers and include all aspects from an analysis of the facility management, to carrying out the procedure at the cage level.[43]

There is a need for clinicians and neuroscientists to collaborate in the development of laboratory animal research. For example, very few clinicians understand the complexities or benefit of the rodent models early childhood adversity models detailed in Laboratory Animal Models of Early Childhood Adversity. Similarly, very few behavioural scientists are likely to have contributed to clinical research trials involving children that have experienced early childhood adversity.[44]

Several drawbacks remain in the ability to translate laboratory rodent research into clinically beneficial data. These include a lack of homological validation value as rodents may not score adequately in the scale of stress reactivity to be valid subjects for early childhood adversity research.[41] Of major relevance to early childhood adversity research is the need to ensure that the test animals have been challenged at all relevant stages of development in order to achieve pathological validity.[3] There is a lack of refinement at experimental design stage of preclinical studies. It is necessary to employ numerous rodent models and tests in order to develop translatable data in terms of drug manipulation for the treatment of depression in humans.[5]

Framework

An animal model of early life adversity must draw on the comparisons between two pathological species, in this case, rodents and humans.[40] The majority of models detailed in the articles reviewed operate on a general framework of a three stage input-output process (Figure 8). There is a transformation process from a healthy organism (Stage 1) to a vulnerable organism (Stage 2) as a result or early life adversity. A trigger factor in adulthood transforms the already vulnerable organism into a pathological organism (Stage 3).[33] In the early life adversity model setting, this trigger factor is an experimentally designed artificial stressor, for example, restraint or social instability paradigms. In humans, homelessness, domestic violence and/or substance abuse are examples of stimuli that represent the trigger factor.[45] The adult rodent behavioural tests, referred to in Laboratory Animal Models of Early Childhood

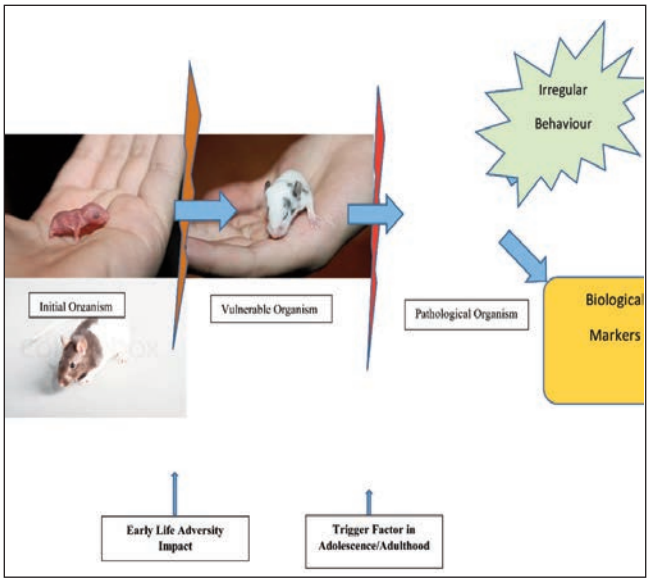


Figure 8. A 3 stage input-output framework for animal models of early life adversity (adapted from Belzung, C. *et al.*,[40] images taken by author/courtesy of Colourbox). Note that the common process by which animals and humans progress from normal organisms through vulnerability and onto pathological conditions is the basis for laboratory animal models of early childhood adversity.

Adversity, determine the presence of irregular behaviour while blood and tissue samples can determine the presence of biological markers of these behaviours.[40]

Intermittent vs Chronic Models of Early Life Adversity

Intermittent Models

MS/MD in rodents, particularly in rats, is the most frequently cited paradigm for the study of early life adversity. In normal mammalian circumstances, the mother provides early life care and this regulates the development of the brain and behaviour in the developing offspring. By altering the rat pup/mother relationship during this crucial period of development, it is possible to experimentally induce susceptibility to adverse psychopathological phenotypes in rodents.[46]

A review of literature has found multiple variations of the MS/MD procedure, for example, the timing of the separation and the age of the pups at separation, differs widely. There are also variables such as distance of the isolated pups from the separated mother, which has implications for sound and smell interference.[29] Handling the pups for the purpose of removing them from the mother has been used as an additional experimental stressor in comparison to the control animals. Interestingly, rat pups that had been handled were shown to have better coping strategies in adulthood when challenged with behavioural tasks, in comparison to the control animals.[27] It is unknown however if the extra attention given by the mother upon return to the handled pups is a factor in the development of the coping strategies.

Most studies are consistent in the view that although short term maternal separation and handling may be beneficial in terms of building coping strategies for future scenarios. Long term maternal separation (3-4 hours per day) are associated with negative outcomes in terms of mental health at adulthood.[47] The MS/MD model is an intermittent model of early childhood stress as there is a relatively short and regular span of disruption to the mother/offspring relationship. However, in cases of extreme poverty, living in war zones, or homes with chronic addiction, the human mother is often present but provides either sub optimal care, or the adversity itself, in the form of abuse and the chronic models of early life adversity are important in this regard.[2]

Chronic Models

The use of MS/MD intermittent stress model detailed above has been challenged, as some would argue that the wild mother rat leaves the pups in the nest for up to three hours to forage.[35] In addition, the stress effects on the rodents in the MS/MD model differs greatly from the experience of some children experiencing chronic adversity. Children experiencing the effects of war, child abuse, parents with chronic addictions etc. are exposed to ongoing chronic stress rather than intermittent stress.[2] Research has also indicated that adversity suffered in the presence of the primary caregiver, is equivalent in terms of neurobehavioral consequences, to trauma or abuse inflicted directly by the caregiver.[4] The impact of such stress in chronic rodent models of early childhood adversity was found to be more profound than the stress experienced in the intermittent models of MS/MD.[16]

A rodent model of chronic stress was developed to research chronic stress caused by early life adversity.[2] This CES model was based on simulating poverty in the rodent cage setting by exposing the mother and pups (from PND 2) old to a barren cage environment, as detailed in the section dealing with Chronic Early-Life Stress (CES) Model. Although the mother rodent carries out a level of care to the best of her ability, the care to each individual animal is fragmented and erratic resulting in chronic unpredictable stress to the developing offspring. Rough manipulations of the offspring by the mother were also noted frequently in the CES model. This is deemed to be a closer representation of the stressful experience of young children living in an abusive household or those living in extreme poverty or war torn areas. This therefore delivers higher construct validity than the MS/MD model as it gives a better representation of the causal effects of early life adversity [19]. The full impact of the stress that is potentially imparted from the mother rat, as a result of being artificially stressed, to the offspring is not detailed in either the intermittent or chronic models cited in the journals and requires further research.

Another model of chronic early life adversity is the Maternal Trauma Witness Model in rats (CES Model section). The American Psychological Association (APA) state that 15.5 million children in the USA witness maternal abuse.[4] The full impact of this abuse on the brain and behaviour is difficult to research in humans and therefore the Early Life Maternal Trauma Witness Model in rats was developed to assess the impact of early life witnessing of traumatic events, (see Laboratory Animal Models of Early Childhood Adversity section). This model also delivers high construct validity as it has high simulation value to early childhood adversity. The attempts of the mother to shield her offspring were also noted in this research and supports the influence of a mediatory role by the mother in situations of early life adversity (discussed further under the section relating to Maternal Mediation Hypothesis.

Conceptual issues

Background

The use of laboratory rodents to model childhood adversity is primarily based on models of attachment

and the relationship between the mother and offspring. The defence mechanism relates to a set of behavioural, physiological and psychological responses that occur in both humans and animals. Even purpose bred laboratory animals express some inter-individual differences and are not a homogenous group.[3] The strategies that individual animals use to deal with adverse situation can be divided into passive and active (similar to the fight or flight response). However rodents could not represent a complete model of early childhood adversity and subsequent potential psychiatric condition due to the diversity in life histories, co-morbidities and neurobiological mechanisms.[48] Nevertheless, the models/hypotheses outlined below are cited by researchers using rodents as models for early childhood adversity in support of their justification.

Diathesis-Stress Model

This model attempts to explain the impact of predispositional vulnerability reacting with life experiences to create a pathological condition. Diathesis (meaning predisposition) can take the form of biological, genetic or psychological factors. The genetic, biological or psychological traits of the developing mammal interacts with environmental stressors to result in either vulnerable or resilient individuals.[49] The model is based on there being a threshold of adversity caused by environmental factors. Below this threshold, both vulnerable and resilient individuals develop in a similar manner. Once the threshold is exceeded, the vulnerable individual will experience worse outcomes than the resilient individual.[50] This model is useful in determining why some individuals, that had shown no symptoms of mental health disorder, unexpectedly develop behavioural problems. As an example, a child from a family with a history of depression and later exposed to a trigger factor, such as social exclusion in adolescence, is more likely to develop a mental health disorder than if that same child developed in a stable and reliable adolescent social group.[40]

Phenotypic Plasticity Model

Despite large volumes of information on the impact of early life adversity, relatively few attempts have been made to develop a rodent model that explains how the effects have evolved in mammals. Some publications have considered the evidence of the mammals' ability to adapt and also an ability to generate age dependent plasticity.[51],[26]. The association between phenotype and plasticity arises if the environmental cues do not accurately represent the environmental state, leading to uncertainty about the most suitable phenotype for the immediate environment. There may be adjustments required to the phenotype, for example behaviour, which incur a physiological cost or perhaps crucial time wasted on a phenotype that will not suit alternative environments. The information gained through environmental cues and the ability to adjust phenotype are closely linked during the early development period. Offspring are likely to be informed by early life information, either from their mother or from direct interaction with the environment, on which phenotype is best suited to their future existence. This predictive adaptive response is dependent on an accurate forecast of the future environment. In cases where a mismatch between the anticipated and subsequent environmental occurs, the individual may suffer adversity as a result of having an inappropriate phenotype.[27] This Phenotypic Plasticity Model is therefore based on the early developing mammal being able to adjust their phenotype in response to environmental cues, in an evolving manner.[52]

The 3 Hit Concept of Vulnerability and Resilience

During early developmental periods, stressful experiences can modulate the behavioural, cognitive and emotional functioning of the developing mammal through modulation of the associated brain circuitry. The 3 Hit Concept of Vulnerability and Resilience (Figure 9) is based on the interaction between multiple genetic influences (Hit 1) and the early life environment (Hit 2) which lead to programmed phenotypes. Depending on the phenotypes and interaction with an adult life trigger factor (Hit 3) there will be either a vulnerability outcome as a result of compromising of mental function, or resilience outcome, resulting in resistance to mental dysfunction.[20]

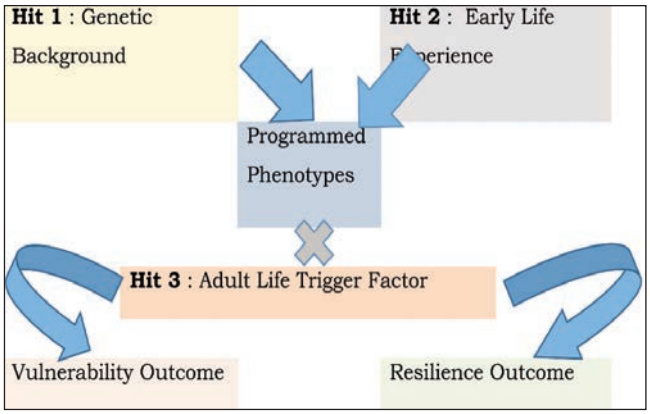


Figure 9. Representation of The 3 Hit Concept of Vulnerability and Resilience, adapted from Daskalakis *et al.*, 2013.[20]

Maternal Mediation Hypothesis

In normal developmental circumstances, the development of the neonate is enhanced by close frequent contact and interaction with the immediate caregiver, normally the mother. In rodents, this is represented by a series of gathering pups into a huddle, licking, and standing over them in a dorsal arch posture. Pups that experience this normative behaviour have been shown to be resilient to stress in later life.[19]

The process of feeding and interaction is thought to be mediated by a complex two-way cue system, between the needs of developing offspring and the maternal instincts of the mother. The mother imparts the stress hormone, corticosterone (equivalent to cortisol in humans) in her milk to the offspring which regulates the stress response.[53] Levels of corticosterone have been recorded as higher in the mother rat in both of the chronic early life stress models detailed in Chronic Early-Life Stress (CES) Model and Early Life Trauma Witness Models, than in the intermittent MS/MD model.[29]

The maternal mediation hypothesis is based on the theory that the mother rat mediates the effects of the artificially induced early life stress. She changes her behaviour to reduce the impact of the stress on the offspring. The offspring are thought to also trigger the mother rodent's alteration in behaviour by emitting signals (for example, vocalisations) indicating discomfort or distress in the presence of the mother. The research indicated that following all stress paradigms detailed in Laboratory Animal Models of Early Childhood Adversity, the mother increased the level of active maternal care when reunited, therefore potentially negating the impact of the stress of being separated.[22] The chronic models of rodent early life adversity detailed in Chronic Early-Life Stress (CES) Model.

Early Life Trauma Witness Model supports this theory in that the mothers were also observed attempting to mitigate the impact of the adversity on the offspring by shielding (in the Witness Trauma Model) and being resourceful (in the CES model).[2,4]

Allostatic Load Model

Allostatic load has been defined as the impact of wear and tear on the brain function of the organism (Figure 10), which results in a predisposition to disease.[54] Most newborn mammals remain in close contact with their mothers for the early developmental stage of life. Under normal circumstances, the mammalian brain, like other essential systems in the body, will strive to maintain normal functioning, known as the allostatic response. Disruption to the mother offspring relationship creates an allostatic load, caused by the disturbances in the stress mediators in the body.[55]

This allostatic load influences the animals' behaviour and neurobiology over the course of a lifetime. Manipulations of this load in rodents, is the basis for laboratory based preclinical *in vivo* research into the impact of early childhood adversity on psychiatric disorders.[28] For example, by removing the mother for prolonged periods during early postnatal developmental days (PND 2-12) the rodent pups experience a sense of helplessness and despair while separated, as measured by subsequent behavioural tests. This elevates allostatic load which creates a disturbance in the physiological, neurological and hormonal make up, changes which have shown to result in long term anxiety and depression.[56]

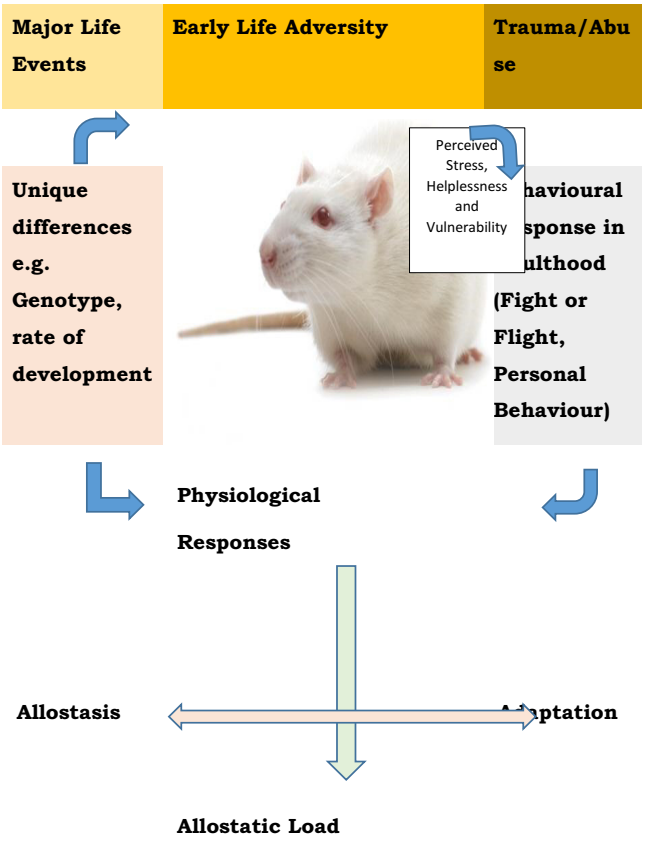


Figure 10. The development of an allostatic load (adapted from Hostinar *et al.* 2013[54]).
Image courtesy of Google Images.

Results

Background

This report analysed in excess of 70 publications in order to research the impact of early childhood adversity and the usefulness of laboratory rodent models in creating models of early childhood adversity.

Findings

There has been a noted shift in the recognition of the limitations of the laboratory animal models of early childhood adversity. In particular, the recognition that human feelings cannot be fully captured in the rodent models is prominent in recent publications.[3] The need for identification of translational genetic and epigenetic factors is essential at the experimental design stage of rodent models for early life adversity.[5] The impact of resilience and the need to factor this into the model, by using specific strains and relevant experimental paradigms was prominent in the recent publications.[3] There is also recognition that the traditional widely used model of early life adversity, such as MS/MD has been re-categorised as a collective term for a number

of extremely different manipulations, many of which have been adapted by behavioural scientists worldwide.[5] Recent publications also suggest that a moderate amount of intermittent early life stress could be beneficial in terms of developing resilience to stress later in life.[25,8] Chronic rodent models of early childhood adversity have been developed to supplement the information gained from intermittent stress of the MS/MD models.[2,4]

There was a noted lack of standardisation of the experimental paradigms throughout the review, with experimental protocols varying widely throughout the studies. The MS/MD model although widely cited, is challenged in terms of validity in several publications.[33,2] As an example, the duration of the separation in the MS/MD models can vary from PND 2 to PND 10, PND 14 or PND 21. Similarly the duration of separation can vary across research groups, ranging from 180 minutes to 24 hours.[46] The variability of the protocols and the lack of quality control, to date, have resulted in a lack of reproducibility and missed opportunities for vital collaborations that would lead to meaningful clinical studies.[5] The understanding of what qualifies as a control group in several studies also varied greatly.[57]

The variability of using different husbandry regimes, inconsistency in the strains being used and little consideration for the impact of biological sex on the pathological condition result in a barrier to the usefulness of early life animal models as models of childhood adversity.[31] While the majority of publications acknowledged the impossibility of developing models equivalent to humans, laboratory rodents are widely acknowledged as indispensable tools for the study of the ethology of early life adversity. Several publications detailed the impossibility of recreating the entire spectrum of human mental health disorders.[2] The optimal use of rodent models for early childhood adversity research lies in the assessment of specific behavioural dimensions of early life adversity.[5]

Discussion

The potential for the use of a rodent model of early childhood stress lies in its ability to achieve reliable, valid and direct comparisons between rodents and humans. The models described in the section Laboratory Animal Models of Early Childhood Adversity are experimentally designed to assess the behavioural, neuroanatomical and biochemical changes that are common to both species in the early life developmental stage. It is increasingly accepted, in the publications reviewed, that the complexities that lie between humans and rodents are too diverse to sufficiently directly compare early life stress/adversity in both species. However the identification of endophenotypes has made certain aspects of human early life adversity possible to model in rodents.[19]

There are opposing opinions on the effects of early life manipulations as a result of variables in the timing, stage of development and type of artificial adversity imposed. Until relatively recently the MS/MD model provided an all-encompassing model of early life adversity but new paradigms that deliver chronic, and potentially more translatable research have been developed to good effect.[2,22] There are contradictory outcomes of many of the published results from the MS/MD procedures leading to a consensus that the currently used MS/MD models are a series of procedures, adapted by the principal researcher to fit with the hypothesis being tested.[2]

The role of the pup in reading the mother's cues and influencing her behaviour has been underestimated in MS/MD models to date.[4] Although there is evidence of alterations in physiology (reduced body temperature, food deprivation and reduced excretory functioning) there is inconsistent evidence that these effects are entirely causal of subsequent behavioural abnormalities.[22] The maternal mediation hypothesis suggests a two-way interaction process between the mother and offspring, allowing for a compensatory effort to restore a normal environment by the mother. There is also little consideration in the publications reviewed for the ability of the developing rodent to modify its phenotype in line with the cues from the mother. This infers that there is an adaptive ability, potentially of genetic or innate origin, in the developing rodent that develops resilience.[24]

There are conflicting publications in relation to the negative impact of early childhood adversity. There is evidence of a potential adaptive advantage in the developing rodent as a result of having experienced moderate stress in early life.[58] Considering that a wild adult mother rat may leave her nest for up to three hours at a time in search of food during her pups' early development,[22] the MS/MD paradigm may not adequately expose the developing laboratory rodent to adversity.[29] In addition, a comparative study involving genetically altered (WKY) rats (that exhibit innately higher stress sensitivity) indicated that early life stress had potential for developing beneficial adaptive response to subsequent stress. This suggests that innate stress reactivity may influence the susceptibility to anxiety-depressive like outcomes in later life.[27]

Any assessment of the validity of the use of rodent models of early life adversity must include an assessment of the reproducibility and translational value of the models. Several publications in this review expressed the need for better designed animal experiments, with a general consensus that the variables must be reduced and quality control at experimental implementation increased.[5,29,39] Stress experiments in rodents are extremely sensitive to variation. There is variability between the behavioural

laboratories in terms of their implementation of protocols in relation to, for example, the MS/MD model.[29] The CES model detailed in Laboratory Animal Models of Early Childhood Adversity, is highly reproducible, as it delivers a robust and repeatable change in maternal behaviour.[2] The CES model also has high translational value as the rodent phenotypes of the offspring rats closely relates to the phenotype of children exposed to various forms of adversity.[29]

There is evidence that maltreatment at early development stages and its associated behavioural abnormalities do not always translate to adult psychiatric illness. This suggests that early life childhood adversity may be contributory and not the primary causal factor in adult mental health diseases.[22] There is increasing evidence of sensitive windows of vulnerability extending into adulthood and not isolated to early childhood adversity.[52] There is also literature that suggests that during adolescence, humans have a switching adaptation which allows an adaptive vs maladaptive response to early life adversity.[22] This argument is frequently used by anti-vivisection groups in relation to the ethical consideration of subjecting rodents to pain and distress involved in the models detailed in the section Laboratory Animal Models of Early Childhood Adversity, as the harm/benefit evaluation is open to refinement.[23]

Hypothesis Accepted or Rejected

The hypothesis of this report is that laboratory animals make valid models for early childhood stress, on which a link to subsequent adult psychiatric illnesses and disorders can be formed.

This hypothesis is accepted on the basis that there is an acknowledgement of the necessity to assess specific symptoms, rather than the whole disorder and create reproducible, quality driven research.

The null hypothesis is that laboratory animals do not make valid models of early life adversity, as animals cannot represent the superiority of the human cognitive processes.

The null hypothesis is rejected, as there is evidence of common endophenotypes that can be researched in animals and humans.

Conclusion

This review has achieved its objectives in that it evaluated the relevance of the use of animal models of early childhood adversity. In the author's opinion the relatively recent inclusion of the chronic Early Life Trauma Witness and CES models have increased the translational and validity value as they represent a more specific stress paradigm than the more widely cited MS/MD paradigm. The strict regulation and compliance, together with effective harm-benefit analysis have also increased validity and therefore relevance of these models.

Concerning the relationship between early childhood adversity and subsequent development of psychiatric disorders: The 3 Hit concept of Vulnerability and Resilience (the interaction between multiple genetic influences (Hit 1) and the early life environment (Hit 2) and depending on the phenotypes and interaction with an adult life trigger factor (Hit 3) provides a balanced representation of the relationship between early childhood adversity and subsequent disorders. The impact of a developing mammal's ability to adapt and become resilient, combined with the influence of genetic makeup are critical factors in determining the individual's trajectory in terms of mental wellbeing.

In terms of conceptual thinking: the various theories and hypotheses and models put forward represent credible reasoning as to why recent publications suggest that the relationship between early life adversity and adult psychiatric disease is not linear. In the author's opinion the Maternal Mediation hypothesis is highly significant for those fortunate to have the experience of the nurturing effects of a caring mother or caregiver. This hypothesis, and the chronic stress models cited in the section, Laboratory Animal Models of Early Childhood Adversity, mirrors the human scenario of a mother compensating and shielding her children in abusive and/or poverty-stricken homes. Offspring reared in the care of a positive maternal environment, despite experiencing adversity in early life, have the capability to adapt to become resilient, because of the maternal protective influences.

Future research

The usefulness of animal models in determining the modulating effects of genetic background and biological sex in relation to resilience and vulnerability has been highlighted as deficient in this review. The factor of biological sex is important because women are twice more likely to develop PTSD or depression than men and yet most animal studies of PTSD use males and not females. The translational value of the extensive findings relating to how early life stress in experimental rodent models can fully benefit our understanding on human childhood brain development, requires further evaluation. There is emerging evidence of the roles of stress mediators, including novel molecules, glucocorticoids and neuropeptides, but their impact at specific sensitive time points requires further research.

Background

The author declares no commercial interest in this research. As stated in the preface, the author's primary, remunerated role is that of Manager of an academic research animal laboratory, with a high

number of ongoing behavioural research projects. The research evaluation roles on the ethical, welfare and genetic engineering committees are voluntary.

Study limitations

This is a highly specialised area of research and it was difficult to find articles specific to laboratory animal models of early childhood adversity.

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