The added value of rodent models in studying parental influence on offspring development: Opportunities, limitations and future perspectives

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The added value of rodent models in studying parental influence on offspring development: opportunities, limitations and future perspectives.

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Highlights

- Rodent models of parental influence on offspring development offer high level of control
- They allow specific timing of both environmental and pharmacological interventions
- In these models, neurobiology can be studied from network to cell to genes
- Improved reporting of methodological details and meta-analyses are needed

Abstract

Over the past decades, the influence of parental care on offspring development has been a topic of extensive research in both human and animal models. Rodent models offer several unique advantages over human studies, allowing for higher levels of environmental control, exploration of interventions, genetic control and examination of underlying neurobiological mechanisms in greater spatiotemporal detail. Although exploitation of these opportunities has led to increased understanding of the neurobiological mechanisms underlying susceptibility to the early-life environment, translation of results to human parenting and child development appears to be challenging. Attuning animal models to the human situation and application of novel structural and functional techniques is therefore of crucial importance to reduce the gap between rodent and human research.

Introduction

Parental care is of vital importance for newborn mammals, including humans, enhancing both survival and development. It is now widely accepted that alterations in parenting during critical early-life periods contribute to long-lasting developmental effects and vulnerability to psychopathology in offspring [1;2]. Animal models offer unique opportunities to study the neurobiology underlying susceptibility to early-life rearing conditions, given the evolutionary conserved mechanisms involved [3]. Moreover, analogue developmental phases create the possibility to relate rodent age to human age [4], although this requires careful interpretation. Against this background, many animal studies of the detrimental effects of adverse early-life experiences have been undertaken, particularly in rodents [5-7]. Rodent models used to study these effects hold a certain face, construct and predictive validity [8]. Yet, future studies could benefit from further integration of human and rodent studies [9;10].

Here, we will discuss contributions of rodent studies to understanding the influence of parental care on offspring development, focusing on four main advantages of animal models: I-A higher level of environmental control; II-Controlled interventions; III-Manipulation of genetic background; IV-Revealing underlying neurobiological mechanisms. For each of these, we will briefly discuss research strategies, some key findings, limitations and suggestions for future research.

Environmental control

Although human studies support an important role of the early-life environment on child development, the complexity of different environmental conditions hampers dissociation of the various contributing factors. In rodent models, substantial environmental control allows for stepwise manipulations. Until weaning around 3 weeks of age, mouse and rat pups spend their life in the nest and their early-life environment is almost exclusively determined by the mother. Therefore, assessment and/or alteration of maternal care -consisting of several well-defined behaviors such as

nest-building, licking/grooming, pup retrieval and nursing- are common approaches to study the influence of early-life environment on offspring development [11••].

The first studies in the field were conducted by Seymour Levine and Victor Denenberg, showing maternal mediation of early life manipulation on the offspring's stress responsiveness in adulthood [6]. Subsequently, numerous studies showed lasting effects of maternal separation (1-8h/day) and maternal deprivation (up to 24h) on the developing hypothalamic-pituitary-adrenal (HPA) axis and adult behavior of the offspring [5;12;13]. Importantly, duration, frequency, and timing [12;14••], as well as (social) environment during separation –e.g. homecage and contact with littermates- influence outcome, underlining the importance of the context in which early life stress takes place [14••].

Meaney and coworkers elegantly demonstrated the importance of quality of maternal care. They showed that naturally occurring variations in licking and grooming (LG) are related to HPA axis development, paralleling changes seen in deprived versus non-deprived rats [11••]. Moreover, not only *between*-, but also *within*-litter variations in received licking/grooming levels predict later-life behavior and neurobiology [11••;15]. These findings are of translational interest, as human parental investment can also vary between children [16]; noteworthy, rodents have large litters (often culled to 6-8 pups), which differs from multiple single births in humans. These rodent studies on natural variations in parental care have led to increased appreciation of the importance of assessing maternal care levels in deprivation/separation experiments.

Providing limited nesting and bedding material is a method to chronically expose dams and pups to adverse environmental conditions. This condition results in fragmented and unpredictable dam-pup interactions, highly relevant for modeling the often chronic adverse rearing conditions in humans [7]. Infant attachment to the mother can also be manipulated in rodents by coupling maternal odour to receiving a shock [17]. Pups maintain their preference for this maternal shock-odour, modelling abusive attachment. Rodent offspring reared in both of these conditions exhibited upregulated

corticosterone levels and developed long-term cognitive, emotional and neuroendocrine abnormalities [7;17] similar to animals that received low levels of LG (Low-LG) or were maternally deprived.

Importantly, early-life stress (ELS) effects represent adaptations to the environment, rather than negative consequences of early-life adversity per se. This view is highlighted in the match/mismatch theory, stating that ELS-induced changes program an individual for optimal performance under similar conditions later in life [18;19]. Accordingly, in cognitive tasks, Low-LG or maternally deprived rats outperformed control animals after moderate stress, although severely stressed animals remained negatively affected in other aspects of brain function, especially in combination with a vulnerable genetic background [20].

A factor lacking in many animal studies is the contribution of paternal care [21], observed in the majority of human cultures. Indeed, human studies indicate the importance of paternal engagement in psychological development [22]. In biparental rodent species, e.g. prairie voles and California mice, paternal deprivation studies have highlighted the importance of paternal care for sex-specific developmental effects in offspring [23]. Although the vast number of genetic techniques used in mice (see genetics section) are not yet available in these species, promising developments are made [24•]. Most mammalian species, including rats and mice, are uniparental and males of these species can be infanticidal. But infanticide by males can be avoided and paternal care can be induced in rats by prolonged exposure of fathers to foster pups [25•] and in mice by post-copulatory cohabitation with a female during gestation and parturition [26], yielding paternal retrieval of pups guided by the mother [27]. Interestingly, father early-life trauma has been shown to affect behavior in male adult offspring via sperm RNAs [28•].

Summarizing, many approaches have been used to elucidate the long-term effects of parental -predominantly maternal- care on offspring development, exploiting the possibility of high environmental control in rodent studies. However, a drawback of attempting to completely control

the environment is the risk of providing impoverished living conditions, devoid of a minimum of external stimuli. Mice that were deprived from normal husbandry provide a striking example of detrimental effects of insufficient stimulation during development [6], but even standard lab settings likely represent impoverished rearing conditions [29]. This underlines the advantages of more naturalistic settings. For instance, co-housing lactating females allows communal nests, with upregulated maternal care levels, enhanced growth rates in pups and increased social competence and resilience to social stress in adult offspring [30•]. In addition, interaction with non-kin caretakers and peers may increase translational value of rodent studies. Hence, approximating naturalistic settings may help to improve the predictive validity of future animal studies [31].

Controlled interventions

Ultimately, the goal of studying early-life environment in relation to developmental disorders is to improve mental and physical health of affected individuals. Preventing ELS is generally difficult in humans, as poor rearing conditions often remain hidden [32]. Moreover, a number of symptoms arise during adolescence [33], years after early-life adversity started. It is therefore of crucial importance to dissect potential windows of interventions throughout development. This can be done in a controlled setting in rodents, after experimentally inducing ELS.

Post-weaning environmental enrichment (EE) is a non-invasive method shown to counteract certain detrimental effects of ELS, notably on adult stress responsivity [34], cognitive function [35], and hippocampal development [36]. For rodents, EE encompasses housing in a larger cage with more cagemates, a shelter, and increased cognitive and physical activity [37]. Thus, providing the appropriate environmental stimuli needed for healthy psychological and physiological development in the peripubertal period might partially reverse ELS effects. Still, disentangling the social, locomotor, cognitive and sensory aspects of EE in reversing developmental deficits is challenging. It has been argued that physical activity in EE is responsible for most effects [38], but this might depend

on outcome measures [39•]. Currently existing variations in design, timing and parameters in EE models indicate the need of a meta-analysis to delineate the contributions of different EE components on a range of developmental outcomes.

Pharmacological treatment is a different approach to explore time-windows and possibilities to improve development following ELS. Altered methylation patterns in the hippocampus after ELS are observed in both rodents [11••] and humans [40]. Interfering with the epigenetic methylation process in low-LG offspring [11••], even in adulthood [41], proved to be effective in reversing low-LG effects on hippocampal expression of the glucocorticoid receptor (GR). Also, brief treatment with the GR-antagonist mifepristone during adulthood or adolescence has been shown to counteract some [42;43•;44], but not all [45] effects of maternal separation. Other neurobiological systems have been targeted too in an attempt to reverse ELS effects, e.g. using antidepressants [46]. When effective, these brief treatments appear to rapidly 'reset' the stress system disturbed after ELS.

In short, although animal intervention models lack the possibility to mimic important aspects of human therapy including verbal instructions, placebo effects and compliance, they demonstrate the promising possibility to reverse several ELS effects by later-life interventions. Future animal studies could help to further fine-tune sensitive periods for intervention.

Genetic control

Detrimental effects of ELS are particularly evident in genetically susceptible individuals [47], underlining the importance of genetic variation in regulating individual responses to the early-life environment. Human evidence suggests a role for several candidate genes involved in the serotonergic system, HPA-axis and neurotrophin system in regulating vulnerability to early-life adversity [48]. Although currently available genetic profiling techniques enable examination of the effects of natural genetic variations in humans, studying causal contributions of specific genes by targeted deletion or overexpression is restricted to animals. Conventional (overall) and conditional

(region-specific and inducible) knock-out (KO) models have been created to test the consequences of altered gene expression. Of note, testing the influence of genes of interest one-by-one is a highly reductionist approach, which does not capture the likely contribution of a multitude of risk genes contributing to ELS susceptibility, each with a very small effect size [49].

Conventional KO animals confirm an important role of the HPA-axis in moderating ELS effects, showing that corticotropin releasing hormone receptor-1 (CRHr1) mediates the corticosterone response following maternal deprivation in mice [50]. Forebrain-specific deletion and overexpression of CRHr1 further specified this receptor's role in cognitive deficits and anxiety-related behavior [51]. Studies focusing on the putative protective role of Mineralocorticoid Receptor (MR) overexpression are ongoing (e.g. [52]).

Animals with a deletion of the serotonin transporter (5-HTT) gene, an important modulator of ELS effects in humans, have also been used to study gene-by-environment interactions. Heterozygous 5-HTT KO *mice* are more vulnerable to negative consequences of reduced maternal care, via molecular mechanisms involving the neurotrophin system [53]. Yet, heterozygous 5-HTT KO *rats* show improved adult stress coping following maternal separation via methylation of the *Crf* gene [54]. These studies suggest a complex network in which candidate genes of the serotonergic system, HPA-axis and neurotrophic system -identified in human studies- together elicit the observed effects. Moreover, the improvements observed in 5-HTT KO rats indicate that developmental effects of genetic polymorphisms in response to ELS are not restricted to detrimental effects per se, in line with the match/mismatch theory.

In addition, human studies suggest that genetic variation could contribute to increased environmental susceptibility 'for better *and* for worse', i.e. differential susceptibility [55;56]. This has been studied in particular for the (loss-of-function) DRD4-7 repeat allele, in which carriers exposed to ELS are more prone to develop disorders such as ADHD in chaotic environments [57]. At the same time, children carrying this allele are more likely to benefit from an intervention creating a more

predictable, rewarding and sensitive environment [58]. For a thorough understanding of the neurobiological mechanisms underlying differential susceptibility in rodents, animals should be exposed to both adverse and stimulating rearing environments and subsequently tested for developmental progress.

Revealing the underlying neurobiology

In humans, neurobiological processes underlying adaptations to early-life environment can be studied e.g. with EEG, MRI or post-mortem dissection. For example, differences in functional connectivity following early-life adversity have been studied in relation to thalamic connectivity [59] and emotion regulatory networks [60]. Most of these techniques are also available for animals, although in contrast to humans, rodent imaging studies are predominantly conducted in anesthetized animals. Recent advancements enable imaging studies in awake animals [61], also applied in ELS studies [62]. However, stress associated with restraining in awake animals can interfere with outcome measures.

Overlapping methodology contributes to direct comparisons between the human and rodent brain; yet, more detailed knowledge on neurobiological changes can presently only be obtained in animal models. This is best illustrated by extensive work on the hippocampus, a brain area consistently affected by ELS [5;63;64]. Hippocampal neuronal cell proliferation and neurogenesis after ELS was found to be increased during adolescence and decreased in adult male animals. At the cellular level, ELS reduced neuronal complexity, as shown by alterations in mossy fiber density and granule cell dendritic morphology. In addition, GR, MR and IL-6 receptor expression, as well as AMPA, NMDA and GABA receptor function and subunit expression were all associated with reductions in maternal care [63;64]. Finally, electrophysiological recordings revealed that moderate to severe ELS usually suppresses the ability to induce synaptic plasticity in the adult hippocampus [65]. A similar focus on other brain regions might shed light on the ensemble of cellular changes responsible for ELS effects.

Linking these cellular measurements directly to behavioral observations will be of critical importance for the translational potential to humans [19]. Promising future directions include sophisticated optogenetic tools, in which light-sensitive ion channels are expressed in targeted neurons, which allows the activation or repression of specific neuronal populations by exposure to a light pulse. This technique enables in vivo examination of causal relations between stimulation, activity of neuronal subpopulations, and behavior at any point in time and has begun to delineate the precise underlying mechanisms of parental care in rodents [25•;66•] and, when applied to offspring, may help to characterize the molecular pathways involved in adaptations to early-life conditions.

Concluding remarks

Despite some limitations, rodent studies offer excellent gene-by-environment control, interventional opportunities and greater spatiotemporal detail in the examination of ELS effects on brain development compared to human studies. Cross-species effects of ELS point to converging mechanisms [3], and human and animal studies both benefit from integrating developments in the respective fields. Obviously, a direct translation from the animal to the human situation and vice versa is impossible; species specific (evolutionary) needs should always be kept in mind, and we should think in endophenotypes rather than modelling human disease [9]. Yet, future studies can and should address some of the shortcomings that currently hamper translational value. Firstly, rodent studies are systematically underpowered, partly because numbers of animals are based on effect sizes, often overestimated because of publication bias [67]. This is hindering reproducibility and stresses the need of reliable effect size estimations. Moreover, improved reporting of procedural details, group size and effect sizes, now often omitted in rodent studies [14••], should facilitate meta-analytic work, often used in human studies, but remarkably absent in the rodent literature.

In addition, it is important to fine-tune techniques used in both humans and animals, allowing direct comparisons between species while complementing human findings with results from experimental approaches that are unique to animal studies. Studies of early-life adversity effects on amygdala-

prefrontal connectivity illustrate the power of this approach [68••]. Here, human experiments were driven by animal studies showing accelerated maturation of amygdala [69] and mPFC neurons [70] after ELS. Similarly, accelerated amygdala-mPFC connectivity maturation was found in previously institutionalized children, in a cortisol-dependent manner [68••].

In conclusion, animal models allow for detailed investigation of the mechanisms through which differences in parental care lead to alterations in offspring's brain development. With some improvements and application of novel techniques, our understanding of parental influence on offspring development will greatly expand.

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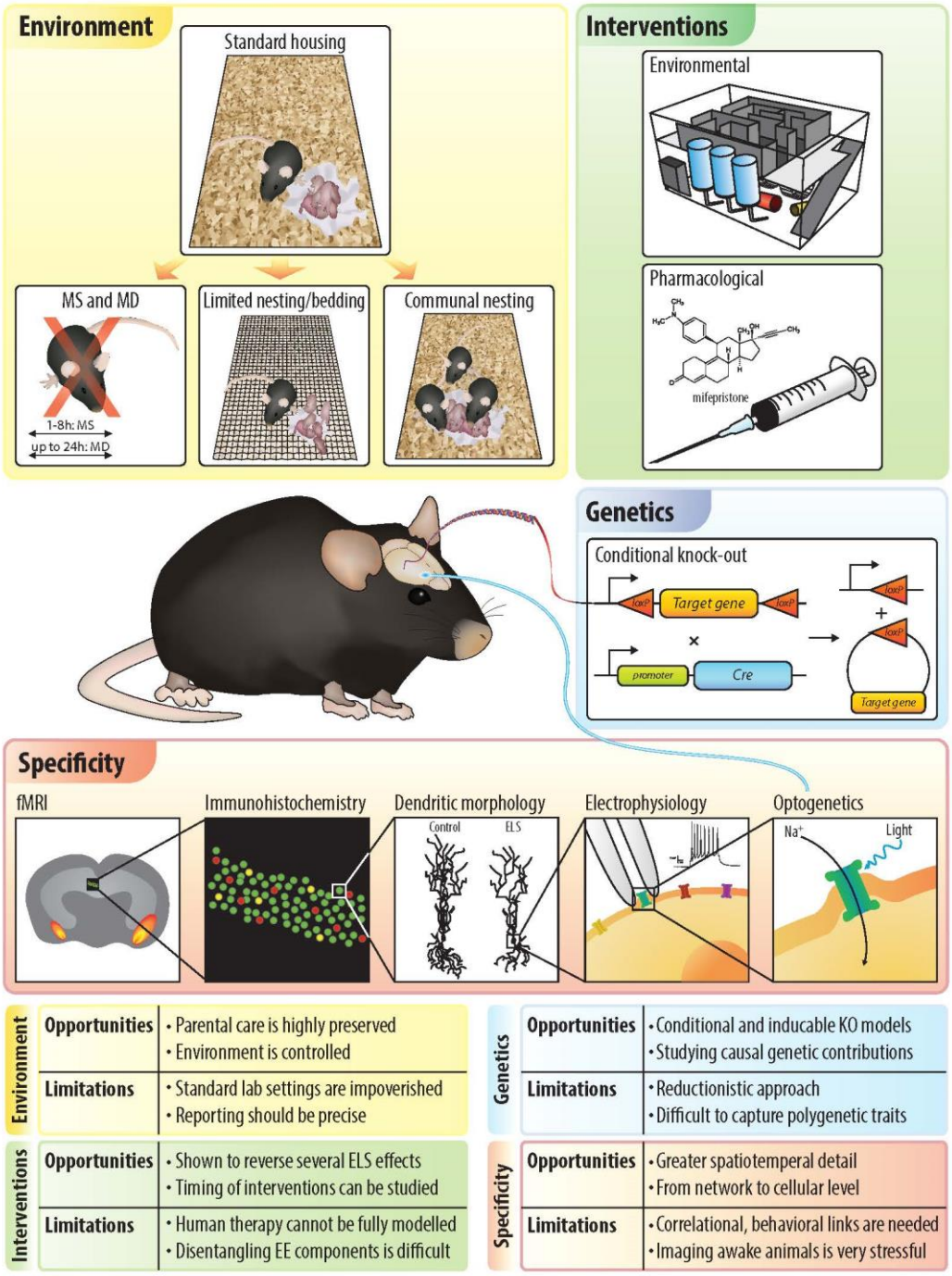

Figure 1

Opportunities for studying parental influence on offspring development in rodent models

Schematic representation summarizing the four domains in which rodent models offer unique advantages for studying parental influences on offspring development compared to human studies. For each aspect, some advantages and points of attention are provided. This figure is not extensive, but illustrates possibilities. MS: maternal separation, MD: maternal deprivation, ELS: early-life stress, fMRI: functional magnetic resonance imaging.