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Bundling the haystack to find the needle: Challenges and opportunities in modeling risk and resilience following early life stress

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ABSTRACT

Various forms of early life adversity (ELA) have been linked with increased risk for negative health outcomes, including neuropsychiatric disorders. Understanding how the complex interplay between types, timing, duration, and severity of ELA, together with individual differences in genetic, socio-cultural, and physiological differences can mediate risk and resilience has proven difficult in population based studies. Use of animal models provides a powerful toolset to isolate key variables underlying risk for altered neural and behavioral maturational trajectories. However, a lack of clarity regarding the unique features of differing forms of adversity, lab differences in the implementation and reporting of methods, and the ability compare across labs and types of ELA has led to some confusion. Here, we highlight the diversity of approaches available, current challenges, and a possible ways forward to increase clarity and drive more meaningful and fruitful implementation and comparison of these approaches.

1. Introduction

Considering the vast number of children worldwide growing up under some form of hardship, the importance of understanding how early life adversity (ELA) influences development is undeniable. ELA is associated with increased vulnerability to affective disorders and externalizing disorders that are often resistant to traditional treatments (Pattwell and Bath, 2017; Ganguly and Brenhouse, 2015; Nemeroff et al., 2003). The sex of an individual, genetic lineage, and the presence of later-life stressors further interact with early life experience to sculpt the biological mechanisms driving mental health (Walker et al., 2017). Clinical neuroscience and psychiatry are therefore sorely in need of precision-based strategies that regard life history together with sex, age, and genetic makeup to guide mechanistic targets for illness prevention and treatment. However, since many of the symptoms resulting from ELA often first emerge later in life (Teicher et al., 2009), intervening variables found in clinical studies make the role that ELA plays in these diseases difficult to interpret. Animal models have therefore been helpful to clarify the causal relationships between ELA and atypical phenotypes, however, they also bring new issues including the translatability to human experience, strain and species differences, and paradigm differences between laboratories. Indeed, the increasingly wide variability in species, strains, and paradigms used to model ELA has shrouded many important discoveries in confusion about which ELA components lead to which outcomes, and how. Importantly, ELA does not characteristically "break" an individual, but rather can alter developmental trajectories to yield either maladaptive outcomes or resilience, depending on the situation or environment. In this perspective, we propose challenges and opportunities inherent to the search for mechanisms of risk and resilience following ELA.

1.1. What is "Early Life Stress"?

The most-oft adopted term for animal models of ELA has been "early life stress." While we ourselves have used this term in our own work, here we have replaced it with "early life adversity" because it is possible that stress is not the primary causal factor of some observed outcomes. Rather, sensory stimuli received from caretakers during early life can profoundly influence the developing organism (Baram et al., 2012). Parental behavior during postnatal life, such as maternal licking and grooming of pups, nursing, and leaving the nest, typically occur in a pattern and sequence that are adaptive for the parent-offspring relationship. Active maternal behaviors, specifically licking and grooming, have been identified as shapers of HPA reactivity (Suchecki et al., 1993) but also of attachment circuits that enable formation of secure relationships (Baram et al., 2012). Baram and colleagues have shown that fragmentation, or disorganized shortened bursts of these caretaking behaviors, results in less hippocampal dendritic complexity,

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cognitive deficits, and increased anxiety-like behaviors later in life. Notably, impoverished and fragmented care does also activate physiological stress responses such as HPA activity (Bath et al., 2016; Bath et al., 2017; Rice et al., 2008), so whether these negative outcomes would occur regardless of whether physiological stress circuitry is activated is not entirely clear. In 1950 Hans Selye proposed the important distinction between the damage/defense associated with a "general adaptation syndrome" to a stressor, and the direct actions of the stimuli making up that stressor, He stated,

"In addition to damage and defence, every stressor also produces certain specific actions … quite apart from their stressor effects. Hence the general adaptation syndrome never occurs in its pure form, but is always complicated by superimposed specific actions of the eliciting stressors. In contemplating any biologic response (e.g., a spontaneous disease, an intoxication, a psychosomatic reaction), it is usually quite difficult to identify individual manifestations as being due respectively to damage, defence, or specific actions of the provocative agent. Only non-specific damage and defence are integral parts of the general adaptation syndrome, but the specific actions of the eliciting stressors modify the course of the resulting general adaptation syndrome."(Selye, 1950)

Taking this into consideration, it is likely that neurodevelopmental and behavioral consequences of ELA can arise from not just stress exposure, but also from the somatosensory stimuli associated with altered maternal signals during a critical period of development. Since the amount, sequence, or quality of caretaker touch may itself impact risk and resilience through its impact on thalamic and prefrontal cortical sensory integration (Molet et al., 2016; Parr et al., 2012), it is important to be mindful of mechanistic distinctions between stress-related mechanisms versus mechanisms related to sensory stimuli during critical periods of development. This distinction will serve to further characterize cause-effect relationships in order to best model mechanisms requiring intervention.

The atypical behaviors that are underpinned by these trauma-evoked mechanisms may importantly serve the individual in some cases, in order to predict or protect against threats that exist in the environment. Indeed, in children with a history of institutionalized care, atypical development of hippocampal-prefrontal connectivity was found to predict later reduction of anxiety (Silvers et al., 2016). In this study, the authors concluded that childhood adversity yielded accelerated development of hippocampal-prefrontal connectivity and enhanced discrimination learning in childhood, and while these factors did not protect against present anxiety, they were associated with resilience to later anxiety. We (HB) have also recently observed in maternally-separated rats that more mature (increased) functional connectivity between the basolateral amygdala and the infralimbic prefrontal cortex in pre-adolescence was correlated with lower anxiety-like behaviors (not yet published). Accelerated development of this circuitry has also been associated with precocious maturation of hippocampal structures and contextual fear learning in mice (Bath et al., 2016), sex selective risk for depressive-like phenotypes (Goodwill et al., 2018), and attentional impairments (Goodwill et al., 2018). At the same time, behavioral consequences of affected corticolimbic connectivity yield deficits in social behavior (Rincón-Cortés and Sullivan, 2016) that predict later depressive-like behavior in rodents. It therefore appears likely that circuits controlling learning, threat appraisal, and attachment are shaped to adapt to adverse early life stimuli, with consequences that can resemble risk or resilience, depending on the type of adversity being experienced, the circuit being probed, and later demands.

Consequences of adaptive or maladaptive alterations in distinct circuits and systems can largely depend on the synchrony between the ELA stimuli and age-related demands of the post-stress environment (Tottenham and Sheridan, 2009). For development of treatments and interventions to provoke resilience to later life disorders, basic research is therefore needed to identify mechanistic links between the experiences of a developing organism and later behavior across the lifespan. Just as clinicians and clinical researchers are met with vast heterogeneity in the types of adversity experienced by children, animal research utilizing models consists of various different paradigms. These paradigms differ in the age at which ELA is imposed, the types of adversity used, species and strains used, and a host of methodological details that are often lab-specific, even within the same general paradigm. We submit here that once this variability is appreciated as inevitable and even desired, the significant variables that truly drive targetable mechanisms can be inspected regardless of investigator-derived paradigms. Convergence onto these significant variables can be achieved through rigorous reporting and a pervasive understanding of representative terminology across laboratories.

2. Guide for investigators

2.1. Species choice

As outlined above, the choice of type and timing of adversity and species being used to model ELA will be critical for the hypothesis being tested. The choice of species will impact the type of adversity that can be modeled, the ability to translate observed effects on brain and behavioral development across species, and granularity at which key variables of interest can be manipulated and measured. A number of factors must be considered in making this choice. Below, some of the more commonly used species to model ELA are described, as well as some of the benefits and limitations of each of these model systems. Here, we highlight a subset of some of the more commonly used models, however this does not represent an exhaustive list of all species used in the study of ELA.

2.1.1. Non-human primate models

To understand the contribution of ELA to human risk for pathology, a number of labs have employed non-human primates (NHPs). NHPs represent an attractive model due to their close genetic relationship with humans, similar developmental time course in terms of brain and behavioral development, the dynamics of mother infant attachment and interaction, complex behavior, and similarities to humans with regard to genetic homology, ontogeny of brain development, and brain morphology. Some of the most famous studies of early adversity tested the impact of maternal presence, warmth, and stimulation through manipulation of the quality of the maternal stimulus in lab reared NHPs on infant development. Manipulating the quality of the maternal stimulus (including substituting the mother with a wire form mother), had significant ramifications for attachment, later maternal behavior, development of the stress response, and behavioral indicators of psychopathology (Arling and Harlow, 1967; Seay and Harlow, 1965; Seay et al., 1962; Seay et al., 1964; Harlow and Suomi, 1971; Young et al., 1973). In more recent studies of free ranging macaques, a more naturalistic form of ELA–natural variation in parental style, was employed. Researchers found variability in levels of parental care, including some mothers that engaged in rough handling, rejection, and dragging (which may represent forms of abuse) on infant development. Infant macaques raised with a mother exhibiting rough handling showed alterations in circulating stress hormone levels, increased emotional reactivity, and alterations in neurodevelopment, including hypertrophy of the amygdala, alterations in white matter development, and biomarkers of accelerated aging (Sanchez, 2006; Howell et al., 2014; Howell et al., 2013; Sanchez et al., 2010; Drury et al., 2017). Still others have used the marmoset NHP model to test the effects of maternal separation and early social behavior on neurogenesis, stress reactivity, and behavior (Mustoe et al., 2014; Taylor et al., 2014; Taylor et al., 2015; Dettling et al., 2002). While these studies have provided important insights into primate attachment, risk for pathological behaviors, and altered brain development, a number of limitations exist in the use of NHPs.

Experiments in NHP's are prohibitive due to cost, space requirements, researcher safety, veterinary staffing for special care of this population, as well as potential ethical considerations. In NHPs, researchers often have limited access to brain and bodily tissues to assess mechanistic underpinning of observed effects. Further, it is difficult to control for genetic variables that may impact risk and resilience. The long time course of development, from birth to adulthood, makes assessing the impacts of ELA on long-term outcomes cumbersome, often providing long time horizon's to determine the consequences of stress on neural and behavioral outcomes. As a privileged and valuable population, NHPs are often enrolled in multiple different studies across their lifespan, impacting the diversity of experiences and exposures that these animals have had. Further, the type and size of enclosures used to house NHPs also vary widely, providing significant differences in the daily activity, degree of enrichment, and social experiences of a given animal, making cross lab comparisons of manipulations difficult. Together, these factors can impact outcome measures and provide additional hurdles to understand the unique consequences of a given form of ELA on behavioral development in NHPs.

2.1.2. Rat models

Rats are one of the most commonly employed species for assessing the impact of ELA on neurobehavioral outcomes. Rats provide the ability to control for genetic variation, through the use of inbred strains, or to test for penetrance of behavioral effects on diverse genetic backgrounds in outbred or hybrid crossed strains. Under laboratory conditions, rats provide the researcher with the ability to precisely control the type, timing, and duration of the adversity experienced. A diversity of ELA manipulations are available in developing rats and can be implemented across a wide range of developmental intervals. Given the rapid development of rats, outcome measures can be collected within a matter of weeks to months instead of years. Rats also provide access to a wide range of behavioral assays to assess endophenotypic outcomes, are highly trainable, and exhibit stabile behavioral profiles relative to other rodent species. The ease of breeding, availability of large number of animals, and short lifespan provides the ability gain direct access to neural and bodily tissues to determine the impact of adversity on neurobiological outcomes, as well as employ and rapidly test the impact of novel interventions. All of these benefits have made rats one of the most useful model systems to assess the impact of ELA on neurobehavioral development.

While there are significant benefits to the use of rats as a model species, there are also some drawbacks. Specifically, when attempting to draw direct translational links to complex human pathological outcomes and timed insults, a number of factors must be considered. This includes the altricial nature of rats at birth relative to humans and other primates, with rats lacking visual input and having highly impoverished auditory inputs during the first week of postnatal life. While there is significant homology with regard to regional brain development and key circuits that regulate complex behaviors, the timing of regional brain development does not scale linearly across species or brain regions (Clancy et al., 2007; Clancy et al., 2007; Clancy et al., 2001), making the effects of timed intervention on global changes in brain development difficult to translate. For example, to match the timing of developmental milestones for hippocampus development, the first week of postnatal life is equivalent to early postnatal development in humans. However, the development of primary visual centers during that same first postnatal week in the rat are analogous to third trimester development in humans/primates. Thus, when interpreting the effects of ELA on development across species, investigators may want to avoid global approximations such as "equivalent to early childhood" and attempt to specify approximate timing for the neural structure or behavior being studied based on common developmental milestones (e.g. period of ocular dominance column formation).

When assessing behavioral outcomes related to emotional processing in rats, researchers must rely upon an endophenotyping approach. To do this, simplistic behaviors with defined and likely common biological underpinnings across species are assayed to identify disturbance in a given domain of function. As there is limited access to the subjective experience of animals, (e.g. emotional states and cognitive processes) researchers are often unable to recapitulate the full constellation of features of pathological conditions and to model many aspects of disorder. Thus, there is a emerging push away from developing animal models of disorder *per se*, and to instead develop animal models that recapitulate core behavioral disturbances that have commonalities to symptoms observed in a given disorder. Similarly, while significant genetic homology exists between rats and humans, researchers also need to take into consideration genetic differences between species that may inhibit the ability to generate translational models of some genetic risk factors. This includes differences in gene sequence, promoters, splice variants as well as gene function. Such differences can impact the physiological response to environmental manipulations, processing and efficacy of pharmacological agents, as well as confer differences in cellular morphology and distribution across brain regions.

On a practical level, laboratory rats are more costly to house than other rodent species, making it more difficult to carry out large scale studies. Housing conditions for laboratory bred rats (including other rodent species) are often similar across institutions, however, they often do not mimic the complexity of the natural ecology of the species, which involves complex social networks and scavenging for resources. Single and pair housing and limited size enclosures may function as impoverished environments, impacting outcomes measures and magnifying effects of adversity manipulations. Finally, while new technologies are emerging for gene editing, genetic manipulation of rats has lagged behind that of other species, making it more difficult to assess select genetic manipulation on risk and resilience, as well as to fully leverage new techniques, including optogenetic approaches in developing animal (which rely upon transgenic lines instead of viruses) and brain clearing techniques.

2.1.3. Laboratory mouse models

Over the past 20+ years, the use of mice in the study of ELA has grown significantly. As with rats, mice provide the opportunity to gain tight control over key early environmental and genetic variables and to precisely control the type and timing of exposure to ELA. As with rats, mice allow for experimental manipulation of a variety of types of ELA, the manipulation of the timing and duration of those experiences, and direct access to brain on bodily tissues. Laboratory mice breed well and mature rapidly, allowing researchers to quickly determine the effect of adversity on brain and behavioral outcomes. The small size of mice, and ability to house at higher densities than other rodent species, allow researchers to scale up studies, increasing throughput and speeding up discovery at a nominal increase in cost and space.

Mice were one of the first vertebrate species to benefit from the genetic revolution. As a consequence, a wide array of genetically engineered lines of animals are available to test the role of key genes on sensitivity to ELA. Many of the mouse models have been deposited at central repositories, allowing for distribution of these animals to the broader scientific community, and testing the generalizability of effects across labs within lines. The use of genetically engineered lines of mice also provides access to a multitude of transgenic tools that allow for cell population specific targeting, developmentally timed genetic manipulation, as well as the full advantage of newly developed optogenetic approaches and genetic modifications to manipulate circuit activity and map functional brain development. The availability of transgenic lines is critical for developmental studies in rapidly maturing species, as analogous viral targeting methods often require weeks for full infection and gene expression, making them difficult to employ when assessing early neurodevelopmental timepoints.

While mice offer a multitude of advantages, they suffer from many of the same limitations as rats (altricial nature, genetic differences,

scalar differences in timing of brain development compared to humans, artificial housing, etc). Mice also carry their own unique drawbacks. For example, depending upon the behavioral outcome being assayed, mice can provide a less stable model for many forms of behavioral assessment, and often require greater care and precise refinement of assays relative to rats. The small size of mice at birth (1–2 g) also makes some forms of early life manipulations difficult to employ (including the use of infusion pumps, surgical manipulation, in vivo physiology, and emerging in vivo imaging approaches) as microtization of these tools continues to progress.

2.1.4. California mice

Unlike common laboratory mice, the *California Mouse* has provided a unique benefit to researchers in the ELA field, in that it represents a biparental species in which researchers can manipulate the loss of a parent, or single parenting on neurodevelopment, while maintaining many of the benefits of small rodent species. This is a key factor, as biparental care (as seen in humans) is rare in mammals, occurring in fewer than 6% of species examined. In California mice, the male engages in many of the same behaviors performed by the dam, including licking and grooming, huddling, nest building, and pup retrieval (Dudley, 1974; Gubernick and Alberts, 1987; Gubernick et al., 1993).

While providing the unique benefit of modeling biparental care and potential buffering effect of this parenting style on outcomes, or the impact of loss of a parent on neurobehavioral development, this line of mice does come with several down sides. Due to the outbred nature of this line of mice, it does not offer the same precise genetic control afforded by heavily inbred mouse strains. Further, it suffers from many of the other hurdles that must be taken into account when working with mice and other small rodents, including the small size and altricial nature. Unlike some lines of laboratory bred rodents (C57Bl/6), behavior of California mice can be more erratic, as they have higher levels of activity and engage in high levels of stereotypic jumping behavior, making use of some traditional behavioral assays more challenging. Further, this line of mice lacks access to many of the readily available tools for genetic manipulation found in other mouse lines.

2.1.5. Octodon degus

A less common, but equally valuable species of rodent used in the study of ELA, is the semi-precocial *Octodon degus*. The degus is unique among rodents in that they are more similar to human infants with regard to sensory development, being born with more functional sensory systems (open ears and eyes), unlike mice and rats that do not show eye opening and unfolding of ears until ~11–15 days following birth. This affords the experimenter the ability to test the impact of environmental or experience-mediated influences on the full suite of sensory, attentional, and physiological development. Further, like California mice, degus engage in biparental care, opening up the ability to manipulate the presence or absence of one of the primary caregivers early development (Braun et al., 2013; Seidel et al., 2011). While there is greater parity with humans in biparental care and sensory development at birth, there are still important differences that need to be taken into account.

As with the California mice, research in degus lacks tight genetic control, access to key genetic tools, and, as an exotic species, genetic sequence data is often lacking and needs to be derived by the research team. While sensory development is more mature at birth in this species relative to other rodents, timing of regional brain development still needs to be considered, as regional differences in the timing of maturation are also found in this species. Further, species selective antibodies that were developed for mouse and rat tissues need to be optimized for histological assessment of degus brain. Despite these drawbacks, similar endophenotyping approaches can be used to measure the effects of ELA on neural and behavioral development (Becker et al., 2007; Helmeke et al., 2008; Seidel et al., 2008; Bock et al., 2012; Kunzler et al., 2015; Bock et al., 2017; Abraham and Gruss, 2010).

2.1.6. Voles

Like California mice and degus, a subset of species of voles engage in monogamous pair-bonding (Wu et al., 2014; Tabbaa et al., 2017), allowing for experimental manipulation of the type and level of early life care. Further, intra-species comparisons with closely related polygamous species of voles can be used to contrast effects of parental care on neurodevelopmental outcomes. Thus, voles provide a model in which intra-species comparisons can be made to determine the impact of different levels of parental investment and differing social structure on neurodevelopment, as well as the impact of parental buffering on response to secondary forms of adversity imposed over early development. As discussed earlier for other rodent species, most species of voles are outbred lines and thus do not afford the same precise control of genetic variation across animals. However, a number of genetic and viral tools have been developed in these species to manipulate neuroendocrine signals (Pitkow et al., 2001; Winslow et al., 1993; Wang et al., 1998). With regard to behavioral outcomes, extensive work has been carried out investigating the impact of early care on social approach and pair-bonding, however, given a slightly more erratic behavioral profile, testing in more complex behavioral assays havelagged behind that of other species.

2.1.7. Hamster

Hamsters provide unique advantages for assaying some forms of ELA on development. Specifically, multiple species of hamsters are believed to be a social isolates, preferring to dwell in single animal burroughs in the wild (Ross et al., 2017). Following weaning and initial social separation, hamsters often need to be housed singly in vivariums to avoid high levels of agonistic behavior and potential harm to cage mates. The high levels of aggression and ability to singly house allows greater control of social experiences as well as manipulation of agonistic experiences across the lifespan. In other common rodent species, isolation rearing would serve as a significant source of distress, which is not the case in hamsters. Unlike most rodent species, both male and female hamsters will engage in high levels of agonism (Huhman et al., 2003), allowing for manipulation of social defeat in females, which has proven difficult in mice and other rodents (Harris et al., 2018). Hamsters show a stereotyped development from play fighting to more mature agonistic behavior and develop stable dominant subordinate relationships that allow for careful manipulation of dominant and subordinate status. As a consequence, hamsters have been used to assess the effects of early agonistic encounters on brain development, later social defeat, weight gain, and ethanol ingestion (Delville et al., 2003; Wommack et al., 2004; Taravosh-Lahn and Delville, 2004; Wommack and Delville, 2007; Bastida et al., 2014; González-Martínez et al., 2017; Rosenhauer et al., 2017; Ferris and Brewer, 1996).

Hamsters share a number of limitations common to other rodents, in that they are born relatively altricial and have impoverished sensory input during early life. Further, their agonistic nature often results in the need to house single animals per cage, increasing the space and cost associated with working with this species. As they are a more “exotic” species of rodent, many genetic and histological tools available in mice and rats are lacking, as is control over genetic variability. While agonistic behavior and some simple choice behavioral manipulations are readily employable in hamsters, behavior can often be difficult to motivate. Thus, significant modifications must be made to common behavioral paradigms to make them more species relevant.

2.2. Choice of early life adversity paradigm

Adversity can come in many forms. For example, work in humans investigating adverse childhood experiences (ACE’s) have found that increased risk for negative health outcomes are associated with exposure to such variable as physical abuse, psychological abuse, sexual abuse, neglect, loss of a parent, drug abuse/exposure, invasive medical procedures, toxicant exposure, and witnessing violence (Felitti et al.,

1998; Dong et al., 2003). Additional variables have been identified through epidemiological studies and correlated with negative outcomes in population based studies, including food insecurity, bullying, homelessness, poverty, etc (Noble et al., 2005; Farah et al., 2006; Lawson et al., 2017; Wolke et al., 2013). Questions have arisen regarding whether each of these forms of adversity may have unique consequences on somatic, physiological, neural and behavioral development and confer risk for pathological outcomes. Work from the ACE's study have taken the approach of disregarding the type and timing of adversity, and merely counting the number and diversity of adverse experiences (Felitti et al., 1998; Dong et al., 2003; Dube et al., 2001; Dube et al., 2003). With nearly 20,000 participants, the ACE's study was powered to have sensitivity to detect elevations in risk, but without drilling down into the data-set, the overarching analyses lacked precision with regard to the types of experiences that conferred risk for select outcomes. While it's true that increasing the number and diversity of ACE's was associated with elevated risk for a variety of negative health outcomes, understanding the unique constellations of symptoms, and risk for specific disorders, likely requires greater precision and an understanding of the timing and type of adversity experienced. However, in humans, variables such as the type, timing, duration, severity, combinatorial effects of these variable, as well as contribution of genetic and cultural variables are difficult to segregate/isolate. This is partially due to the imprecise nature of recall of adverse events from childhood (including specific timing, number, and duration of events), differences in the subjective experience and real differences in severity of those experiences, presence or absence of stigma or cultural support, and genetic variation in the population. Model systems allow far more precision to control the type, timing, duration, and severity of many of forms of adverse early life experiences as well as key environmental and genetic factors. Below, we highlight some of the more commonly employed models of ELA and the type of experience they are proposed to model. We provide a subset of some of the more common models, however, this does not represent an exhaustive list. Further, for many manipulations, there is lab specific variation in the application of these paradigms (e.g. differences in timing, severity, duration, sex, etc.) that may represent important variables for determining outcomes.

2.2.1. Limited bedding (LB)

In the limited bedding manipulation, dams give birth to a litter, and shortly thereafter, the dam and litter is placed in a new cage with no (or limited) bedding and limited access to nesting material. This manipulation typically occurs for ~1 week and is generally restricted to the first weeks of postnatal life. The manipulation was originally designed to simulate stress associated with loss of caregiving resources, and may serve as a model of extreme poverty, homelessness, or refugee situations, where only the bare essential for shelter and nutrition are available. In this model, removal of bedding leads to significant distress in the dam, as evidenced by elevations in basal serum corticosterone levels, changes in CRF, and a fragmentation in the care that is delivered to the pup (Walker et al., 2017; Bath et al., 2016; Rice et al., 2008; Heun-Johnson and Levitt, 2016; Bath et al., 2017). In previous reports, using a total of 1–4 h of video sampling, researchers observed a fragmentation of care (indexed by increased sorties into and out of the nest) which was interpreted as a possible model of neglect, fragmentation in the predictability of provided care, or loss of sustained bouts of care (Rice et al., 2008; Heun-Johnson and Levitt, 2016). In more recent analysis of home cage behavior carried out by our lab (KGB), in which 24-hour video monitoring across the entire manipulation was used, we further believe that this manipulation is driving a form of hypervigilant augmented care. We base this interpretation on the observation that in addition to the elevations in sorties to and from the nest, we found that dams repeatedly return to interact with the pups, and showed an elevation in the total time on nest compared with control housing conditions (Gallo et al., in revision). Thus, this manipulation may model deficits in the sustained/predictability of care and stress level of the caregiver, while either not impacting or increasing the total care provided to the pups.

This manipulation has been employed in both rats and mice, and the core phenotype (increased nest sorties) has been replicated across labs in the U.S., Canada, Europe, and Australia. As with many manipulations, a single paradigm can come in multiple iterations, depending on the lab. For a more detailed discussion of the different variations of this paradigm see (Walker et al., 2017). In brief, different labs have manipulated the exact amount of materials provided, the presence or absence of the wire mesh floor, and the time during which the manipulation is implemented (e.g. postnatal days 2–9 versus 4–11). A significant benefit of this paradigm is its ease to employ and the limited contact between the animal and the experimenter. LB requires limited staffing as animals are transitioned to a new limited bedding environment at a single time, are left undisturbed for 7 days, and then returned to normal housing. Thus, potential impact of experimenter gender, coarseness of handling, odor, sensory experience, and other experimenter associated variables are diminished. While this manipulation limits experimenter contact, which may decrease inter-lab variability, some factors still need to be taken into account. A subset include: the type of housing (static, passive air, or forced air), type of wire mesh (gauge, diameter of openings, coating), elevation of mesh from the floor, ambient room temperature and lighting, and room traffic (amount of activity). Each of these factors may impact the implementation and or severity of this manipulation. As an example, Baram and colleagues have directly manipulated the amount of nesting material provided to dams and found a dose dependent effect on weight gain and pup mortality (Rice et al., 2008). Thus, while not meant to be prescriptive in how a given lab employs this procedure, many of these factors should be reported in methods sections to allow for future comparison of disparate outcomes between labs to determine relevant variables for observed outcomes.

2.2.2. Scarcity adversity

The scarcity adversity model has a number of similarities to the LB paradigm, in that the primary manipulation is limiting access of the dam to resources. However, there are some important differences between this paradigm and LB, which lead to unique consequences on maternal behavior. Specifically, Sullivan, Roth, and colleagues developed a less severe form of impoverished bedding that is implemented from P8-P12 (Raineki et al., 2010; Moriceau et al., 2009; Raineki et al., 2015) or intermittently from P1-7 (30 min- 1 h per day) (Blaze and Roth, 2013; Doherty et al., 2017; Roth et al., 2009; Roth and Sullivan, 2005) and occasionally includes the use of temporary care by foster dams. In this manipulation, the investigators have used paradigms in which individual litters can be split between conditions, by placing animals with foster dams that have access to either adequate bedding or scarce bedding for a defined amount of time, followed by return to their birthing dam. Thus, these studies can investigate effects of adversity using a within litter design, diminishing potential confounds of prenatal environment and potential long term effects of adversity on maternal care beyond the intended duration of the manipulation. Dams in scarce bedding conditions exhibit what appears to be significant distress and elevations in the expression of rough handling, biting, dragging, and kicking of the pups. Distribution of abusive behaviors appear to be preferentially directed toward female pups over males (Keller et al., 2019). As a consequence, this particular variant of scarcity of resources may provide insights into the effects of distress associated with loss of resources to care for young, alterations in the predictability of care, as well as an increase in what appear to be abusive behaviors. However, care should be taken in calling the observed rough handling behaviors abuse, as this term connotes intentionality of the rats, to which the experimenter does not have access. This paradigm also offers the benefit that both control and scarcity exposure can be tested within litter, however, questions arise regarding the impact of mixing scarcity adversity pups with control pups. Mixing of pups that experienced

disparate levels of maternal care with a single dam could alter the amount or quality of maternal provided to pups, relative to unmanipulated groups (McCarty, 2017). For example, pups from high abuse dams may engage in high level of vocalization and activity, altering maternal behavior. This concern is tempered by reports showing a limited impact of cross-fostering on maternal behavior maternal behavior (van der Veen et al., 2008).

2.2.3. Brief handling

One of the earliest forms of environmentally induced adversity was the model of maternal handling, adapted by Seymore "Gig" Levine in the 1950's (Wiener and Levine, 1983; Wiener and Levine, 1978; Weinberg et al., 1978; Levine, 1967; Levine, 1956; Erskine et al., 1975; Weinberg and Levine, 1977; Weinberg et al., 1978; Weininger, 1953). In this model, Levine demonstrated that even brief bouts of handling by the experimenter (3–4 min) was capable of accelerating the maturation of the somatic stress response, with long-term consequences for HPA reactivity in adulthood (Suchecki, 2018). This work was some of the first to demonstrate the importance of early contact and care on HPA programming and was critical in overturning dogma that infants were insensitive to stress during the early developmental period, and unable to mount a somatic response to stress. Levine and colleagues expanded on this work to demonstrate the importance of early maternal contact on development in both rodents and NHPs and to develop additional models of maternal separation and maternal deprivation to better understanding the impact of maternal signals on the developing offspring. Curiously, in subsequent work, maternal handling was actually shown to represent a mixed model, in which following the return of pups from the handling manipulation, dams provided elevated levels of pup-directed maternal care, including increased anogenital licking relative to unmanipulated controls, possibly serving as a means to both induce distress and subsequently enrich or augment the quantity and quality of maternal care. Further studies repeatedly showed that animals exposed to brief handling typically had an attenuated or better modulated HPA response to stressors later in life, as well as faster recovery to basal hormone levels following termination of stress (Stern et al., 2010; Levine, 1957). Therefore, brief handling is often expected to confer resilience to stress-related dysfunction, though the mechanisms underpinning the long-term effects of brief handling are still under investigation.

2.2.4. Maternal separation

Harlow and Zimmermann (Harlow and Zimmermann, 1959) and others (Huot et al., 2001; Levine et al., 1985) found that, in contrast to the protective effect of early handling, depriving pups of maternal contact for long periods during postnatal development had negative effects on their ability to face future stressors. The maternal separation paradigm entails the removal of the mother from her offspring for at least three hours/day, and is meant to model impact of either loss of or diminished access to parental care, with consequential disruption of the infant-parent relationship. This loss is considered maladaptive since in the wild, mothers typically leave the nest to forage for no longer than 1–2 h (Grota and Ader, 1969). Benefits of the maternal separation paradigm are its long history with strong characterization and validation, and its ability to apply to virtually all mammalian species. However, the paradigm lends itself to a high degree of inter-lab variability, since implementation can vary in duration and number of separations, separation of pups from each other versus keeping the litter together, and location of the dam relative to the pups. To this point, several reviews (Nylander and Roman, 2013; Molet et al., 2014; Schmidt et al., 2011; Tractenberg et al., 2016) describe inconsistencies in the literature concerning definitions of maternal separation procedures and provide a summary of methodological definitions. Regardless of these variations, it is important to monitor and/or control nutritional and thermoregulation factors, since maternal contribution to nest temperature and nutrition is altered during separations. Importantly, several ELA paradigms including LB and maternal separation reportedly affect pup weight gain (Walker et al., 2017; Grassi-Oliveira et al., 2016) and nest temperature (Walker et al., 2017), therefore deprivation of the mother is not alone in these impacts on physiology.

Effects of maternal separation on maternal behavior have been repeatedly investigated, and while behavioral changes can differ depending on the species and strain, generally disorganized behaviors have been observed, with different temporal distributions of nursing bouts (Macri et al., 2004) and with elevated active maternal behaviors immediately following reunion (Macri et al., 2004; Bailoo et al., 2014). Due to the variabilities discussed above, no one maternal behavior profile exists for "maternal separation" as a whole. Taken together, the maternal separation paradigm continues to provide important diversity to ELA research, but is not immune to criticism and its heavy use has led to a need for renewed inspection for further clarity.

2.2.5. Maternal deprivation

The maternal deprivation model is meant to test the impact of protracted separation from the dam on neural and behavioral development of the pup. This form of separation was first developed to determine the impact of loss of nutrition and maternal contact on the development of the HPA response (Suchecki, 2018). Unlike the maternal separation paradigms, which are relatively short in duration, lasting for 3–4 h on successive days, maternal deprivation often includes the use of a 24-hour long continuous bout of separation of the pup from the dam. The use of this extended separation (deprivation) paradigm has been highly relevant in understanding the impact of temporary loss of nutrition, maternal contact and licking, and thermoregulation on brain and behavioral development (for review see (Suchecki, 2018). However, due to the extended nature of the separation, it must be employed with great care. For example, careful attention should be paid to vivarium conditions and to developmental timing. For example, very young rodents are poor at thermoregulating themselves prior to P12. Thus, temperature of the pups must be maintained through elevating the ambient temperature to avoid hypothermia. Further, in many rodent models, the first 24–48 h is critical for attachment. Thus, implementation of this paradigm during the very early postnatal period can increase the risk of pup rejection or cannibalize following return to the home cage for some species of rodents. As with maternal separation, there is significant interlab variation in the precise employment of this paradigm, with a renewed need to gain clarity with regard to variables of interest.

2.2.6. Paternal deprivation

While many labs focus on the impact of changes in maternal care or maternal stress level on neurobehavioral development, a number of models provide the ability to investigate the impact of bi-parental care or the loss of a single parent on development. These models are meant to both simulate the loss of a caregiver in humans (e.g. death, incarceration, or separation of a parent) as well as understand how manipulating the absolute quantity of care provided in biparental species impacts development. For example, in California mice, degus, and some species of voles, the father engages in high levels of pup directed care, including huddling, simulated arched back nursing, pup retrieval, and pup directed licking and grooming. In these models, a number of labs have begun to investigate the impact of the loss of the male on pup outcomes (Braun et al., 2013; Seidel et al., 2011; Wu et al., 2014; Tabbaa et al., 2017; Glasper et al., 2018). While a subset of those studies have focused on the impact of loss of the quantity of care provided following the removal of the male from the home cage, more recent studies have begun to also measure the impact of the loss of the male on stress in the dam, and the compounding effects of a loss in the quantity of care and maternal distress on the type and quality of care provided. While critically important for understanding the impact of the loss of a caregiver, this model requires the use of monogamous biparental species, limiting the majority of studies to a small subset of rodents and

NHPs. As mentioned previously, only 6% of mammals engage in biparental care. Further, in this paradigm the manipulation is almost solely used to investigate the impact of the loss of the male, as removal of the dam provides limited means of providing nutrition without significant experimenter intervention. While the principle manipulation is meant to alter levels of care, it is important to provide quantitation of key metrics of maternal behavior, including the impact of manipulations on pup contact, nursing, stress level of the dam, and possible nutritional effects on outcomes.

2.2.7. Natural variation in maternal care

While the majority of studies of ELA rely upon experimenter induced changes in the early environment to alter parental care or stress reactivity in the pup, other labs have relied upon monitoring natural variations in the quality of early care. One of the most famous examples of this comes from the work of Meaney and colleagues, where they monitored levels of pup-directed licking and identified high and low licking and grooming rats. In assessing outcomes, they identified significant differences in the development of anxiety and depressive-like behaviors, future maternal behavior, and identified epigenetic changes associated with variation in care that appeared to program future outcomes (Liu et al., 1997; Fish et al., 2004; Bredy et al., 2003; Champagne and Meaney, 2001; Caldji et al., 2000; Liu et al., 2000; Caldji et al., 1998; Anisman et al., 1998; Francis et al., 2000; van Hasselt et al., 2012). More recent studies in NHPs have employed a similar approach to investigate the impact of natural variation in maternal care, including the impact of mothers that engage in rough handling on neurodevelopmental outcomes (Howell et al., 2014; Sanchez et al., 2010). These models provide a more naturalistic manipulation, relying upon natural tendencies of animals rather than response to experimenter directed changes. However, such approaches are relatively labor intensive to quantify, can be variable in their expression and duration, and can lead to selection criteria that exclude all animals except those on the distal ends of the distribution of maternal care. Thus, much larger groups of animals may need to be assayed to account for the high degree of variability in the type and quality of maternal care, with regression analyses being employed to attempt to relate levels of care with observed outcomes. Alternatively, individual animals can be selectively bred for these core features, however, in implementing such approaches the inclusion of cross-fostering is critical in dissociating genetic from early environmental effects on outcome measures.

2.2.8. Food insecurity

Loss of access to nutritional resources is known to induce significant distress and has long-term, even transgenerational, consequences for cognitive, affective, and health outcomes. Some of the most compelling data for such effects come from the study of victims of the dutch famine (Ruemmele and Garnier-Lengliné, 2012; Roseboom et al., 2011; Heijmans et al., 2008). With increasing levels of poverty and food deserts, the impact of malnutrition on brain and behavioral development as a form of adversity is a growing concern. A number of models have been developed to determine the impact of food insecurity on somatic, physiological and behavioral development. Some earlier examples are the work of Levine and colleagues who tested the impact of nutritional instability associated with maternal deprivation on outcomes in rodent models (Wiener and Levine, 1983; Wiener and Levine, 1978). In more recent years, work has also investigated the impact of food restriction of the dam on intra-uterine growth restriction and malnutrition on immune activation and HPA reactivity (Wiener and Levine, 1983; Wiener and Levine, 1978; Léonhardt et al., 2002; Lesage et al., 2002; Lesage et al., 2006; Grissom et al., 2017; Belluscio et al., 2016; Belluscio et al., 2014). Still others have tested the impact of malnutrition during the prenatal or peri-partum period through significant caloric restriction (in some cases reducing intake to 50% of free feeding calories) (Seimon et al., 2013). While these manipulations have provided new and important insights into the impact of nutritional instability on development, they often also profoundly impact maternal behavior and maternal stress levels. Thus, these additional variables are often difficult to isolate and must be taken into consideration when determining the underlying causal factors contributing to a given outcome.

2.2.9. Immune challenge

In recent years, a significant role for immune activation has been identified in the sculpting of neural development, sexual differentiation of the brain, and potential risk for later pathology development (VanRyzin et al., 2018; McCarthy et al., 2017; McCarthy, 2019; Cowan and Petri, 2018; Thion et al., 2018; Macht and Reagan, 2018; Bilbo and Stevens, 2017; Kentner et al., 2018). Exposure to adversity, activation of the HPA-axis, and illness all engage the immune system, which impact the physiology of the organism and have consequence on brain and behavioral development. A variety of models have been developed to determine the impact of immune challenge during the prenatal and/or postnatal periods on neural development. These include the use of lipopolysaccharide (LPS) challenge, polyinosinic:polycytidylic acid (poly (I:C)), monitoring cytokine response to other forms of adversity, genetic elimination of select immune cell populations, and the use of anti-inflammatory agents (Li et al., 2018; Kolmogorova et al., 2018; Kane and Ismail, 2017; Guidolin et al., 2018; Hanamsagar and Bilbo, 2017). The use of this diversity of approaches have shed light on the impact of early illness, illness during pregnancy, anti-inflammatory exposure, etc. on neurodevelopmental events and lifetime risk for a variety of forms of pathology. Further, it has identified potential interactions between what were previously believed to be solely peripheral signals on immune signaling in the CNS. As with previous paradigms, many of the manipulations used to model immune activation can impact maternal and pup behavior, including through induction of sickness behavior, thermal challenge and risk for febrile seizures, and food consumption. Additionally, as immune cells have been implicated in sculpting the sexual differentiation of the brain there may be greater potential for sex differences in response to this manipulation. Thus, in addition to the impact of these manipulations on immune markers, a number of additional variables should be accounted for to better understand the mechanisms driving given outcomes.

3. Proposal for investigators

Considering the breadth of paradigms researchers use to study the impact of ELA, variability in outcomes–rather than reproducibility–-should be the expectation. That said, as long as intra-group variability is minimized and as long as early life experiences are operationally defined appropriately, domain-specific trauma-evoked mechanisms can be identified. Animal models allow precise manipulation of the timing of adversity (e.g., intermittent vs. chronic, age of exposure), manipulation of pre and post-stress environment, and the characterization of parental caretaking. Once stratified into these domains, outcomes related to neuroendocrine signaling, neurocircuitry, and behavior have been quite reproducible (detailed below). We present two approaches to best organize various lines of evidence and address the current obstacles to individualistic treatments in ELA-exposed individuals: standardized environment reporting and standardized experience reporting.

3.1. Standardized environment reporting

Rather than attempting to reconcile the countless differences between paradigms in different laboratories, we propose reporting of a finite set of independent variables that contribute to the developing animal's environment in all ELA research reports. Such standardized "checklists" have been proposed for other related research fields, such as a recent guideline for maternal immune activation modeling (Kentner et al., 2018), and may be adapted and useful for enhancing rigor and reproducibility, as well as allowing identification of

environmental domains. As evident above in the discussion of various methodologies, there are myriad opportunities for inter-lab and intra-lab variability, even within the same named paradigm. Several independent variables separately contribute to biobehavioral outcomes, therefore reliable reporting could help form new subclasses of translational models for ELA.

Early life neurodevelopment of humans and animals hinges upon caretaking with temporal regularity and environmental consistency (Molet et al., 2016; Wachs, 1993). Parental care is a major source of such reliability, yet environmental factors including lighting, temperature, caging, noise, crowding, and surrounding activity can all impact development both directly and indirectly through their effects on caretakers. Reporting of illumination levels, ambient noise levels in the rearing environment, caging systems that can influence airflow, climbing opportunities, and olfactory cues (e.g., open-top, filter-top, individually ventilated) (Kentner et al., 2018; Kallnik et al., 2007), and cage cleaning schedules (Rosenbaum et al., 2009) are therefore all important factors that are often unreported yet could help explain variable outcomes in otherwise identical paradigms.

In addition to the early life environment of the offspring, pregnant dams purchased from differing vendors have likely been exposed to slightly varied illumination, light/dark schedules, and noise regimes, which can impact the behavior of the mother as well as the prenatal environment (Smith, 1979). It is important to note that the same vendor will often ship from different facilities depending on availability unless this is explicitly prohibited by the purchaser. Therefore, due diligence is necessary in order to allow for useful environment reporting.

Another factor that can influence offspring development but is often unknown to the reader, if not even to the investigator, is the age of the parents at time of breeding. Vendors often breed females as early as 7–8 weeks old, or 9–10 weeks old in males. Offspring of late adolescent mothers this age have been shown to differ in cognitive function in adulthood (Zemunik et al., 2003), suggesting that neurodevelopment can differ in subjects within the same laboratory depending on the age at which pregnant dams were either provided by vendors or bred in-house. While some labs exclusively use primiparous females, a number of labs allow for repeated breeding of dams, impacting the amount of maternal experience, which should additionally be accounted for. There is often a lack of clarity regarding these factors, which have the potential to impact the quality of care, sensitivity of these animals to the manipulation, and potentially drive disparities in outcome. For example, we (HB) have observed measurable differences in morning maternal behavior in the first week after birth between dams purchased at gestational day 15 and those mated in-house. Further, for some studies, investigators attempt to control for levels of maternal care through culling of litters to a common size and sex distribution, however, not all labs engage in this approach, instead allowing for a more natural distribution in litter size and composition and sampling across multiple litters.

More obvious details that certainly impact physiological and behavioral outcomes involve the paradigm-specific manipulations themselves. Timing of ELA exposure is one particularly critical variable that is sufficiently reported yet deserves highlighting. For maternal separation and maternal deprivation paradigms in particular, oft-omitted details include whether the pups are separated together or individually, whether the pups are separated in the same room as the dam (in which case the dam is able to hear their vocalizations and has access to their olfactory cues), whether the pups are maintained at nest temperature, and whether pups are separated in fresh bedding or in home bedding consisting of maternal odor.

Due to the potentially critical impact of diverse experimental variables on heterogeneity in outcomes, it will be crucial to provide more detailed and standardized reporting of key variables, even if labs choose to not match across sites for these variables. As important as standardized environment reporting is to rigor and reproducibility, it will also allow establishment of stronger causational relationships between environmental factors and the experience of the developing animal. Importantly, males and females often differentially experience or respond to their environments differently, both during development (VanRyzin et al., 2018; Farrell et al., 2016) and in adulthood (VanRyzin et al., 2018; Gruene et al., 2015; Wellman et al., 2018; Rubinow and Schmidt, 2019). Therefore, using sex as a biological variable is another essential contributor to parsing mechanistic targets for intervention in ELA-exposed populations.

3.2. Standardized experience reporting

A second approach involves the assessment of experiences that are either intentional or unintentional consequences of each ELA model used. The stimulation of, or wear-and-tear on, systems developing to control affective and cognitive processes may be best classified through the measured impact on the individual. Utilizing continua of mediators that reflect how an animal experiences ELA can reveal domain-specific impacts that are generalized across any given number of paradigms. Mediators we will discuss here are parent-offspring interaction, developmental milestone achievement, and neuroendocrine stress responsivity, however there are likely others that will be important targets of further investigation.

Maternal buffering is consistently confirmed across human and non-human animals as a source of resilience, even in the face of severe trauma (Suomi, 2006; Hennessy, 2014; Gee et al., 2014). Recent measurements in rats have substantiated the hypothesis that maternal presence has immediate effects on cortical activity in the pups (Courtiol et al., 2018), which can impact plasticity of developing corticolimbic circuits. Importantly, studies in precocial species such as guinea pigs (Hennessy, 2014) and NHP (Suomi, 2006) have revealed that maternal presence and filial attachment, despite a lack of patterns of active maternal care that are common in rodents, are necessary for emotional and cognitive health. For an excellent overview of such maternal behavior domains in NHP, see (McCormack et al., 2015). In contrast, deviations from the unique species typical pattern of caregiving early in life can shape a developing animal's nervous system towards less-desired outcomes. High and low levels of maternal licking and grooming (LG) were characterized by Meaney, Levine, Baram and others as mediators of hippocampal plasticity and activity, and of neuroendocrine and behavioral stress responsivity in adulthood (Baram et al., 2012; Liu et al., 1997; Liu et al., 2000; Levine and Lewis, 1959). Hofer (Hofer, 2006) further characterized several domains of experiences that shape the mother-infant attachment, and explained that warmth, sensorimotor stimulation, and nutrition each have distinct influences on separate physiological systems and circuits. Therefore, using measures of active maternal behaviors, proximity to the nest/litter, nest temperature, and nutrition as mediators of later outcomes could each provide guiding cross-paradigm avenues for investigation.

Consistent with a "bedside to bench, and back again" approach, we further turn to clinical observations dating back to Erikson (Erikson, 1959) that developmental milestones marking critical periods for affective and cognitive maturation can predict later function. More recently, longitudinal studies in humans have revealed that positive adaptation in the face of adversity (a.k.a., resilience) over the lifespan can be predicted by childhood trajectories of executive function related to future orientation (Oshri et al., 2018). Other examples of specific predictors of later cognitive or emotion-regulation deficits reportedly include failure to meet language development milestones by 24 months (Peyre et al., 2017) and delayed bedwetting cessation after 4 years (Liu et al., 2001), respectively. Since the timing and sequential pattern of milestone acquisition form an important marker of neurological integrity, these measures can provide additional domains for ELA characterization.

In animal models, age-typical achievement of thermoregulation (Mrdalj et al., 2014), pubertal maturation (Grassi-Oliveira et al., 2016), motor coordination and reflexes (Mesquita et al., 2007), and ultrasonic

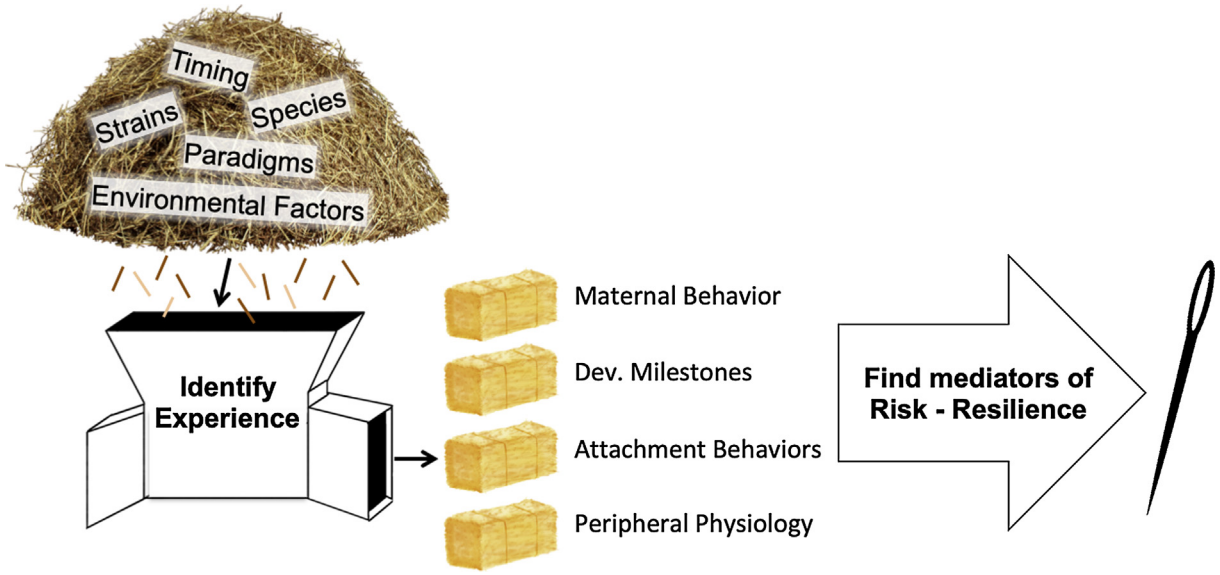

Fig. 1. Varied forms of early life adversity (ELA) are associated with increased risk for negative health outcomes, but can also confer resilience in certain circumstances. A variety of approaches are available to model ELA, identify differences in early experiences, and track outcome measures in these models. By identifying and quantifying these early life experiences, researchers should aim to identify key variables mediating risk and resilience to ELA.

vocalization calls (Hunt et al., 1976), can all be affected by ELA and have often also been associated with later functional outcomes. For example, infant monkeys undergoing maternal separation show hypothermia in their inactive phase (Reite et al., 1978). Following reunion, a phase delay and a reduced circadian amplitude were observed and, notably, these circadian changes preceded a behavioural depressive like state (Reite et al., 1982). In rats as well, postnatal maternal separation or juvenile stress alter circadian body temperature (Yee et al., 2011; Yoshihara et al., 2005), with subsequent alterations of circadian rhythmicity in adulthood (Mrdalj et al., 2014). Evidence in humans and from animal models suggests that pubertal timing can also be associated with affective psychopathology in adolescence and adulthood due to common risk factors. Late menarche has been independently associated with lower risk of adult-onset affective disorders in women (Gaysina et al., 2015), while early pubertal maturation is associated with increased risk of depressive and anxiety symptoms in girls (Jones et al., 2007; Reardon et al., 2009). Animal models also reveal early initiation of puberty in rodent models of ELA (Grassi-Oliveira et al., 2016), and have associated early pubertal maturation with affective alterations later in life, as well as delays in pubertal maturation in different ELA paradigms (Manzano Nieves et al., 2019). While animal models are typically useful for establishing causal mechanisms, to date the evidence associating ELA, developmental milestone achievement, and later behavior are largely descriptive. Future identification of directional pathways will guide research towards mechanistic targets. This may require experimental manipulation of the maturation itself, such as disrupting the HPG axis (Castellano and Tena-Sempere, 2016) or interfering with sensorimotor systems. In the meantime, simple assessment of developmental milestones with each ELA paradigm would facilitate domain-based interpretations.

As discussed throughout, the form, timing, and duration of adversity can vary widely across paradigms and model systems as well as consequences for neurodevelopment. However, significant questions remain with regard to how to compare outcomes across the various forms of ELA, species used, and lab specific modifications of a given manipulation. To provide greater clarity with regard to key variables, there has been a push to develop a checklist, as has been done in immune activation models (Kentner et al., 2018). One possibility is to leverage this already existing checklist, which was developed in a way to be easily adaptable to other paradigms, and apply these to other forms of ELA. Further, it may be useful to provide a single common measure at the behavioral and physiological level across paradigms to use as a benchmark (even if only applied within a species). For example these could include measures as simple as general locomotor activity and morning basal plasma corticosterone/cortisol (cort) levels. For behavioral measures, environmental parameters would need to be prescribed and controlled for across labs, controlling for size, age, lighting, time of day, etc, and with precise reporting of species, sex, strain, and manipulation. For physiological measures, one possible metric could be the collection of basal cort levels, possible through the measure of fecal cort. Use of fecal cort would provide a relatively stable measure of HPA tone, would be less circadian dependent, and not induce additional stress on the animal for sample collection. As with the behavioral measures, additional factors, including age, sex, and weight of the animal, time since completion of stress, etc. would need to be taken into account and reported. Further, as there is variability in the sensitivity of assays (RIA and various ELISAs), a common approach to sample preparation and measurement would be needed. The choice of cort is not meant to infer that elevations in this measure are central or causal to any of the proposed outcomes, but would at least provide a starting point for comparison of ELA effects across labs on at least one common measure. In the future alternative metrics may emerge as more causally linked to outcomes, and the proposed behavioral and physiological measure may change. However, as we move forward and attempt to understand the relationship between the varied forms of ELA and outcomes, some common metrics may serve as an important starting point for comparison across labs and or paradigms.

4. Conclusion

Here, we have provided a synopsis of some of the ways in which ELA is implemented, some of the challenges facing the field, and some of the factors that impact rigor and reproducibility in this area (Fig. 1). We have proposed a direction for ELA research, in order to strengthen the translational value of current animal models. As individualized medicine is increasingly valued in psychiatry, the field is challenged to determine the best way to treat Individual A, who was abused at age 4 and now struggles with drug addiction, or how to treat Individual B, who was neglected as an infant and now struggles with depression. Importantly, these kinds of decisions will require questions about why only some individuals who experiences ELA go on to develop trauma-related pathologies later in life, and why pathological outcomes take

different forms over development and across individuals. Currently, the synthesis of research to understand risk and resilience following ELA is hindered by a lack of standard operational definitions of ELA and a lack of consensus on the appropriate implementation and reporting of this work. Perhaps even more importantly, individualized interventions and treatments will require better understanding of how early life experience can interact with developmental stage, sex, and genetic makeup to confer adaptive or maladaptive outcomes. Further, a recognition that there are a diversity of paradigms that will each provide unique insights into factors mediating risk and resilience and embracing the diversity of approaches will enhance our understanding of the impact of these diverse experiences in risk and resilience. We posit that basic ELA research is poised to answer these important questions, and will benefit significantly from increased clarity with regard to the methods being employed, standardization of outcomes being measured, setting the kinds of goals discussed here.

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