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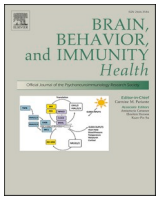

Pitfalls and potential: Translating the two-hit model of early life stress from pre-clinical non-human experiments to human samples

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ABSTRACT

Exposure to early life stress (ELS) has been linked to at least double the risk of psychopathology as well as higher morbidity and earlier mortality across the lifespan. For this reason, the field of developmental psychopathology has spent decades identifying factors that explain which individuals are at risk for negative health outcomes. Preclinical experiments in this field commonly test the "two-hit hypothesis", which explores how ELS potentiates vulnerability to pathogenic physiological and behavioral outcomes when an individual is exposed to a stressor later in development. Yet, translation of the two-hit hypothesis to humans is conceptually and practically challenging, thus impeding progress in the field. This review summarizes the two-hit hypothesis used in preclinical experiments as it pertains to two putative pathways linking ELS to psychopathology: the innate immune and neuroendocrine systems. This review also identifies important considerations when translating this model to humans and provides several recommendations. Specifically, attention to the "biological salience" of different forms of ELA and the concordance of that salience with later probes of the system are needed. Further, the consequences of ELS may be context-specific rather than ubiquitous, at least among young people. Within this conceptualization, "second hits" may be best operationalized using standardized acute challenges to the innate immune and neuroendocrine systems (e.g., psychosocial stress). Third, more explicit reporting of sex differences in the human literature is needed. Finally, preclinical experimental designs that more accurately reflect the natural occurrence of ELS in community samples will more effectively advance the understanding of developmental mechanisms that occur as a consequence of ELS.

Early life stress (ELS) is a broad term used to describe both malicious and non-malicious experiences during childhood that range from maltreatment and neglect to living in poverty or witnessing violence. While exposure to some adversity is common, between as many as 17% of individuals will be exposed to 4 or more forms of ELS (Centers for Disease Control and Prevention, 2021; Chapman et al., 2007, 2004; Dube et al., 2003). This group comprises at least one third of all individuals living with a psychiatric disease (Green et al., 2010; Kessler et al., 2010; McLaughlin et al., 2010). Specifically, they experience a 4-fold greater risk for depressive disorders and a 12-fold greater suicide risk (Kessler et al., 2010; McLaughlin et al., 2010), report less benefit from existing mental health treatments (Lewis et al., 2010), and attain less social capital (e.g., adult income, educational attainment) (Chang and Kuhlman, 2022; Copeland et al., 2021; Metzler et al., 2017). Mitigating this lifelong trajectory of compounding mental health disparity has the potential to profoundly enhance health equity and reduce the overall disease burden in the United States of America and globally (Bellis et al., 2019; Kessler et al., 2010; Nelson et al., 2020). Yet, ELS is not deterministic (Baldwin et al., 2021); more individuals exposed to ELS will be "resilient" than susceptible to psychiatric and stress-related diseases. Thus, efforts to better understand stress susceptibility are critical to understanding who is at risk and how to intervene.

Because ELS is neither necessary nor sufficient to lead to psychopathology, preclinical experiments have employed paradigms that test models of stress resilience and susceptibility. Preclinical experimental models are essential to understanding how ELS affects human development because causality in humans can, at best, be inferred statistically. One of these models is the "two-hit hypothesis". The two-hit hypothesis became prominent in the field of developmental neuroscience after the finding that variability in maternal care can shape susceptibility to stress via epigenetic programming (Meaney, 2001). This model has dominated preclinical models of schizophrenia in particular

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Table 1
Common forms of early life stress (ELS) in non-human animals and humans.

Non-human	Human
Maternal separation	Physical or emotional neglect
Limited Bedding and nesting	Physical, emotional, or sexual abuse
Diesel exhaust exposure	Family member incarceration
Tail shock stress	Parental divorce or separation
Bacterial infection	Caregiver mental illness or substance abuse
Maternal immune activation	Witnessing domestic violence
Social isolation	Witnessing community violence or combat

(Deslauriers et al., 2013; Maynard et al., 2001), but has also been adopted more broadly to study transdiagnostic factors such as anhedonia (Duque-Quintero et al., 2022). In the past two decades, the two-hit hypothesis has been tested using a wide range of early life stressors including but not limited to a mimicked bacterial or viral infection (Deslauriers et al., 2013; Lorusso et al., 2022; Monte et al., 2017; Mouihate et al., 2010; Rincel et al., 2019; Rymut et al., 2020; Yee et al., 2011), toxin exposure (Bolton et al., 2012), limited bedding and nesting material (Bolton et al., 2022; Peña et al., 2019), and maternal separation (Gildawie et al., 2020, 2021; Hennessy et al., 2019; Jaric et al., 2019; Lesse et al., 2017; Peña et al., 2019). Many of these experiments sought to better understand the role of stress susceptibility in neuroimmune dysregulation, which has been repeatedly linked to the maintenance of psychiatric disorders and their symptoms (Bower and Kuhlman, 2023; Dantzer et al., 2008; Dooley et al., 2018; Pariante, 2015). Table 1 provides an overview of the common ways to operationalize ELS in non-human and human subjects.

The prototypical experiment testing a two-hit hypothesis is depicted in Fig. 1. Many of these experiments focus on a behavioral phenotype as the primary outcome, but more often the primary outcome is a putative neuroimmune or neuroendocrine process associated with the behavioral phenotype of interest. I have made four broad observations about this literature in my repeated attempts to replicate and extend their results in human subject research which can be summarized as 1) biological salience of the "first hit" to the developing organism is often neglected, 2) interpretation of responses to the "second hit" is not made explicit within and between studies, 3) the importance of reporting sex differences, and 4) the need to adapt pre-clinical models to reflect naturalistic occurrence of ELS in humans. Table 2 includes the details of a selected subset of the pre-clinical literature on the two-hit hypothesis. This table illustrates the variability in methodological approaches to testing the two-hit hypothesis among contemporary experiments with respect to the species, types of "hits", as well as the timing of both the hits and measurement of their consequences.

1. Biological salience of the "first hit" to the developing organism

First, experiments vary with respect to the nature and heterogeneity of the "hits" administered to the animals. For example, studies have often mixed a predominantly physiological first "hit" [i.e., poly(I:C)] with a non-physiological second "hit" (i.e., unpredictable stress) later in development (Monte et al., 2017), or a non-physiological first "hit" (i.e., maternal separation) with a physiological second "hit" (i.e., LPS) (Gildawie et al., 2020). Table 2 identifies first and second hits as either physiological (Phys.) or psychosocial (Psychosoc.). Adversity that is relevant to the developing innate immune system (e.g., pain, injury, infection) may sensitize the individual to immune-relevant challenges in the future (e.g., bacterial challenge), whereas adversity that threatens an animal's social integration may sensitize the individual to future experiences of social threat or rejection. This is the basic premise of the ELS theory on biological salience (See Kuhlman et al., 2017 for review); different forms of adversity vary in their relevance to different psychophysiological systems *within* a developing individual, and varying forms of adversity may shape the development of that system as well as sensitize the individual to similar contexts. To this point, my team and I have shown that ELS is associated with differential HPA axis and inflammatory responses to standardized psychosocial stress that vary by type and timing of the ELS (Kuhlman et al., 2015a, 2015b, 2022). Pre-clinical experiments designed to determine the specific vs. generalized developmental implications of ELS are needed to advance understanding of the heterogeneity in biobehavioral outcomes among adversity-exposed individuals.

2. Nature and interpretation of the "second hit"

Moving beyond the match or mismatch between the first and second hit, experiments also vary in how responses to a second hit are operationalized. Pre-clinical studies vary with respect to whether their outcomes are measured in the immediate aftermath of the "second hit", on the order of hours or days, or after a delay of weeks to months. Measurement of primary outcomes are identified on this dimension in Table 1, where the primary outcomes are categorized as either "Immediate" or "Delayed" and the timing of those measures are reported in postnatal days (PD) or days since birth. Importantly, the link between ELS and inflammatory biology at rest is not consistently observed in non-human animals (e.g., Hameete et al., 2021) or only reliably present in the immediate aftermath of a "second hit" (Bonapersona et al., 2019). By contrast, studies using human participants are more likely to report the psychophysiological correlates of multiple adversity exposures *at rest* and without regard for when the most recent stressor occurred. A greater focus in human samples on characterizing links between ELS and acute psychophysiological reactivity to standardized challenges are needed to

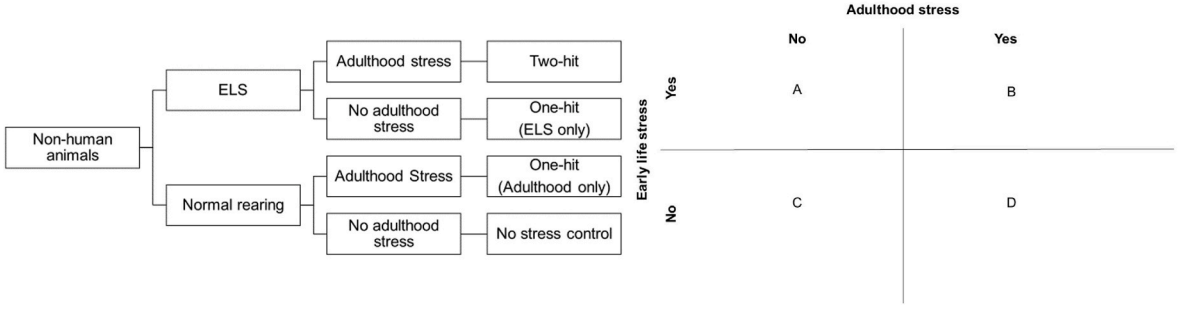

Fig. 1. Two-hit model of early life stress
Figure caption: Experiments testing a two-hit hypothesis randomizes animals to either normal rearing conditions or one form of ELS, then randomizes animals again to either receive a subsequent challenge or a control condition. This creates comparable groups of animals, some of which had perturbations during early development (A and B), some of which experienced a physiological or non-physiological challenge as adults (B and D), some of which had both (B), and some of which had neither (C).

Table 2
Methodological details of a selected subset of preclinical studies testing the two-hit hypothesis of early life stress.

Primary Author	Publication Year	Subjects	First hit: Early life exposure (type, timing)	E. Second hit (type, timing)	Primary outcomes	Result
Peña	2019	Male and female C57BL/6 J mice	**Psychosoc**. - Combination of maternal separation and LBN at PD10–PD20	**Phys**. - Adulthood (males - chronic social defeat stress (CSDS); females – subthreshold variable stress (on 3 consecutive days, female mice experienced: 100 random mild foot shocks at 0.45 mA for 1 h (10 mice to a chamber), a tail suspension stress for 1 h, and restraint stress in a 50 mL conical tube for 1 h)	**Immediate** -Depression-like behaviors (composite) testing began 1 day after final stressor; RNA-seq in ventral tegmental area, nucleus accumbens, and prefrontal cortex 1 day after final behavioral test	✓ ELS combined with variable stress in adulthood was associated with increase in depressive-like behaviors, particularly latency to eat; adulthood stress results in different transcriptional control pathways in reward circuitry depending on ELS
Bolton	2013	Male and female C57BL/6 mice	**Phys**. - 2.0 mg/m^3 of Diesel exhaust at GD9-GD17	**Phys**. - High fat diet at ~>PD120 for 6 weeks	**Immediate** - Anxiety-like behavior via open-field test; weight gain	✓ Diesel exposure can increase susceptibility to diet-induced weight gain and neuroinflammation in adulthood in a sex-specific manner.
Fonken	2018	Male and female Sprague Dawley rats	**Phys**. - Tail shock stress at ~ PD90	**Phys**. - LPS challenge (10 μg/kg) at ~ PD91	**Immediate** - Behavioral anhedonia assessed via sucrose preference; social exploratory behaviors at ~ PD91	✓ Stress exposure potentiated increases in anhedonia and decreases in exploratory behavior Stress exposure prior to LPS challenge inhibited anti-inflammatory mechanisms in the CNS in both male and female animals though mechanisms differed by sex
Jaric	2019	Male and female C57BL/6 mice	**Psychosoc**. -Maternal separation at PD1-PD14	**Psychosoc**. - Adolescent social isolation at PD35-PD56	**Immediate** - Depressive-like and anxiety-like behaviors assessed via open-field test; elevated plus maze; sucrose preference; and forced swim test at PD57-PD68	MS reduced sucrose preference and immobility in forced swim, particularly among females. ✓ Combined MS and social isolation were associated with larger reductions in body weight and increased locomotor activity during the open field test. For other outcomes, combined SI may have mitigated the effect of MS on behavior. MS impedes the protective role of estrogen on behavioral phenotype
Hennessy	2019	Male and female Albino Hartley guinea pigs	**Psychosoc**. - Maternal separation twice for 3 h at PD19 and PD22	**Phys**. - LPS at PD31	**Immediate** – depressive-like behavior and neuroendocrine and inflammatory response to LPS	✓ Maternal separation blunted the response of prostaglandin synthesizing enzymes (COX-2 and mPGES) and chemokines (CXCL-1 and MCP-1) 120 min following injection with LPS and isolation in a novel cage.
Gildawie	2020	Male and female Sprague Dawley rats	**Psychosoc**. - Maternal separation (4 h daily) at PD2 – PD20	**Phys**. - 0.1 lg/kg of LPS at either PD20 (pre-weaning) or PD40 (adolescence)	**Immediate** - Microglia density in PFC and prelimbic regions 4 h after LPS administration	✓ Effect of maternal separation on microglia morphology (size of cell body and number of processes) varies by sex and age (pre- vs. post-adolescence)
Mouihate	2010	Male and female Sprague Dawley rats	**Phys.** – 100 μg/kg of LPS at PD14	**Phys. –** 50 μg/kg of LPS at > PD60	**Immediate –**Hormones, mRNA, and circulating proteins associated with HPA axis activation, COX-2, and TLR4 expression	✓ Post-natal LPS sensitizes peripheral tissues to activate the HPA axis via TLR4 and COX-2.
Deslauriers	2013	Male and female C57BL/6	**Phys**. - poly (I:C) at GD 12	**Psychosoc**. -restraint stress for 2 h, for three consecutive days at PD33-PD35	**Immediate** - Prepulse inhibition of acoustic startle response at PD35	✓ Combined prenatal poly (I:C) + juvenile restraint stress induced prepulse inhibition deficit. PPI deficit correlated with increase in neural dopamine D2 receptor.
Rymut	2020	Male and female PIC Camborough pigs	**Phys**. - MIA via Porcine reproductive and respiratory syndrome virus at GD76	**Physical** - 1 mg/kg bodyweight Poly (I:C) at PD60	**Immediate** - sickness, locomotor, and social behaviors were measured 1–3 h after Poly (I:C)	✓ MIA exaggerated the effects of Poly (I:C) on social behavior among females
Bolton	2022	Male and female C57BL/6 mice	**Psychosoc**. – LBN at PD2-PD10	**Psychosoc**. - Acute, complex stress (1 h of restraint, bright lights, loud noise, peer discomfort, and physical	**Immediate** - Synaptic pruning processes by microglia	✓ ELA attenuates microglia pruning of CRH + neurons leading to greater excitatory synapses in stress sensitive neural circuits

(*continued on next page*)

Table 2 (*continued*)

Primary Author	Publication Year	Subjects	First hit: Early life exposure (type, timing)	E. Second hit (type, timing)	Primary outcomes	Result
				jostling) in adulthood (>PD59)		
Castillo-Gómez	2017	Male GIN and THY1 mice	**Phys**. - NMDAR antagonist at PD7	**Psychosoc**. - Juvenile social isolation at PD21-90	**Immediate** -Locomotor activity, exploratory behavior at PD90	✓ SI related to anxiety-related behavior, brain volume, neuronal structure, and the expression of molecules related to plasticity and excitatory/inhibitory. Effects of SI on outcomes were potentiated by NMDAR antagonism during early development.
Gildawie	2021	Male and female Sprague-Dawley rats	**Psychosoc**. -maternal separation at PD2-PD20	**Psychosoc**. - juvenile social isolation at PD21-PD35	**Delayed** - Locomotor activity, risk assessment, structural integrity of PFC at P70–P85	MS was related to increased activity and risk assessment among females. ✓ MS + SI was associated with reduction in PFC structural integrity.
Lesse	2017	Male C57BL/6 mice	**Psychosoc**. – chronic stress (daily maternal separation at PD1-PD21 + social isolation at PD22+)	**Psychosoc**. - forced swimming (FS) in young adulthood at PD 62–64	**Delayed** - oxytocin receptor (OxtR) and arginine vasopressin receptor type 1a (AvpR1a) gene expression in the hippocampus (HC) (PD 100)	✓ Only combined stress exposure (chronic stress + forced swim), but neither alone, resulted in increased gene expression of OxtR in HC. AvpR1a expression was decreased in both adult forced swim and combined stress exposed animals.
Monte	2017	Male and female Wistar rats	**Phys**. - Poly (I:C) at PD5-PD7	**Psychosoc**. - Unpredictable stress at PD 30-45	**Delayed** - Prepulse inhibition (PPI) of the startle response), working memory (Y-maze test), and locomotor activity (open field test) at PD60	✓ Combined immune challenge and juvenile stress was associated with memory deficits. Effects of two-hit on oxidative stress mechanisms were more evident in females
Rincel	2019	Male and female Gestant C3H/HeNRj mice	**Phys**. - MIA via LPS at GD17	**Psychosoc**. – Combined uncontrollable maternal stress and maternal separation from PD2 -PD14	**Delayed** - Social interaction, anxiety-like behavior via elevated plus maze and marble burying, and HPA reactivity to tail suspension (PD60-PD150)	✓ Multi-hit ELA associated with greater social impairment in males and greater anxiety-like behavior in females. Multi-hit ELA associated with greater increases in gut permeability in males.
Yee	2011	Male and female Sprague Dawley rats	**Phys**. - poly (I:C) at GD15	**Psychosoc**. - predator-scent stress at PD 27-PD29	**Delayed**- Anxiety-like behavior on the elevated plus maze at PD69	∅ Both MIA and predator scent stress independently increase anxiety-like behavior in adulthood; no evidence of a sensitizing effect of one on the other.
Lorusso	2022	Male and female Wistar rats	**Phys**. – MIA (10 mg/kg bodyweight poly (I:C)) at GD 15	**Psychosoc**. - LBN at PD1-PD10	**Unspecified** - Memory (novel object recognition)	✓ MIA, as well as MIA + LBN were associated with higher risk of performance deficits on the memory task

GD = gestational day; LBN = Limited-bedding and nesting; LPS = lipopolysaccharide; MIA = maternal immune activation; MS = Maternal separation; NMDAR = N-methyl-D-aspartate receptor; Phys. = Physiological; Poly (I:C) = polyinosinic-polycytidylic acid; PD = postnatal day; Psychosoc. = psychosocial; TLR4 = toll-like receptor 4.

*Note: This table is not a comprehensive reflection of the literature or approaches to testing the two-hit hypothesis in non-human experiments. This table predominantly features studies published in the past ten years and aimed to highlight the sources of variability between studies that impede faster translation.

better understand biobehavioral pathways linking ELS to its well-established health sequelae. To this point, ELS changes how the brain responds to subsequent stressors (Peña et al., 2019). Indeed, psychological correlates of acute activation of the HPA axis and the innate immune system are more pronounced among both clinical and non-clinical samples with greater ELS (Kuhlman et al., 2020b, 2021a, 2021b).

Resolving discrepancies between outcome measurement in preclinical and clinical studies could also be achieved by selecting more stable measures that can be used across species. Many studies, in both preclinical and clinical, use a single biological marker as their primary outcome as a representative of an entire dynamic system (e.g., cortisol for HPA axis function, IL-6 for immune function). Identification and adoption of more stable biological markers that reflect meaningful variability in system function is needed. One promising but underutilized approach to this has been the use of biological composites derived using multi-omics (c.f. transcriptomics: Cole et al., 2012; epigenomics: Provençal and Binder, 2015).

Increased attention to the association between ELA and immediate psychophysiological responses to stress would help to address the underexplored possibility that ELS alters the way in which individuals respond to different types of psychophysiological challenge. For example, the Trier Social Stress Test for Children (TSST-C) uses uncontrollable socially-evaluative threat and cognitive demand to induce psychophysiological stress. Individual differences in response to the TSST-C may provide insight into how ELS shapes functioning in academic settings but may not be indicative of how individuals respond to all forms of stress or challenge (e.g., physical assault, injury). At least among adolescent samples, the association between ELA and psychophysiological responses to stress appears to be context-specific. For example, my lab has shown that ELS is related to differences in the immune (Kuhlman et al., 2022) and HPA axis (Kuhlman et al., 2015a) response to socially-evaluative stress, but not the innate immune response to mild immune activation using the annual flu vaccine (Kuhlman et al., 2020b).

Table 3
Early adversity exposure among three community samples of adolescents.

Study name	Age (years) M (SD)	n	% female (n)	Number of adversities M (SD)	Number of adversities -Range
Research on Adolescents with Anxiety and Depression (RAAD)	12.76 (2.28)	138	42.8 (59)	2.28 (2.73)	0–18
UCLA Vaccine Study	18.50 (0.72)	47	72.3 (34)	4.68 (3.98)	0–16
Teen Resilience Project	13.91 (1.60)	97	46.4 (45)	8.19 (5.04)	1–25

Indeed, the preclinical literature clearly shows that disrupted caregiving environments alter memory, promoting stress-related memory systems and impairing others (Bonapersona et al., 2019), suggesting that psychosocial stress may be an important context in which to examine the residual consequences of ELS. This may be why, in humans, ELS is not consistently associated with elevated circulating markers of inflammation until adolescence (Kuhlman et al., 2020a) and continues to increase throughout adulthood (Chiang et al., 2022). In children and adolescents, evidence of an association between ELS and dysregulated immune function may largely be context specific (e.g., social threat but not infection), and become more generalized and persistent across the life course. Garnering a better understanding of this potential context specificity may be critical to prevention of the insidious trajectory associated with ELS.

3. Importance of reporting sex differences

Sex differences are abundant in the preclinical literature (Bolton et al., 2012; Bonapersona et al., 2019; Fonken et al., 2018; Gildawie et al., 2020, 2021; Jaric et al., 2019; Monte et al., 2017; Rincel et al., 2019), but often ignored in the human literature. Males and females differ substantially in the incidence of psychiatric disorders (Zahn-Waxler et al., 2015) and understanding the role of sex differences in susceptibility to stress has long been identified as critical to understanding the pathogenesis of psychiatric diseases like depression (Hammen, 2005, 2015; Rutter et al., 2003). There is a growing understanding within the preclinical literature that the mechanisms leading to the same psychiatric diseases differ for male and female animals, with major implications for treatment (Hodes and Kropp, 2023). Sample sizes are often a limiting factor in human studies; studies often lack the statistical power to test or interpret additional interactions by sex. However, the field would still benefit from studies providing supplemental results stratified by sex to better understand mechanistic differences in humans.

4. Naturalistic occurrence of ELS

Finally, in my opinion, the greatest challenge to translating the two-hit hypothesis to humans is the naturalistic occurrence of ELS itself. Few people experience two hits. Data best illustrates this point. My team has collected data on ELS using the Early Trauma Inventory (Bremner et al., 2000, 2007) in three community samples: Research on Adolescents with Anxiety and Depression (Project RAAD) (Kuhlman et al., 2015a, 2015b), the UCLA Flu Vaccine Study (Kuhlman et al., 2018, 2020b), and the Teen Resilience Project (TRP) (Kuhlman et al., 2022). Table 3 provides basic descriptive information about ELS in each of these samples. Fig. 2 shows the average number of adversities participants reported by each year of life (left axis) as well as the percent of individuals that had been exposed to at least one (white bars) or at least two (grey bars) ELS by age (right axis). Based on this aggregate data, 80% of 12-year-olds had been exposed to at least one ELS, 65% had been exposed to at least two, and the average 12-year-old had been exposed to just under 4 ELS. Taking the two-hit hypothesis into consideration, this graph clearly shows that the average child reported exposure to two adversities by age 8, more than half of the children reported exposure to some adversity by age 5, and more than half reported exposure to two or more different adversities by age 9. As can be seen in Table 2, it is common in pre-clinical experiments to administer the first hit during gestation or early postnatal development and the second hit in adolescence or adulthood. In its most common form, the factorial experimental design that randomizes non-human animals to receive either no, early, late, or early and late stressors (See Fig. 1), leaves both the average and the most affected children out of the model. Addressing this discrepancy between experimental non-human models and human experience may be as simple as adopting combined stressors as the first hit (e.g., Peña et al., 2019) or administering multiple stressors sequentially (Rincel et al., 2019), importantly before adolescence begins (PD21 in mice; Brust et al.,

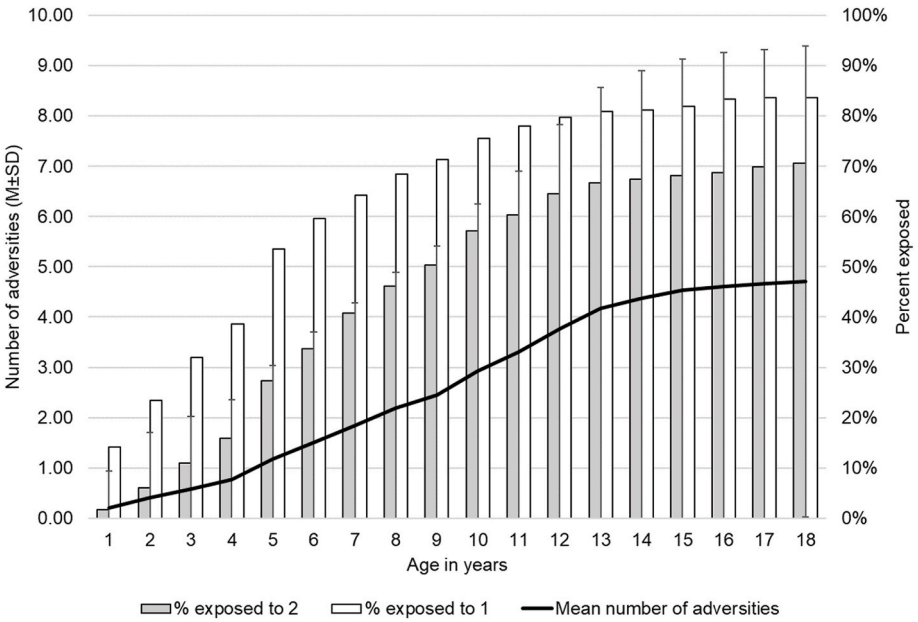

Fig. 2. Early life adversity exposure by age across three community samples ($n = 282$).

2015).

5. Conclusions

The two-hit model of ELS that is commonly employed in preclinical experiments clearly demonstrates that the pleiotropic consequences of both physiological and non-physiological challenges that occur during sensitive periods of neural or immune development are evident well into adulthood, particularly in the immediate aftermath of subsequent stressors. Yet, clinically meaningful translation of this literature to humans requires several specific changes to ELS conceptualization and methodological approaches. First, investigators should consider the nature of the first hit within a developmental framework that acknowledges the potential biological salience and, importantly, whether that salience is concordant with the system being challenged by the "second hit." Second, more human studies are needed which characterize the links between ELS and psychobiological responses to different types of acute, standardized challenges. Second hits may not be additional adversities but rather psychosocial contexts in which the developmental consequences of ELS are observable. Further, attention to the contexts these challenges probe (e.g., interpersonal conflict, academic evaluation, infection) is essential to understanding how ELS-related sensitization to stress may manifest in a person's daily life. Third, sex differences in human studies need to be more directly addressed, either by reporting results stratified by sex or, where appropriate, testing whether reported results are moderated by sex. Using the more heterogeneous and frequent naturalistic occurrence of ELS in humans to inform preclinical experimental design would also aid in translation and potential clinical impact. Mitigating the lifelong health disparities associated with ELS depends upon identification of modifiable biobehavioral mechanisms associated with disease pathogenesis. Given the cost-prohibitive, time-intensive nature of collecting data that can be used in causal inference models of ELS in human subject research, taking these and other steps to maximize the translational utility of preclinical experiments is crucial.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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