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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Reference List

1. Ehlert U: **Enduring psychobiological effects of childhood adversity**. *Psychoneuroendocrinology* 2013, **38:**1850-1857.

2. de Baca TC, Ellis BJ. **Early stress, parental motivation, and reproductive decision-making: Applications of life history theory to parental behavior.** *Curr Op in Psychol* in press, this issue.

3. Callaghan BL, Sullivan RM, Howell B, Tottenham N: **The international society for developmental psychobiology Sackler symposium: Early adversity and the maturation of emotion circuits: cross-species analysis**. *Developmental psychobiology* 2014, **56:**1635-1650.

4. Dutta S, Sengupta P: **Men and mice: relating their ages**. *Life sciences* 2016, **152:**244-248.

5. Krugers HJ, Joëls M: **Long-lasting consequences of early life stress on brain structure, emotion and cognition**. In *Behavioral Neurobiology of Stress-related Disorders*. Springer; 2014:81-92.

6. Levine S: **Developmental determinants of sensitivity and resistance to stress**. *Psychoneuroendocrinology* 2005, **30:**939-946.

7. Molet J, Maras PM, Avishai-liner S, Baram TZ: **Naturalistic rodent models of chronic early–life stress**. *Developmental psychobiology* 2014, **56:**1675-1688.

8. Schmidt MV, Wang XD, Meijer OC: **Early life stress paradigms in rodents: potential animal models of depression?** *Psychopharmacology* 2011, **214:**131-140.

9. Andersen SL: **Exposure to early adversity: Points of cross-species translation that can lead to improved understanding of depression**. *Development and psychopathology* 2015, **27:**477-491.

10. Haller J, Harold G, Sandi C, Neumann ID: **Effects of Adverse Early–Life Events on Aggression and Anti–Social Behaviours in Animals and Humans**. *Journal of neuroendocrinology* 2014, **26:**724-738.

••11. Curley JP, Champagne FA: **Influence of maternal care on the developing brain: Mechanisms, temporal dynamics and sensitive periods**. *Frontiers in neuroendocrinology* 2016, **40:**52-66.
Overview of the neurobiological effects of natural variations in maternal care, limited nesting and communal nesting, with special emphasis on the temporal aspect. The authors conclude that studying the timing of sensitivity to alterations in maternal care is crucial for understanding the mechanisms of maternal influence on offspring development.

12. Lehmann J, Feldon J: **Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing?** *Reviews in the neurosciences* 2000, **11:**383.

13. Marco EM, Llorente R, López-Gallardo M, Mela V, Llorente-Berzal Á, Prada C, Viveros MP: **The maternal deprivation animal model revisited**. *Neuroscience & Biobehavioral Reviews* 2015, **51:**151-163.

••14. Tractenberg SG, Levandowski ML, de Azeredo LAj, Orso R, Roithmann LG, Hoffmann ES, Brenhouse H, Grassi-Oliveira R: **An overview of maternal separation effects on behavioural outcomes in mice: Evidence from a four-stage methodological systematic review**. *Neuroscience & Biobehavioral Reviews* 2016, **68:**489-503.
This systematic review critically discusses methodological inconsistencies in maternal separation mice studies. The authors conclude that depressive-like behaviors and memory performance are most consistently affected by ELS, most notably in the BALB/c mouse strain. In addition, methodological recommendations are provided to improve consistency in future maternal separation mice studies.

15. Claessens SE, Daskalakis NP, van der Veen R, Oitzl MS, de Kloet ER, Champagne DL: **Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences**. *Psychopharmacology* 2011, **214:**141-154.

16. Simpson JA, Belsky J: **Attachment theory within a modern evolutionary framework**. In *Handbook of attachment: Theory, research, and clinical applications, 3rd ed*. Edited by Cassidy J, Shaver P. New York, NY, US: Guilford Press; 2016:91-116.

17. Rincón-Cortés M, Sullivan RM: **Early Life Trauma and Attachment: Immediate and Enduring Effects on Neurobehavioral and Stress Axis Development**. *Front Endocrinol (Lausanne)* 2014, **5:**33.

18. Nederhof E, Schmidt MV: **Mismatch or cumulative stress: toward an integrated hypothesis of programming effects**. *Physiology & behavior* 2012, **106:**691-700.

19. Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, Joëls M, Krugers H: **Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress**. *The Journal of Neuroscience* 2008, **28:**6037-6045.

20. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER: **The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome**. *Psychoneuroendocrinology* 2013, **38:**1858-1873.

21. Rilling JK, Mascaro JS. **The neurobiology of fatherhood.** *Curr Op in Psychol* in press, this issue.

22. Sarkadi A, Kristiansson R, Oberklaid F, Bremberg S: **Fathers' involvement and children's developmental outcomes: A systematic review of longitudinal studies**. *Acta paediatrica* 2008, **97:**153-158.

23. Bales KL, Saltzman W: **Fathering in rodents: neurobiological substrates and consequences for offspring**. *Hormones and Behavior* 2016, **77:**249-259.

•24. Katayama M, Hirayama T, Horie K, Kiyono T, Donai K, Takeda S, Nishimori K, Fukuda T: **Induced pluripotent stem cells with six reprogramming factors from Prairie Vole, which is an animal model for social behaviors**. *Cell transplantation* 2016.
Promising basis for the introduction of genetic tools in socially monogamous animals. As prairie voles exhibit paternal care and similar oxytocin expression patterns as humans, establishing conditional genetic models may contribute to better understanding of the neurobiology underlying social behaviours such as paternal care.

•25. Dulac C, O'Connell LA, Wu Z: **Neural control of maternal and paternal behaviors**. *Science* 2014, **345:**765-770.
This review describes the neural circuitry regulating parental care across a variety of species, emphasising the antagonistic role of certain brain areas in regulating both parental care and infant-directed aggression. The paper describes optogenetic studies targeting galanin expressing neurons in the medial preoptic area (MPOA). Ablation of these neurons impaired parental behavior in mothers and fathers and induced pup-directed aggression in virgin female mice, whereas activation in virgin males suppressed pup-directed aggression.

26. Tachikawa KS, Yoshihara Y, Kuroda KO: **Behavioral transition from attack to parenting in male mice: a crucial role of the vomeronasal system**. *The Journal of Neuroscience* 2013, **33:**5120-5126.

27. Liu HX, Lopatina O, Higashida C, Fujimoto H, Akther S, Inzhutova A, Liang M, Zhong J, Tsuji T, Yoshihara T, *et al*: **Displays of paternal mouse pup retrieval following communicative interaction with maternal mates**. *Nat Commun* 2013, **4:**1346.

•28. Gapp K, Bohacek J, Grossmann J, Brunner AM, Manuella F, Nanni P, Mansuy IM: **Potential of environmental enrichment to prevent transgenerational effects of paternal trauma.** *Neuropsychopharmacology* 2016, **41**:2749-2758.
Parental effects on offspring development are not restricted to behavioural influence alone, as transgenerational effects can also be transmitted via altered methylation patterns in the germline. Using a mouse model of maternal separation and unpredictable stress, male adult

mice and their male offspring showed altered coping behavior. This was accompanied by altered GR promoter methylation in the hippocampus and sperm cells. The effects of paternal trauma on offspring were prevented by environmental enrichment, indicating plasticity of the epigenome across life.

29. Würbel H: **Ideal homes? Housing effects on rodent brain and behaviour**. *Trends in neurosciences* 2001, **24:**207-211.

•30. Branchi I, Cirulli F: **Early experiences: building up the tools to face the challenges of adult life**. *Developmental psychobiology* 2014, **56:**1661-1674.
Depending on the environment, up to 80% of feral mouse populations raise their young in communal nests. This results in increased levels and various styles of maternal care and peer-interactions, leading to a variety of neurobiological adaptations. In this review, these effects are discussed and communal nesting is argued to represent a more naturalistic rearing setting.

31. Bales KL. **Parenting in animals.** *Curr Op in Psychol* in press, this issue.

32. Gilbert R, Widom CS, Browne K, Fergusson D, Webb E, Janson S: **Burden and consequences of child maltreatment in high-income countries**. *The lancet* 2009, **373:**68-81.
33. Andersen SL, Teicher MH: **Stress, sensitive periods and maturational events in adolescent depression**. *Trends in neurosciences* 2008, **31:**183-191.

34. Francis DD, Diorio J, Plotsky PM, Meaney MJ: **Environmental enrichment reverses the effects of maternal separation on stress reactivity**. *The Journal of Neuroscience* 2002, **22:**7840-7843.

35. do Prado CH, Narahari T, Holland FH, Lee H-N, Murthy SK, Brenhouse HC: **Effects of early adolescent environmental enrichment on cognitive dysfunction, prefrontal cortex development, and inflammatory cytokines after early life stress**. *Developmental psychobiology* 2015.

36. Hui Jj, Zhang Zj, Liu Ss, Xi Gj, Zhang Xr, Teng GJ, Chan KC, Wu EX, Nie Bb, Shan Bc, *et al*: **Hippocampal neurochemistry is involved in the behavioural effects of neonatal maternal separation and their reversal by post-weaning environmental enrichment: A magnetic resonance study**. *Behavioural Brain Research* 2011, **217:**122-127.

37. Van Praag H, Kempermann G, Gage FH: **Neural consequences of enviromental enrichment**. *Nature Reviews Neuroscience* 2000, **1:**191-198.

38. Pietropaolo S, Feldon J, Alleva E, Cirulli F, Yee BK: **The role of voluntary exercise in enriched rearing: a behavioral analysis**. *Behavioral neuroscience* 2006, **120:**787.

•39. Rogers J, Vo U, Buret LS, Pang TY, Meiklejohn H, Zeleznikow-Johnston A, Churilov L, Van Den Buuse M, Hannan AJ, Renoir T: **Dissociating the therapeutic effects of environmental enrichment and exercise in a mouse model of anxiety with cognitive impairment**. *Translational psychiatry* 2016, **6:**e794.
It is often difficult to disentangle the role of different aspects in environmental enrichment. This study adressed this issue by exposing wild-type and 5-HT1AR KO mice to either running wheels (Ex) or environmental enrichment without exercise (EE). Differences in anxiety and cognitive behaviours between Ex and EE groups were observed, independent of genotype. Certain cognitive EE effects were shown to be mediated by serotonin signalling.

40. Zhang TY, Labonté B, Wen XL, Turecki G, Meaney MJ: **Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans**. *Neuropsychopharmacology* 2013, **38:**111-123.

41. Weaver IC, Meaney MJ, Szyf M: **Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood**. *Proceedings of the National Academy of Sciences of the United States of America* 2006, **103:**3480-3485.

42. Loi M, Koricka S, Lucassen PJ, Joëls M: **Age- and sex-dependent effects of early life stress on hippocampal neurogenesis**. *Front Endocrinol (Lausanne)* 2014, **5:**13.

•43. Arp JM, ter Horst JP, Loi M, den Blaauwen J, Bangert E, Fernández G, Joëls M, Oitzl MS, Krugers H: **Blocking glucocorticoid receptors at adolescent age prevents enhanced freezing between repeated cue-exposures after conditioned fear in adult mice raised under chronic early life stress**. *Neurobiology of learning and memory* 2016, **133**:30-8.
This study explored the possibility to counteract ELS effects by brief mifepristone (antiglucocorticoid) treatment during early adolescence in mice. Freezing behavior was affected by the limited nesting paradigm, only in male offspring. A 3-day mifepristone treatment from postnatal day 28-30 normalized freezing in cue-off periods that represent a potentially safe context.

44. Aisa B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ: **Effects of maternal separation on hypothalamic–pituitary–adrenal responses, cognition and vulnerability to stress in adult female rats**. *Neuroscience* 2008, **154:**1218-1226.

45. Kentrop J, van der Tas L, Loi M, Van IJzendoorn M, Bakermans-Kranenburg MJ, Joëls M, van der Veen R: **Mifepristone treatment during early adolescence fails to restore maternal deprivation-induced deficits in behavioral inhibition of adult male rats**. *Frontiers in behavioral neuroscience* 2016, **10**.

46. El Khoury A, Gruber SH, Mørk A, Mathé AA: **Adult life behavioral consequences of early maternal separation are alleviated by escitalopram treatment in a rat model of depression**. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2006, **30:**535-540.

47. Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE: **Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits**. *Focus* 2010, **8:**398-416.

48. Nugent NR, Tyrka AR, Carpenter LL, Price LH: **Gene–environment interactions: early life stress and risk for depressive and anxiety disorders**. *Psychopharmacology* 2011, **214:**175-196.

49. Montalvo-Ortiz JL, Gelernter J, Hudziak J, Kaufman J: **RDoC and translational perspectives on the genetics of trauma–related psychiatric disorders**. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 2016, **171:**81-91.

50. Schmidt M, Oitzl MS, Müller MB, Ohl F, Wurst W, Holsboer F, Levine S, De Kloet ER: **Regulation of the developing hypothalamic–pituitary-adrenal axis in corticotropin releasing hormone receptor 1-deficient mice**. *Neuroscience* 2003, **119:**589-595.

51. Wang X–D, Labermaier C, Holsboer F, Wurst W, Deussing JM, Müller MB, Schmidt MV: **Early–life stress–induced anxiety–related behavior in adult mice partially requires forebrain corticotropin–releasing hormone receptor 1**. *European Journal of Neuroscience* 2012, **36:**2360-2367.

52. Kanatsou S, ter Horst JP, Harris AP, Seckl JR, Krugers HJ, Joëls M: **Effects of mineralocorticoid receptor overexpression on anxiety and memory after early life stress in female mice**. *Frontiers in behavioral neuroscience* 2015, **9**.

53. Carola V, Frazzetto G, Pascucci T, Audero E, Puglisi-Allegra S, Cabib S, Lesch KP, Gross C: **Identifying molecular substrates in a mouse model of the serotonin transporter x environment risk factor for anxiety and depression**. *Biological psychiatry* 2008, **63:**840-846.

54. van der Doelen RH, Arnoldussen IA, Ghareh H, van Och L, Homberg JR, Kozicz T: **Early life adversity and serotonin transporter gene variation interact to affect DNA methylation of the corticotropin-releasing factor gene promoter region in the adult rat brain**. *Development and psychopathology* 2015, **27:**123-135.

55. Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, van IJzendoorn MH: **Differential susceptibility to the environment: An evolutionary-neurodevelopmental theory**. *Development and psychopathology* 2011, **23:**7-28.

56. Belsky J, van IJzendoorn MH. **Genetic Differential Susceptibility to the Effects of Parenting**. *Curr Op in Psychol* in press, this issue.

57. Ebstein RP, Benjamin J, Belmaker RH: **Behavioral genetics, genomics, and personality**. In *Behavioral genetics in the postgenomic era*. Edited by Plomin R, DeFries JC, Craig IW, McGuffin P. Washington, DC, US: American Psychological Association; 2003:365-388.

58. Bakermans-Kranenburg MJ, van IJzendoorn MH: **The hidden efficacy of interventions: Gene x environment experiments from a differential susceptibility perspective**. *Annual Review of Psychology* 2015, **66:**381-409.

59. Philip NS, Tyrka AR, Albright SE, Sweet LH, Almeida J, Price LH, Carpenter LL: **Early life stress predicts thalamic hyperconnectivity: a transdiagnostic study of global connectivity**. *Journal of psychiatric research* 2016, **79:**93-100.

60. Cisler JM, James GA, Tripathi S, Mletzko T, Heim C, Hu XP, Mayberg HS, Nemeroff CB, Kilts CD: **Differential functional connectivity within an emotion regulation neural network among individuals resilient and susceptible to the depressogenic effects of early life stress**. *Psychological medicine* 2013, **43:**507-518.

61. Martin C: **Contributions and complexities from the use of in vivo animal models to improve understanding of human neuroimaging signals**. *Frontiers in neuroscience* 2014, **8:**211.

62. Brydges NM, Whalley HC, Jansen MA, Merrifield GD, Wood ER, Lawrie SM, Wynne SM, Day M, Fleetwood-Walker S, Steele D, Marshall I, *et al*: **Imaging Conditioned Fear Circuitry Using Awake Rodent fMRI**. *PloS one* 2013, **8:**e54197.

63. Fenoglio KA, Brunson KL, Baram TZ: **Hippocampal neuroplasticity induced by early-life stress: functional and molecular aspects**. *Frontiers in neuroendocrinology* 2006, **27:**180-192.

64. Huang LT: **Early-life stress impacts the developing hippocampus and primes seizure occurrence: cellular, molecular, and epigenetic mechanisms**. *Frontiers in molecular neuroscience* 2014, **7**.

65. Joëls M, Sarabdjitsingh RA, Karst H: **Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes**. *Pharmacological reviews* 2012, **64:**901-938.

•66. Scott N, Prigge M, Yizhar O, Kimchi T: **A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion**. *Nature* 2015, **525:**519-522.
This study shows that optogenetic activation and ablation of tyroxine hydroxylase (TH)-expressing neurons in a specific neuronal population of the hypothalamus results in enhanced and impaired maternal care, respectively. Activation of this system in males also suppressed inter-male aggression. The neuronal population was linked to oxytocin secretion. It illustrates how optogenetics can be used to study social behaviours in parents, and potentially in their offspring.

67. Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, Munafò MR: **Power failure: why small sample size undermines the reliability of neuroscience**. *Nature Reviews Neuroscience* 2013, **14:**365-376.

••68. Gee DG, Gabard-Durnam LJ, Flannery J, Goff B, Humphreys KL, Telzer EH, Hare TA, Bookheimer SY, Tottenham N: **Early developmental emergence of human amygdala–prefrontal connectivity after maternal deprivation**. *Proceedings of the National Academy of Sciences* 2013, **110:**15638-15643.
This describes how maternal deprivation studies in rats, in which accelerated maturation of amygdala-prefrontal connectivity was observed, guided studies in humans. They used a human sample of previously institutionalised children, resembling rodent deprivation studies, and found that these children exhibit early emergence of an adult-like neural phenotype. Importantly, this was mediated by cortisol, confirming an important role of the HPA-axis, similar to findings in rodents.

69. Ono M, Kikusui T, Sasaki N, Ichikawa M, Mori Y, Murakami-Murofushi K: **Early weaning induces anxiety and precocious myelination in the anterior part of the basolateral amygdala of male Balb/c mice**. *Neuroscience* 2008, **156:**1103-1110.

70. Muhammad A, Carroll C, Kolb B: **Stress during development alters dendritic morphology in the nucleus accumbens and prefrontal cortex**. *Neuroscience* 2012, **216:**103-109.

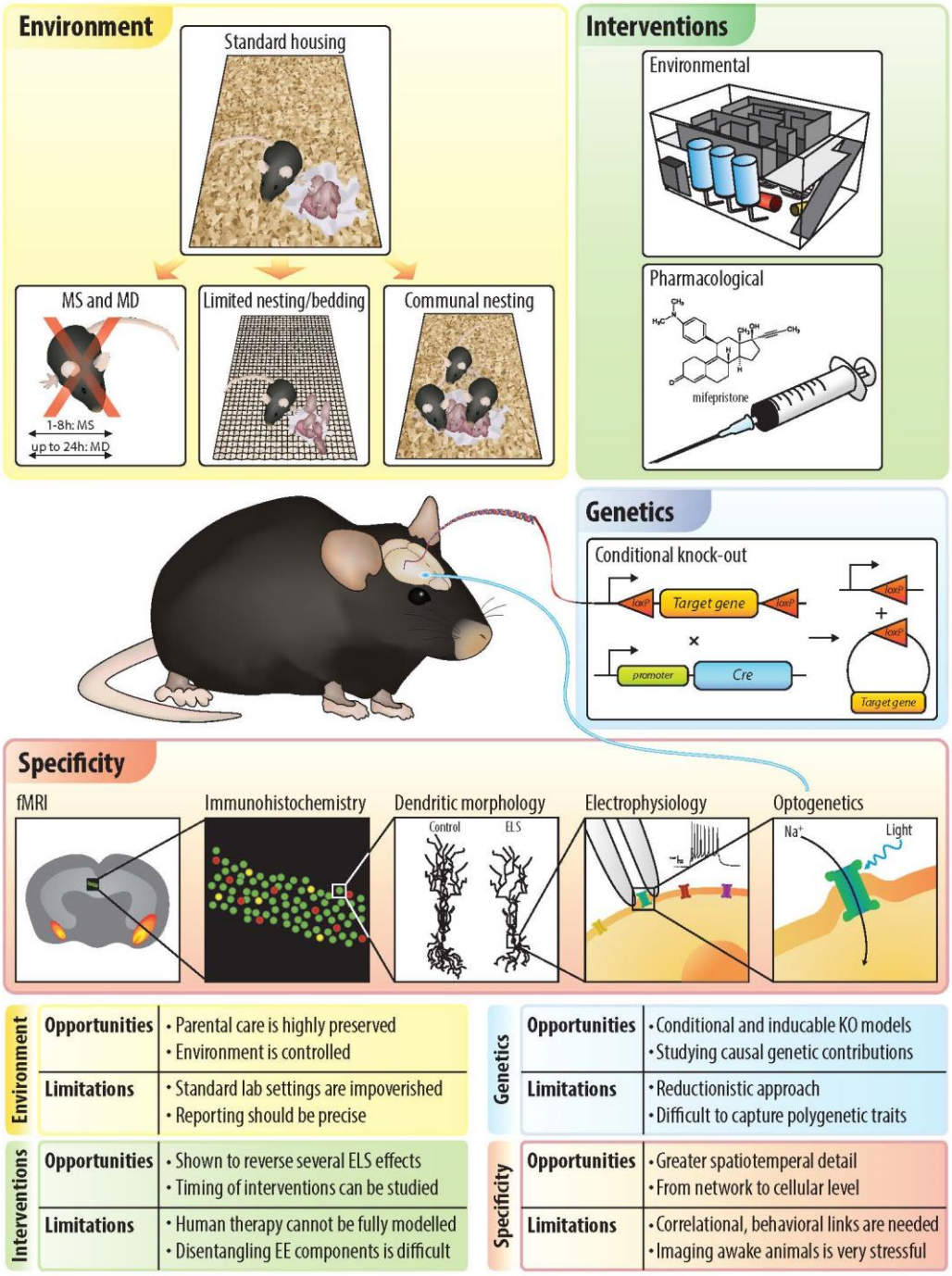

Figure 1

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.