Title: Effects of early life adversity on immediate early gene expression: systematic review and 3-level meta-analysis of rodent studies

Short title: Early life adversity and immediate early genes

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16 **Abstract**

17 Early-life adversity (ELA) causes long-lasting structural and functional changes to the brain, rendering
 18 affected individuals vulnerable to the development of psychopathologies later in life. Immediate-
 19 early genes (IEGs) provide a potential marker for the observed alterations, bridging the gap between
 20 activity-regulated transcription and long-lasting effects on brain structure and function. Several
 21 heterogeneous studies have used IEGs to identify differences in cellular activity after ELA;
 22 systematically investigating the literature is therefore crucial for comprehensive conclusions. Here,
 23 we performed a systematic review on 39 pre-clinical studies in rodents to study the effects of ELA on
 24 IEG expression. Females and IEGs other than cFos were investigated in only a handful of
 25 publications. We meta-analyzed publications investigating specifically cFos expression. ELA increased
 26 cFos expression after an acute stressor only if the animals (control and ELA) had experienced
 27 multiple negative life events. At rest, ELA increased cFos expression irrespective of other life events,
 28 suggesting that ELA creates a phenotype similar to naïve, acutely stressed animals. We present a
 29 conceptual theoretical framework to interpret the unexpected results. Overall, ELA likely alters IEG
 30 expression across the brain, especially in interaction with other negative life events. The present
 31 review highlights current knowledge gaps and provides guidance to aid the design of future studies.

32 Introduction

33 Synaptic connections in the brain are continuously altered, including through gene
 34 expression, to accommodate experiences, thereby preparing the organism to deal with future
 35 events [1–3]. This potential for adaptation, called neuronal or synaptic plasticity, is prominently
 36 present during critical periods early in life [4]. For this reason, adverse experiences throughout
 37 childhood - such as physical, sexual or emotional abuse - have far-reaching effects on an individual's
 38 brain function and structure, and consequently on cognition and behavior [5–7]. It is therefore not
 39 surprising that *early-life adversity* (ELA) is consistently associated with an increased risk for
 40 psychopathologies later in life, including major depressive disorder (MDD), post-traumatic stress
 41 disorder (PTSD), and schizophrenia [8, 9].

42 To investigate the mechanisms underlying the effects of ELA on brain and behavior, several
 43 models of alteration of maternal care in rodents have been developed [10, 11]. These models
 44 consistently show that ELA leads to fundamental remodeling of stress-sensitive brain regions, which
 45 in turn may be linked to altered function [12, 13]. For example, ELA has been reported to modify the
 46 regulatory response of the *hypothalamic-pituitary-adrenal* (HPA) axis, an essential part of the
 47 organism's stress response system [14, 15]. Furthermore, rodents exposed to ELA display a robust
 48 behavioral phenotype characterized by enhanced anxiety-like behavior, changes in memory
 49 formation, and decreased social behavior [16]. Overall, this evidence highlights that ELA leads to
 50 structural, functional and behavioral alterations in the rodent brain, yet the events giving rise to said
 51 alterations remain unclear.

52 Immediate-early genes (IEGs), such as *cFos* (alias *Fos*), *Egr1* (alias *Zif-268*, *NGFI-A*, *Krox-24*)
 53 and *Arc* (alias *Arg3.1*), provide a potential link between experience-induced cellular activity in the
 54 brain and the resulting long-term changes in neurons and synapses. IEGs are immediately and
 55 transiently expressed in response to extracellular calcium influx, as occurs when an action potential
 56 is fired [17]. Among the IEG, *cFos* is most often studied; it forms the activator protein-1 (AP1) by

dimerization with a *Jun*-family transcription factor [18]. The AP1 complex initiates the transcription of other late genes, which result in long-lasting changes of cellular physiology. Consequently, a strong relationship between IEG expression and neuronal activity is observed, with increases in neuronal activity being accompanied by increased IEG expression [17]. For decades, IEGs have been a prominent tool for mapping neuronal activity in rodents by means of immunohistochemistry (IHC) and in-situ hybridization (ISH) due to their brain-wide expression. More recently, IEGs have been increasingly investigated for their protein properties, in particular with respect to synaptic plasticity [19].

Whereas the downstream products of IEGs are diverse (*e.g.*, transcription factors, postsynaptic proteins, secretory factors), their functions are surprisingly homogeneous and can mostly be related to cellular processes, such as dendrite and spine development; synapse formation, strength and elimination; and regulation of the excitatory/inhibitory balance ([3]; *Fig. 1*). In line with this functional similarity, knockouts (KOs) of several different IEGs affect behavior and synaptic plasticity in a similar manner. More specifically, system-wide *Arc*-KO and *Egr1*-KO, as well as central nervous system-specific *cFos*-KO mice all display behavioral impairments in learning and memory as well as deficits in long-term potentiation or depression, underscoring the necessity of IEGs for memory formation and retention [20–22]. In addition, many neuropsychiatric disorders characterized by memory impairments, such as major depressive disorder, post-traumatic stress disorder and schizophrenia, have also been shown to feature a dysregulation of activity-dependent transcription [23]. Interestingly, the risk to develop any of these disorders is increased by exposure to ELA, further indicating a potential causal interaction between ELA, IEGs and mental health [8, 9].

THE EFFECTS OF ELA ON IEG EXPRESSION

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Fig 1. Mechanisms of IEG activation. A) Action potentials induced by glutamate signaling result in membrane depolarization, which in turn results in opening of L-type voltage calcium channels (LVCCs). The resulting Ca^{2+} influx induces calcium-dependent signaling pathways. These cascades further result in the recruitment of existing transcription factor, such as CREB, which in turn lead to the expression of IEGs. Once transcribed, IEGs act as B) transcription factors in the nucleus or C) regulators of synaptic plasticity at the synapse as, for example, post-synaptic proteins. B) The transcription factors of the *Fos* family bind to a transcription factor of the *Jun* family to form the AP1 complex, whereas *Egr1* acts independently. *Egr1* and *cFos* are transiently expressed, whereas Δ FosB accumulates over time in the nucleus. C) *Arc* acts at the post-synaptic density by reducing the number of surface AMPA receptors. Therefore, increased *Arc* expression results in reduced synaptic strength by AMPA receptor endocytosis.

While numerous studies have used IEGs to identify differences in cellular activity after ELA, the study designs are heterogeneous, and findings are seemingly discrepant. Reviewing the available literature will provide a clearer picture of the effects of ELA on IEG expression and will aid future development of study designs by identifying sources of heterogeneity within and between experiments. To that end, we performed a systematic review to synthesize the available evidence and explore outcomes in a sex-, gene- and region-specific manner. A meta-analysis was then conducted on a subset of the data based on *a priori* determined thresholds. We hypothesized that ELA exposure leads to an exaggerated increase in IEG expression after an acute stress challenge,

98 further amplified by exposure to additional stressors in life, in line with the multiple-hit concept of
99 vulnerability [24].

100

101 **Methods**

102 Search strategy, protocol and risk of bias assessment of the present review were performed
103 in line with SYRCLE (SYstematic Review Center for Laboratory animal Experimentation) guidelines
104 [25–27]. We adhered to the PRISMA checklist for reporting [28] (Supplementary Information). The
105 protocol (S1.1) and the PRISMA checklist are openly accessible at <https://osf.io/qkyvd/>.

106

107 **Study selection and data extraction**

108 We conducted a systematic literature search with the search engines PubMed and Embase
109 on the 3rd of April 2019 to select experiments investigating differences in IEG expression between
110 control and ELA exposed rodents. The terms ‘*mice and rats*’ and ‘*postnatal ELA*’ were used to
111 construct the search string (S1.2). For the purpose of this review, ELA was defined as models altering
112 maternal care. We included the ELA models of maternal separation and deprivation, isolation,
113 limited bedding and nesting, as well as licking and grooming. Study selection was performed in
114 Rayyan [29] in alphabetical order and any disagreements between investigators were resolved by
115 discussion until unison was reached. An overview of the study selection procedure is displayed in
116 *Figure 2*.

Fig 2. Flow-chart of study selection process.

A complete list of final inclusion and exclusion criteria can be found in the protocol (S1.1). First, titles and abstracts were screened by at least three blinded investigators (HS, VB, EK, DvN, LvM) for the following exclusion criteria: 1) not a primary experimental publication, 2) not using adult (>8 weeks) mice or rats which are younger than 1 year, 3) not using a postnatal model of ELA as specified in S1.1. Eligibility was then determined by full-text screening of the remaining studies by at least two blinded investigators (HS, EK, LvM), with a random subset screening performed by a fourth (VB), blinded investigator to confirm agreement. Publications were deemed non-eligible

based on the following criteria: 1) not measuring an IEG product in the brain, 2) deviation from *a priori* determined criteria concerning the background of the animals, interventions, or outcomes, 3) control and experimental groups differed at more aspects than just ELA exposure. Lastly, reference sections of eligible publications were screened for articles missed by the search string, but none were added through this procedure.

Data from eligible studies were extracted into a combined dataset using *a priori* determined sets of variables to comprehensively capture experimental design, methods and results with minimal subjectivity (*S1.3*). Differently from the original protocol, we extracted also measurements without acute stress to have an appropriate control, baseline condition. Outcome data for each comparison (*i.e.* group-based mean and variance) were extracted in the following order of preference: 1) from numbers provided in the text or tables; 2) from graphs by using WebPlotDigitizer (v4.3 [30]; or 3) from statistical test results. A comparison is defined as the difference in expression of a specific IEG in a specific brain area at rest or after acute stress exposure in ELA-exposed animals and controls. To compare the results on a systematic review level, we performed an independent samples *t*-tests on the extracted summary statistics. The results were interpreted dichotomously as significant / not significant, with $p < 0.05$ used as a criterion. We chose this approach to equalize the statistical method used for analysis across publications.

Meta-analysis

Data selection. We performed a meta-analysis on outcomes that were assessed by at least three independent comparisons (*i.e.*, at least one comparison from three independent publications). During analysis coding, the investigators were blinded to the outcome by randomly multiplying half of the effect sizes by -1.

To account for potential sex differences, we planned to perform separate meta-analyses for males and females. However, only few comparisons were reported for female rodents, and their

study designs were strongly heterogeneous. We therefore restricted our quantitative synthesis to outcomes from male rodents, with female data being evaluated qualitatively only. Furthermore, only comparisons using either IHC, immunocytochemistry (ICC), or ISH to quantify IEG expression were included on the meta-analytic level. While both methods differ in the type of molecule being assessed, quantification and analysis procedures largely overlap. To confirm this, we investigated whether the choice of quantification method affects the outcome. PCR based methods and western blots were evaluated qualitatively only.

Based on the aforementioned threshold and restrictions, the meta-analysis was performed on comparisons of cFos expression in the amygdala, thalamus, hippocampal formation, hypothalamus, prefrontal cortex and midbrain at rest and after acute stress experiences. Smaller subregions were grouped into larger structures (S1.4) in line with the Allen Mouse Brain Atlas (©2004, Allen Institute for Brain Science) to allow for comparisons between studies.

Statistical analysis. For comparisons included in the meta-analysis, we calculated the standardized mean difference Hedge's g as a measure of effect size. If only the standard error of the mean (SEM) was reported, the standard deviation (SD) was calculated as $SEM * \sqrt{n}$, where n = the number of animals per group. If the total number of animals was reported, this was distributed equally across groups. If the number of animals was reported as a range (e.g. 6-8 animals/group), we used the mean (e.g. 7 animals/group). If the same control group was used as control of multiple experimental groups (e.g. different ELA models), the sample size of the control group was divided by the number of experimental groups and the adjusted sample size was used for the calculation of the effect size [31]. Heterogeneity was assessed with Cochran's Q -test [32]. Influential outliers were determined in accordance with Viechtbauer and Cheung [33] and removed from quantitative synthesis.

A three-level mixed-effects model was built to capture variance not only between publications (Level 1), but also between experiments (Level 2) and outcomes (Level 3), thereby

taking into account the statistical dependency of outcomes acquired from the same animals within the same publication [34–36]. Moderators of the multilevel model were presence of an acute stress challenge, presence of a multiple hits and brain area.

We tested whether ELA effect sizes at rest or after acute stress challenges are significantly different from zero to understand the effects of ELA on *cFos* expression under each of these conditions. Subsequently, a subgroup analysis was performed to investigate whether the effects are moderated by the experience of single (ELA) or multiple negative life experiences (hits). The presence of multiple hits was classified with previously determined criteria [16]. Finally, we explored the effects of type of acute stressor (i.e. *mild* versus *severe*, S1.4), novelty of stress experience, and brain region using subgroup analyses.

Bias assessment and sensitivity analyses. We followed SYRCLE guidelines on risk of bias assessment, with items not reported being coded as ‘unclear’ [27]. To detect publication bias, funnel plot asymmetries for each outcome variable were evaluated [27]. Due to the uneven frequency of the number of studies, we performed sensitivity analyses (rather than subgroup as specified in the protocol) on the type of ELA model, and difference between mRNA and protein. Since these analyses were not initially included, the results were only qualitatively assessed and were in line with the interpretation of the main results. All analyses can be found at our repository (<https://osf.io/qkyvd/>).

Software and data availability. All analyses were performed in R (v3.6.1; [37]). The following R packages were used: metafor (v 2.1.0; [38]), tidyverse (v1.2.1; [39]). Data are presented as the standardized mean difference Hedge’s *g* and standard error of the mean ($g[\pm\text{SEM}]$). The significance level α was set to 0.05. Multiple testing correction on the planned analysis was performed using the Holm-Bonferroni method [40]. The full dataset as well as the code for analysis are openly accessible at <https://osf.io/qkyvd/>.

Results

Study selection and characteristics

A total of 1019 animals reported in 39 publications were included in the review. The animals were predominantly male (72.5%); rats (76.3%) were used more often than mice; and protein (77.4%) rather than mRNA was more frequently assessed as outcome. The IEG *cFos* was investigated in the majority of studies (88.7%), and maternal separation was the most frequently used ELA model (90.6%). *Figure 3* shows a graphical overview of the study characteristics.

Fig 3. Study characteristics reported as percentage of experiments. *Fem* = females.

Systematic review of *cFos* and ELA

A total of 31 publications reported *cFos* expression in control and ELA animals (*Table 1*). IEG expression was reported to be significantly affected by ELA in 72 (45.8%) comparisons, of which 33 (59.6%) displayed upregulation and 39 comparisons (54.2%) reported downregulation.

THE EFFECTS OF ELA ON IEG EXPRESSION

12

218 **Table 1. Overview of study designs and findings of reviewed publications reporting on *cFos* expression in ELA**
219 **and control animals.**

Author (Year)	MA	Model (PNDs)	Species	Sex	Notes	AS	Effect	Area(s)
Auth (2018) [41]		MS (2-15)	Mouse	F	Dark-light box	✓	↔	BLA, LA, CEA, PVN
					Open-field test	✓	↔	BLA, LA, CEA, PVN, dIPAG, vIPAG
					Two independent naive cohorts	x	↑	dIPAG
							↔	BLA, CEA, PVN, vIPAG, LA
						x	↑	LA
Banqueri (2018) [42]		MS (1-10)	Rat	F	Morris water maze	✓	↑	CA1, DG
							↓	ACA
							↔	avTN, amTN, IL, PL
					Morris water maze	✓	↑	DG
							↓	IL, PL, ACA
Benner (2014) [43]		MS (2-15)	Mouse	M	Competitive dominance task	✓	↑	BLA
							↓	CA1
							↔	ACA, CEA, DG, IL, PL
							↔	ACA, CEA, DG, IL, PL
							↔	ACA, CEA, DG, IL, PL
Chung (2007) [44]	✓	MS (2-14)	Rat	M	Colorectal distension	✓	↑	ACA
							↔	CEA, cmTN, PAG, PVT, vmHN
					-	x	↑	ACA
							↔	CEA, cmTN, PAG, PVT, vmHN
							↔	CEA, cmTN, PAG, PVT, vmHN
Clarke (2013) [45]	✓	MS (10-11)	Rat	M	Small litter (12 pups); Restraint stress	✓	↑	mPPVN
							↔	vBNST, MGPVN, IPPVN, dPPVN
					Small litter	x	↓	dPPVN
							↔	mPPVN, MGPVN, IPPVN, vBNST
					Large litter (20 pups); Restraint stress	✓	↔	mPPVN, MGPVN, IPPVN, dPPVN, vBNST
Cohen (2013) [46]	✓	LBN (2-21)	Mouse	M	Novel environment	✓	↔	BLA
							↔	BLA
					MS pups remained in HC; re-exposure to fearful context	✓	↑	MEA
							↔	BLA, CEA
					MS pups placed in NC; re-exposure to fearful context	✓	↑	BLA, MEA
Daskalakis (2014) [47]		MS (3-5)	Rat	M			↔	CEA
							↔	CEA
							↔	CEA
							↔	CEA
							↔	CEA
Desbonnet (2008) [48]	✓	MS (2-14)	Rat	M	Forced swim test	✓	↔	PVT, CEA, PVN, BNST, DG
					-	x	↔	PVT, CEA, PVN, BNST, DG
					F Forced swim test	✓	↔	PVT, CEA, PVN, BNST, DG
					-	x	↔	PVT, CEA, PVN, BNST, DG
							↔	PVT, CEA, PVN, BNST, DG
Felice (2014) [49]	✓	MS (2-12)	Rat	M	Open-field test	✓	↔	BLA, CEA, rostral & caudal ACA, IL, PL
							↔	BLA, CEA, rostral & caudal ACA, IL, PL
					Colorectal distension	✓	↑	rostral & caudal ACA, IL, PL
							↔	BLA, CEA
					-	x	↔	BLA, CEA, rostral & caudal ACA, IL, PL
Gardner (2005) [50]		MS (2-14)	Rat	M	Social defeat paradigm; cFos counts summed across 4 slices	✓	↔	DRN
						x	↔	DRN
							↔	DRN
							↔	DRN
							↔	DRN
Gaszner (2009) [51]		MS (8-14)	Rat	M	Restraint stress	✓	↔	EW
					-	x	↔	EW
					F Restraint stress	✓	↔	EW
					-	x	↔	EW
							↔	EW
Gaszner (2009) [51]		MS (8-14)	Rat	M	Restraint stress	✓	↔	EW
					-	x	↔	EW
					F Restraint stress	✓	↑	EW
							↔	EW
							↔	EW

THE EFFECTS OF ELA ON IEG EXPRESSION

13

Author (Year)	MA	Model (PNDs)	Species	Sex	Notes	AS	Effect	Area(s)
Genest (2004) [52]	✓	MS (3-12)	Rat	M	Novel environment	✓	↔	EW
				F	Novel environment	✓	↑	PVN
Hidaka (2018) [53]		MS (2-14)	Mouse	M	Three chamber test	✓	↔	PVN
James (2014) [54]	✓	MS (2-14)	Rat	M	Restraint stress	✓	↔	ACA, IL, PL
				F	Restraint stress	✓	↓	mPPVN
				M	Restraint stress	✓	↔	PVT
Loi (2017) [55]		MS (3-4)	Rat	M	Rodent Iowa gambling task	✓	↔	mPPVN, PVT
						✓	↓	rCA1, rCA3, leAI, leIL
							↔	r&leDG, r&leACA, r&lePL, leCA1, leCA3, r&le dISX, r&le mISX, r&le AI, r&le NAcc Shell&Core, rIL, r&le vOFC, r&le mOFC, r&le cOFC
Menard (2004) [56]	✓	LG	Rat	M	Shock-probe burial task with electrified probe	✓	↓	dISX, vISX, vSUB, dPAG, vPAG
							↔	vDG, dDG, mSX, CA1, CA3, aHN, CEA, BLA, LC, NAcc shell
O'Leary (2014) [57]		MS (1-14)	Mouse	F	Restraint stress	✓	↓	dDG, vCA3
							↔	dCA1, dCA2, PVN, dCA3, vDG, NAcc, VTA, IL, PL, ACA, LA, BLA, CEA, DRN
Ren (2007) [58]		MS (2-21)	Rat	M	Colorectal distension	✓	↔	DRN
Renard (2010) [59]	✓	MS (1-21)	Rat	M	Perfusion 24h after last day of chronic variable stress	×	↔	mPPVN
				F	-	×	↔	mPPVN
				M	-	×	↔	mPPVN
				F	-	×	↔	mPPVN
Rincel (2016) [60]	✓	MS (2-14)	Rat	M	Open-field test	✓	↓	PVN
Rivarola (2008) [61]		MS (1-21)	Rat	F	Perfusion 24h after last day of chronic variable stress	×	↑	adTN
				-		×	↑	adTN
Rivarola (2009) [62]		MS (1-21)	Rat	F	Perfusion 24h after last day of chronic variable stress	×	↑	RSP
				-		×	↔	adTN, MMN
				-		×	↔	adTN, RSP
				-		×	↔	MMN
Shin (2018) [63]	✓	MS (1-14)	Mouse	M	Social interaction after 1d social isolation	✓	↑	ISX, VTA
				-		×	↔	mPFC, NAcc, vPAL, AHA, VH
				-		×	↔	ISX, VTA, mPFC, NAcc, vPAL, AHA, VH
Tenorio-Lopes (2017) [64]	✓	MS (3-12)	Rat	M	Novel Environment	✓	↔	BLA, CEA, MEA, DMH, PVN
Troakes (2009) [65]	✓	MS (5-21)	Rat	M	Elevated plus maze	✓	↓	PIR
				-		×	↔	ACA, SSb, ISX, PVN, CEA, MEA, dCA1, vCA1, dCA2, vCA2, dCA3, vCA3, dDG, vDG, CP, DRN, Pontine region, CB
				-		×	↔	ACA, SSb, PIR, ISX, PVN, CEA, MEA, dCA1, vCA1, dCA2, vCA2, dCA3, vCA3, dDG, vDG, CP, DRN, Pontine region, CB
Trujillo (2016) [66]	✓	MS (1-21)	Rat	M	Perfusion 24h after last day of chronic variable stress	×	↑	MEA
				-		×	↔	CA1, CA2, CA3, PVN
				-		×	↑	CA1, CA2, CA3, MEA
				-		×	↔	PVN
van Hasselt (2012) [67]		LG	Rat	P	Rodent Iowa gambling task; results reported as correlation with %LG	✓	↑*	NAcc Shell, AI
							↔*	mOFC, vOFC, IOFC, ACA, PL, IL, dISTR, dmSTR, NAcc Core, CEA, BLA, DG, CA1
Vivinetto (2013) [68]	✓	MS (1-21)	Rat	M	Foot shock in step-down inhibitory avoidance task	✓	↔	CA1, CA3, DG
Yajima (2018) [69]		MS (2-14)	Mouse	M	-	×	-	HPF
Zhang (2009) [70]	✓	MS (2-14)	Rat	M	Colorectal distension	✓	↑	cmTN
				-		×	↔	ACA, vplTN, PVT
				-		×	↑	vplTN
				-		×	↔	ACA, cmTN, PVT
Zhao (2013) [71]	✓	MS (2-14)	Rat	M	Chinese language	×	↑	PVN

THE EFFECTS OF ELA ON IEG EXPRESSION

14

Author (Year)	MA	Model (PNDs)	Species	Sex	Notes	AS	Effect	Area(s)
publication								
<p>Header: MA – whether some or all comparisons from this study are included in the meta-analysis (✓) or on systematic review level only; Model(PNDs) – which ELA model (MS – maternal separation, LBN – limited bedding and nesting, LG – licking and grooming) was applied during which postnatal days (PNDs); Sex – animals were female (F) or male (M); Notes – indicates how experiments (nests) differed, if – then rest/no manipulation; AS – if acute stress challenge was present (✓) or not (×); Effect – if ELA significantly increased (↑), decreased (↓) or did not alter (↔) IEG expression as based on independent t-tests; * = t-test could not be performed and effects are shown as reported in the original publication; Areas – brain areas as identified in publication, with position (lowercase, if identified) and area acronym as follows:</p> <p>Position: a – anterior; c – central; d – dorsal; l – lateral; le – left; m – medial; p – posterior; r – right; v – ventral.</p> <p>Area acronyms (in alphabetical order): ACA – anterior cingulate area; AHA – anterior hypothalamic nucleus; AI – agranular insular cortex; BLA – basolateral amygdala; BNST – bed nuclei of the stria terminalis; CB – cerebellum; CEA – central amygdala; CP – caudate putamen; CTX – cortex; DG – dentate gyrus; DRN – dorsal raphe nucleus; EW – Edinger-Westphal nucleus; HN – hypothalamic nucleus; HPF – hippocampal formation; IL – infralimbic area; DMH – dorsomedial hypothalamic nucleus; LA – lateral amygdala; LC – locus coeruleus; MEA – medial amygdala; MGPVN – magnocellular part of the PVN; MMN – mammillary nucleus; NAcc – nucleus accumbens; OFC – orbital-frontal cortex; PAG – periaqueductal gray; PAL – Pallidum; PFC – prefrontal cortex; PIR – Piriform cortex; PL – prelimbic area; PPVN – parvocellular part of the PVN; PVN – paraventricular nucleus of the hypothalamus; PVT – paraventricular nucleus of the thalamus; RSP – retrosplenial cortex; SSb – somatosensory barrel cortex; STR – striatum; SUB – subiculum; SX – septum; TN – thalamic nucleus; VH – ventral hypothalamic nucleus; VTA – ventral tegmental area.</p>								

Overall, of the 322 comparisons within these studies, 140 comparisons ($n_{pub} = 20$) qualified for further meta-analysis in male rodents after removal of influential outliers ($n_{comp} = 1$); these are analyzed quantitatively in the following section. Comparisons were excluded from quantitative review because of brain area ($n_{comp} = 40$), acute stressor type ($n_{comp} = 49$) or unspecified or pooled sex ($n_{comp} = 15$). The excluded comparisons are subject to a qualitative review in the Supplementary Materials.

Given fundamental biological differences between males and females [72], we *a priori* chose to evaluate female cFos data separately from males'. However, only ten publications reported on cFos expression in female rodents ($n_{comp} = 77$). Given the limited amount of studies, with variable designs, we had to abandon the separate meta-analytical evaluation of female rodents. Qualitatively, the majority of the studies with females found no significant differences between cFos levels of ELA versus controls at rest or after an acute stress challenge ($n_{comp} = 55$ [48, 51, 52, 54, 59]). A more detailed description is supplied in the Supplementary Material.

Systematic review of ELA and other IEGs

We here only summarize the main findings on IEGs other than cFos. In general, the number of studies on these IEGs compared to cFos was very limited. For a more elaborate description and discussion we refer to the Supplementary Material.

Arc is a post-synaptic protein, which plays an essential role in regulating the homeostatic scaling of AMPA receptors, thereby directly modifying plasticity at the synapse [73]. *Arc* expression was investigated in only five publications under varying conditions in male and female mice and rats (see Table 2 and Supplementary Material).

Table 2. Overview of study designs and findings of reviewed publications reporting on *Arc* expression in ELA and control animals.

Author (Year)	Model (PNDs)	Species	Sex	Notes	AS	Effect	Area(s)
Benekareddy (2010) [74]	MS (2-14)	Rat	M	-	x	↔	mPFC
Benner (2014) [43]	MS (2-15)	Mouse	M	Competitive dominance task	✓	↔	ACA, BLA, CEA, CA1, DG, IL, PL
McGregor (2018) [75]	MS (2-14)	Rat	M	Juvenile restraint stress	x	↑*	dSTR
				-	x	↑*	dSTR
Rincel (2019) [76]	MS (2-14)	Mouse	M	-	x	↓*	mPFC
			F	-	x	↑*	mPFC
Solas (2010) [77]	MS (2-21)	Rat	M	-	x	↓	CA1, CA3, DG

Header: *Model(PNDs)* – which ELA model (MS – maternal separation) was applied during which postnatal days (PNDs); *Sex* – animals were female (F) or male (M) or not specified (NS); *Notes* – indicates how experiments (nests) differed, if – then rest/no manipulation; *AS* – if acute stress challenge as present (✓) or not (x); *Effect* – if ELA significantly increase (↑), decreased (↓) or did not alter (↔) IEG expression as based on independent t-tests; * = t-test could not be performed and effects are shown as reported in the original publication; *Areas* – brain areas as identified in publication, with area acronym as follows:
Area acronyms (in alphabetical order): ACA – anterior cingulate area; BLA – basolateral amygdala; CEA – central amygdala; DG – dentate gyrus; IL – infralimbic area; mPFC – medial prefrontal cortex; PL – prelimbic area; dSTR – dorsal striatum.

Early-growth response (Egr) proteins are a family of transcription factors with a zinc-finger motif, which allows all Egr factors to connect to identical DNA binding sites [78]. We identified only three studies investigating Egr expression after ELA exposure at rest (Table 3 and Supplementary Material); specifically, one investigated Egr-1 [79], another investigated Egr-4 only [76], and one other investigated Egr-2 and Egr-4 [75].

THE EFFECTS OF ELA ON IEG EXPRESSION

16

Table 3. Overview of study designs and findings of reviewed publications reporting on expression of the Egr-family in ELA and control animals.

Author (Year)	Model (PNDs)	Species	IEGs	Sex	Notes	AS	Effect	Area(s)
McGregor (2018) [75]	MS (2-14)	Rat	Egr-2	M	Juvenile restraint stress	x	↔*	dSTR
					-	x	↑*	dSTR
			Egr-4	M	Juvenile restraint stress	x	↑*	dSTR
					-	x	↑*	dSTR
Navaillès (2010) [79]	MS (2-15)	Mouse	Egr-1	M	Balb/c strain	x	↓	CTX
							↔	DG, CA1, CA2, CA3
					C57BL/6 strain	x	↔	CTX
Rincel (2019) [76]	MS (2-14)	Mouse	Egr-4	M	-	x	↓*	mPFC
				F	-	x	↑*	mPFC

Header: *Model(PNDs)* – which ELA model (MS – maternal separation) was applied during which postnatal days (PNDs); *Sex* – animals were female (F) or male (M) or not specified (NS); *Notes* – indicates how experiments (nests) differed, if – then rest/no manipulation; *AS* – if acute stress challenge as present (✓) or not (✗); *Effect* – if ELA significantly increase (↑), decreased (↓) or did not alter (↔) IEG expression; * = t-test could not be performed and effects are shown as reported in the original publication; *Areas* – brain areas as identified in publication, with area acronym as follows:

Area acronyms (in alphabetical order): CTX – cortex; DG – dentate gyrus; dSTR – dorsal striatum; mPFC – medial prefrontal cortex.

FosB is an IEG of the *Fos* family, and - similarly to *cFos* - it binds to members of the *Jun* family to form the AP1 transcription factor [80]. Of particular interest in stress research is its isoform Δ *FosB*, whose extended half-life makes it an exceptional marker for chronic stress [80]. Three publications reporting on the expression of Δ *FosB* at rest in ELA and control animals were identified (see Table 4 and Supplementary Material).

Table 4. Overview of study designs and findings of reviewed publications reporting on Δ FosB expression in ELA and control animals.

Author (Year)	Model (PNDs)	Species	Sex	Notes	AS	Effect	Area(s)
Kim (2015) [81]	MS (1-14)	Rat	F	-	x	↓	NAcc
Lippmann (2007) [82]	MS (2-14)	Rat	M	-	x	↔*	CTX, NAcc, STR
	Handling (2-14)	Rat	M	-	x	↔*	CTX, NAcc, STR
Wang (2016) [83]	MS (1-15)	Rat	NS	-	x	↑	mPFC

Header: *Model(PNDs)* – which ELA model (MS – maternal separation) was applied during which postnatal days (PNDs); *Sex* – animals were female (F) or male (M) or not specified (NS); *Notes* – indicates how experiments (nests) differed, if – then rest/no manipulation; *AS* – if acute stress challenge as present (✓) or not (✗); *Effect* – if ELA significantly increase (↑), decreased (↓) or did not alter (↔) IEG expression; * = t-test could not be performed and effects are shown as reported in the original publication; *Areas* – brain areas as identified in publication, with area acronym as follows:

Area acronyms (in alphabetical order): CTX – cortex; mPFC – medial prefrontal cortex; NAcc – nucleus accumbens; STR – striatum.

Meta-analysis of cFos in male rodents.

For cFos, our survey yielded sufficient data to carry out a meta-analysis, next to the systematic review. In comparison to control animals, rodents with a history of ELA displayed significantly increased cFos levels at rest ($g[SEM] = 0.421[\pm 0.18]$, $t = 2.35$, $p_{adj} = 0.041$), but not after acute stress exposure ($g[SEM] = 0.133[\pm 0.166]$, $t = 0.805$, $p_{adj} = 0.422$; Fig. 4a). To gain a deeper understanding of these findings, we performed subgroup analyses to investigate the experience of multiple hits, i.e. an additional negative life event. Of note, the control and experimental groups always differed only in the presence/absence of ELA. Therefore, in the ‘multiple hits’ comparisons, both control and ELA animals experienced multiple negative life events. This was particularly important for cFos expression after acute stress, where the effects of ELA were pronounced only in synergy with multiple hits (Fig. 4b, acute_{no hit}: $g[SEM] = -0.193[\pm 0.135]$, $z = -1.436$, $p_{adj} = 0.151$; acute_{mult hits}: $g[SEM] = 0.442[\pm 0.159]$, $z = 2.784$, $p = 0.016$; at rest_{no hit}: $g[SEM] = 0.475 [\pm 0.16]$, $z = 2.976$, $p < .012$; at rest_{mult hits}: $g[SEM] = 0.344[\pm 0.153]$, $z = 2.253$, $p = 0.049$; the analyses were conducted comparing the effect size between control and ELA animals against 0). Lastly, we performed an exploratory analysis to investigate potential interactions with acute stressor severity on the effect sizes. Acute stressor severity was not a significant moderator ($Q_M(3) = 4.35$, $p = 0.226$). Of note, no publication investigated cFos expression after a mild acute stressor in animals that experienced multiple hits ($n_{comp} = 0$). The findings of our main analysis do not confirm our hypothesis that cFos expression is higher in ELA animals compared to control particularly after acute stress; rather, the results indicate that cFos expression is increased after ELA already at baseline, i.e. at rest. Moreover, the results highlight the relevance of investigating multiple versus single negative life events.

Fig 4. Main and subgroup analyses. Effects of ELA on cFos expression in male rodents A) at rest and after an acute stressor. B) Subgroup analysis for absence (Single) or presence (Multiple) of additional negative life events (hits). Of note, control and experimental animals always differed only in the presence/absence of ELA. Therefore, in the 'multiple hits' comparison, also control animal experienced the additional negative life events. * $p < 0.05$.

Next, we tested whether the effects of ELA on cFos expression differed across brain regions important for the stress reaction (Fig. 5), only taking into account those datasets with sufficient observations ($n_{\text{publications}} > 3$). Brain region was not a significant moderator ($Q_M(12) = 13.908$, $p = 0.307$) of the effects of ELA on cFos expression. Exploratory subgroup analysis suggests that at rest all brain areas show a comparable increase in cFos expression. After an acute stress challenge, the effects appeared more variable across brain areas than at rest. We then performed an additional exploratory analysis to investigate whether brain areas after acute stress differed after ELA with / without the experience of additional life events. The results of this analysis suggested that the prefrontal cortex may be specifically affected; however, since this effect was supported by those studies unevenly represented in the funnel plot, these results may not be reliable due to presumed publication bias.

Fig 5. Effects of ELA on *cFos* expression across brain areas at rest and after an acute stressor.

Despite significant contribution of the moderators ($Q_M(23) = 40.089$, $p = 0.015$), residual heterogeneity between studies remained significant ($Q_E(117) = 167.95$, $p = 0.001$). Study of the distribution of variance showed that remaining variance is mainly attributable to differences between experiments (Level 2) and not to differences within experiments (Level 3). Concerning potential bias, while reporting risk of bias was incomplete in all publications (*S2.1a*), 46% of studies reported adequate randomization and blinding procedures ($n_{pub} = 10$). Visual assessment of the funnel plot for the studies qualifying for quantitative synthesis suggests the presence of publication bias (*S2.1b*), which was also supported by a significant Eggers' test ($z = 4.6903$, $p < .0001$). We identified two studies [49, 66] which were mainly responsible for the bias.

Discussion

In this review, we synthesized the evidence of 39 publications investigating the effects of ELA on IEG expression in mice and rats. Due to low number of animals used in preclinical research,

studies are commonly underpowered [84], rendering results of individual studies vulnerable to confounding effects of the chosen study design. In order to circumvent this limitation, we systematically reviewed the available literature on several IEGs in males and females. We meta-analyzed a subset of our male data to quantify cFos expression following ELA exposure and to identify potential moderators of the observed effects. Using a three-level mixed effects model, we observed an increase in cFos expression after an acute stress exposure due to ELA only in combination with one or more other negative life events. This suggests that ELA creates a vulnerable phenotype that is manifested only when sufficiently triggered. If rodents had ‘only’ experienced ELA, we report – contrary to our expectations – an increase in cFos expression already at rest, suggesting that the situation normally seen (in naïve rodents) after acute stress is already visible at rest when the animals have been exposed to early life adversity. These findings led us to propose a new model as outlined in *Figure 6*.

At rest, ELA animals compared to controls show increased IEG expression. Since raw values of IEG expression are either not reported or of incomparable scales, we could only investigate effect sizes and not absolute values of IEG expression. This has a direct effect on the interpretation of the results. Specifically, if IEG levels in control animals were low, effect sizes could be inflated. If IEG levels in control animals were high, the results should be interpreted not as “rest” but rather as “mildly aroused”, since IEG levels are expected to be minimal for control, naïve animals. Nonetheless, we observed a consistent, positive standardized mean difference in cFos effects sizes after ELA across five out of the six quantitatively investigated brain regions. This suggests a small, but stable brain-wide effect. Previous studies showed that IEG expression matches the transcriptional activity from early environment and experiences [85]. In control animals, this is likely to result in a minimal IEG expression. However, in ELA animals, the expression observed may be the result of long-lasting ELA effects on brain structure and chemistry [86]. Indeed, the transcriptional activity of ELA mice at rest is comparable to that of acutely-stressed control mice [87]. Increased activity-regulated transcription at rest after ELA could be indicative of an overall synaptic

sensitization, in accordance with increased anxiety-like behavior and reduced memory performance under neutral conditions [16]. Functionally, increased IEG expression at rest could reflect a differential, less adaptive way of processing previous experiences as well as an overall increased transcriptional activity as a result of synaptic sensitization. Intuitively, considering the relationship between IEGs and synaptic plasticity, we would suspect that ELA results in increased synaptic plasticity. In line with this idea, it has been shown that ELA leads to increased LTP in freely-behaving adult, male rats as compared to controls [88]. Taken together, this evidence suggests that differences we report in IEG expression after ELA at rest may underlie long-lasting effects on transcriptional activity, pushing the system towards an “activated” state similar to acute stress.

Fig 6. Summary interpretation of the results. Cartoon image of how to interpret effect sizes in absolute terms (y-axis, cFos expression, e.g. number of cFos+ cells). Significance levels identify the difference between control and ELA groups that we identified in our analysis (Fig. 3). Of note, cFos expression levels are expected to be higher after acute stress than at rest, although this cannot be studied in the current meta-analysis.

The model proposed in *Figure 6* relies primarily on the quantitative and qualitative analysis of *cFos* data, as there are only few publications investigating the effects of ELA on the expression of the IEGs *Arc*, *ΔFosB*, and IEGs of the *Egr*-family. Nonetheless, the available evidence suggests a sensitization effect of ELA on IEG expression (and, more generally, synaptic plasticity) at rest. Although IEGs overlap in function and overall expression pattern, they have specific and independent roles [3, 17, 20–22, 90]. *cFos* and *Egr*-family members are transcription factors, while *Arc* is a post-synaptic protein modifying dendritic AMPA receptors, and *ΔFosB* is a less transient marker of neuronal activity [80, 91, 92]. With technological advances in the field of immunohistochemistry and bioinformatics it becomes increasingly feasible to investigate and interpret multiple IEGs within one animal, thereby also allowing for the investigation of interactions between IEGs and their downstream effects. In the future, the study of different IEGs could be used as a proxy to more thoroughly understand ELA-induced changes in gene-regulated synaptic plasticity [93].

On a systematic review level, effects in females appear more limited than in males. Whether this is a true biological effect is unclear. For example, it could be due to the comparatively low number of female publications, or to a male-biased experimental methodology [16, 72]. While it has been shown that acute stress exposure increases IEG expression in both sexes in the hippocampus [94], it is possible that effects of ELA on IEG expression will be more subtle in females than in males due to model characteristics. Of note, among the 39 publications included in this review, only 5 investigated males and females under the same experimental conditions.

Lastly, given the expected heterogeneity in study designs, we restricted our meta-analysis to adult animals only. It is possible, that during different developmental stages different patterns of IEG expression associated to ELA exposure emerge. Therefore, the current analysis does not generalize to developing and juvenile rodents.

To conclude, we systematically provided a general overview on the relationship between ELA and IEG expression and highlighted current knowledge gaps. Despite subject-specific and

390 methodological limitations, the outcomes of the meta-analysis were robust and suggest a
391 sensitization of activity-regulated transcription in ELA rodents at rest while changes after acute
392 stress only became apparent in combination with multiple ELA hits. Recent advances in the fields of
393 immunostaining, live cell imaging and bioinformatics may help close the described voids, yielding a
394 more comprehensive picture on the complex relationship between IEGs, ELA and psychopathologies.

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402

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 406 the manuscript; M.J. contributed with the conceptualization, funding acquisition, project
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411 **Competing interests:**

412 The authors have no competing interests that might be perceived to influence the results and/or
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References

- [1] Burns SB, Szyszkowicz JK, Luheshi GN, et al. Plasticity of the epigenome during early-life stress. *Semin Cell Dev Biol* 2018; 77: 115–132.
- [2] Citri A, Malenka RC. Synaptic plasticity: Multiple forms, functions, and mechanisms. *Neuropsychopharmacology* 2008; 33: 18–41.
- [3] Yap EL, Greenberg ME. Activity-Regulated Transcription: Bridging the Gap between Neural Activity and Behavior. *Neuron* 2018; 100: 330–348.
- [4] Hübener M, Bonhoeffer T. Neuronal plasticity: Beyond the critical period. *Cell* 2014; 159: 727–737.
- [5] Lang J, Kerr DM, Petri-Romão P, et al. The hallmarks of childhood abuse and neglect: A systematic review. *PLoS One* 2020; 15: e0243639.
- [6] Lippard ETC, Nemeroff CB. The devastating clinical consequences of child abuse and neglect: Increased disease vulnerability and poor treatment response in mood disorders. *Am J Psychiatry* 2020; 177: 20–36.
- [7] Nemeroff CB. Paradise Lost: The Neurobiological and Clinical Consequences of Child Abuse and Neglect. *Neuron* 2016; 89: 892–909.
- [8] Herzog JI, Schmahl C. Adverse Childhood Experiences and the Consequences on Neurobiological, Psychosocial, and Somatic Conditions Across the Lifespan. *Front Psychiatry* 2018; 9: 420.
- [9] Merrick MT, Ports KA, Ford DC, et al. Unpacking the impact of adverse childhood experiences on adult mental health. *Child Abuse Negl* 2017; 69: 10–19.
- [10] Liu D, Diorio J, Tannenbaum B, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic- pituitary-adrenal responses to stress. *Science (80-)* 1997; 277: 1659–1662.
- [11] Plotsky PM, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Mol Brain Res* 1993; 18: 195–200.

- 440 [12] McEwen BS, Nasca C, Gray JD, et al. Stress Effects on Neuronal Structure: Hippocampus,
441 Amygdala, and Prefrontal Cortex. *Neuropsychopharmacol Rev* 2016; 41: 3–23.
- 442 [13] Molet J, Maras PM, Kinney-Lang E, et al. MRI uncovers disrupted hippocampal microstructure
443 that underlies memory impairments after early-life adversity. *Hippocampus* 2016; 26: 1618–
444 1632.
- 445 [14] Chen Y, Baram TZ. Toward understanding how early-life stress reprograms cognitive and
446 emotional brain networks. *Neuropsychopharmacology* 2016; 41: 197–206.
- 447 [15] Van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the hypothalamic-pituitary-
448 adrenal axis by early life stress exposure. *Front Cell Neurosci* 2017; 11: 87.
- 449 [16] Bonapersona V, Kentrop J, Van Lissa CJ, et al. The behavioral phenotype of early life
450 adversity: A 3-level meta-analysis of rodent studies. *Neurosci Biobehav Rev* 2019; 102: 299–
451 307.
- 452 [17] Okuno H. Regulation and function of immediate-early genes in the brain: Beyond neuronal
453 activity markers. *Neurosci Res* 2011; 69: 175–186.
- 454 [18] Kovács KJ. c-Fos as a transcription factor: A stressful (re)view from a functional map.
455 *Neurochem Int* 1998; 33: 287–297.
- 456 [19] Yap EL, Pettit NL, Davis CP, et al. Bidirectional perisomatic inhibitory plasticity of a Fos
457 neuronal network. *Nature* 2021; 590: 115–121.
- 458 [20] Fleischmann A, Hvalby O, Jensen V, et al. Impaired long-term memory and NR2A-type NMDA
459 receptor-dependent synaptic plasticity in mice lacking c-fos in the CNS. *J Neurosci* 2003; 23:
460 9116–9122.
- 461 [21] Plath N, Ohana O, Dammermann B, et al. Arc/Arg3.1 Is Essential for the Consolidation of
462 Synaptic Plasticity and Memories. *Neuron* 2006; 52: 437–444.
- 463 [22] Jones MW, Errington ML, French PJ, et al. A requirement for the immediate early gene Zif268
464 in the expression of late LTP and long-term memories. *Nat Neurosci* 2001; 4: 289–296.
- 465 [23] Gallo FT, Kathe C, Morici JF, et al. Immediate Early Genes, Memory and Psychiatric

- 466 Disorders: Focus on c-Fos, Egr1 and Arc. *Front Behav Neurosci* 2018; 12: 79.
- 467 [24] Daskalakis NP, Bagot RC, Parker KJ, et al. The three-hit concept of vulnerability and resilience:
468 Toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology*
469 2013; 38: 1858–1873.
- 470 [25] de Vries RBM, Hooijmans CR, Langendam MW, et al. A protocol format for the preparation,
471 registration and publication of systematic reviews of animal intervention studies. *Evidence-*
472 *based Preclin Med* 2015; 2: e00007.
- 473 [26] Leenaars M, Hooijmans CR, van Veggel N, et al. A step-by-step guide to systematically identify
474 all relevant animal studies. *Lab Anim* 2012; 46: 24–31.
- 475 [27] Hooijmans CR, Rovers MR, de Vries RBM, et al. SYRCLE's risk of bias tool for animal studies.
476 *BMC Med Res Methodol*; 14. Epub ahead of print 26 March 2014. DOI: 10.7507/1672-
477 2531.20140206.
- 478 [28] Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline
479 for reporting systematic reviews. *Int J Surg*; 88. Epub ahead of print 1 April 2021. DOI:
480 10.1016/j.ijsu.2021.105906.
- 481 [29] Ouzzani M, Hammady H, Fedorowicz Z, et al. Rayyan-a web and mobile app for systematic
482 reviews. *Syst Rev* 2016; 5: 210.
- 483 [30] Rohatgi A. WebPlotDigitizer, <https://automeris.io/WebPlotDigitizer> (2020).
- 484 [31] Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: A practical
485 guide. *J Neurosci Methods* 2014; 221: 92–102.
- 486 [32] Chochran WG. The comparison of percentages in matched samples. *Biometrika* 1950; 37:
487 256–266.
- 488 [33] Viechtbauer W, Cheung MWL. Outlier and influence diagnostics for meta-analysis. *Res Synth*
489 *Methods* 2010; 1: 112–125.
- 490 [34] Assink M, Wibbelink CJM. Fitting three-level meta-analytic models in R: A step-by-step
491 tutorial. *Quant Methods Psychol* 2016; 12: 154–174.

- 492 [35] Aarts E, Verhage M, Veenvliet J V., et al. A solution to dependency: Using multilevel analysis
493 to accommodate nested data. *Nat Neurosci* 2014; 17: 491–496.
- 494 [36] Cheung MWL. Modeling dependent effect sizes with three-level meta-analyses: A structural
495 equation modeling approach. *Psychol Methods* 2014; 19: 211–229.
- 496 [37] R Core Team. R: A Language and Environment for Statistical Computing, <https://www.r-project.org>
497 (2019).
- 498 [38] Viechtbauer W. Conducting meta-analyses in {R} with the {metafor} package. *J Stat Softw*
499 2010; 36: 1–48.
- 500 [39] Wickham H. tidyverse: Easily Install and Load the ‘Tidyverse’, [https://cran.r-](https://cran.r-project.org/package=tidyverse)
501 [project.org/package=tidyverse](https://cran.r-project.org/package=tidyverse) (2017).
- 502 [40] Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat* 1979; 6: 65–70.
- 503 [41] Auth CS, Weidner MT, Popp S, et al. Differential anxiety-related behaviours and brain
504 activation in Tph2-deficient female mice exposed to adverse early environment. *Eur*
505 *Neuropsychopharmacol* 2018; 28: 1270–1283.
- 506 [42] Banqueri M, Méndez M, Arias JL. Why are maternally separated females inflexible? Brain
507 activity pattern of COx and c-Fos. *Neurobiol Learn Mem* 2018; 155: 30–41.
- 508 [43] Benner S, Endo T, Endo N, et al. Early deprivation induces competitive subordination in
509 C57BL/6 male mice. *Physiol Behav* 2014; 137: 42–52.
- 510 [44] Chung EKY, Zhang XJ, Li Z, et al. Neonatal maternal separation enhances central sensitivity to
511 noxious colorectal distention in rat. *Brain Res* 2007; 1153: 68–77.
- 512 [45] Clarke M, Cai G, Saleh S, et al. Being suckled in a large litter mitigates the effects of early-life
513 stress on hypothalamic-pituitary-adrenal axis function in the male rat. *J Neuroendocrinol*
514 2013; 25: 792–802.
- 515 [46] Cohen MM, Jing D, Yang RR, et al. Early-life stress has persistent effects on amygdala function
516 and development in mice and humans. *Proc Natl Acad Sci U S A* 2013; 110: 18274–18278.
- 517 [47] Daskalakis NP, Diamantopoulou A, Claessens SEF, et al. Early experience of a novel-

- 518 environment in isolation primes a fearful phenotype characterized by persistent amygdala
519 activation. *Psychoneuroendocrinology* 2014; 39: 39–57.
- 520 [48] Desbonnet L, Garrett L, Daly E, et al. Sexually dimorphic effects of maternal separation stress
521 on corticotrophin-releasing factor and vasopressin systems in the adult rat brain. *Int J Dev*
522 *Neurosci* 2008; 26: 259–268.
- 523 [49] Felice VD, Gibney SM, Gosselin RD, et al. Differential activation of the prefrontal cortex and
524 amygdala following psychological stress and colorectal distension in the maternally separated
525 rat. *Neuroscience* 2014; 267: 252–262.
- 526 [50] Gardner KL, Thiruvikraman K V, Lightman SL, et al. Early life experience alters behavior during
527 social defeat: Focus on serotonergic systems. *Neuroscience* 2005; 136: 181–191.
- 528 [51] Gaszner B, Jensen KO, Farkas J, et al. Effects of maternal separation on dynamics of urocortin
529 1 and brain-derived neurotrophic factor in the rat non-preganglionic Edinger-Westphal
530 nucleus. *Int J Dev Neurosci* 2009; 27: 439–451.
- 531 [52] Genest SE, Gulemetova R, Laforest S, et al. Neonatal maternal separation and sex-specific
532 plasticity of the hypoxic ventilatory response in awake rat. *J Physiol* 2004; 554: 543–557.
- 533 [53] Hidaka C, Kashio T, Uchigaki D, et al. Vulnerability or resilience of motopsin knockout mice to
534 maternal separation stress depending on adulthood behaviors. *Neuropsychiatr Dis Treat*
535 2018; 14: 2255–2268.
- 536 [54] James MH, Campbell EJ, Walker FR, et al. Exercise reverses the effects of early life stress on
537 orexin cell reactivity in male but not female rats. *Front Behav Neurosci* 2014; 8: 244.
- 538 [55] Loi M, Sarabdjitsingh RA, Tsouli A, et al. Transient prepubertal mifepristone treatment
539 normalizes deficits in contextual memory and neuronal activity of adult male rats exposed to
540 maternal deprivation. *eNeuro*; 4. Epub ahead of print 2017. DOI: 10.1523/ENEURO.0253-
541 17.2017.
- 542 [56] Menard JL, Champagne DL, Meaney MJP. Variations of maternal care differentially influence
543 ‘fear’ reactivity and regional patterns of cFOS immunoreactivity in response to the shock-

- 544 probe burying test. *Neuroscience* 2004; 129: 297–308.
- 545 [57] O’Leary OF, Felice D, Galimberti S, et al. GABAB(1) receptor subunit isoforms differentially
- 546 regulate stress resilience. *Proc Natl Acad Sci U S A* 2014; 111: 15232–15237.
- 547 [58] Ren TH, Wu J, Yew D, et al. Effects of neonatal maternal separation on neurochemical and
- 548 sensory response to colonic distension in a rat model of irritable bowel syndrome. *Am J*
- 549 *Physiol - Gastrointest Liver Physiol* 2007; 292: 849–856.
- 550 [59] Renard GM, Rivarola MA, Suárez MM. Gender-dependent effects of early maternal
- 551 separation and variable chronic stress on vasopressinergic activity and glucocorticoid
- 552 receptor expression in adult rats. *Dev Neurosci* 2010; 32: 71–80.
- 553 [60] Rincel M, Lépinay AL, Delage P, et al. Maternal high-fat diet prevents developmental
- 554 programming by early-life stress. *Transl Psychiatry* 2016; 6: e966–e966.
- 555 [61] Rivarola MA, Dalmasso C, Valdez DJ, et al. Early maternal separation and chronic variable
- 556 stress as adults differentially affect fos expression in the anterodorsal thalamic nuclei. *Int J*
- 557 *Neurosci* 2008; 118: 735–748.
- 558 [62] Rivarola MA, Suárez MM. Early maternal separation and chronic variable stress in adulthood
- 559 changes the neural activity and the expression of glucocorticoid receptor in limbic structures.
- 560 *Int J Dev Neurosci* 2009; 27: 567–574.
- 561 [63] Shin S, Pribyl H, Lilascharoen V, et al. Drd3 Signaling in the Lateral Septum Mediates Early
- 562 Life Stress-Induced Social Dysfunction. *Neuron* 2018; 97: 195–208.
- 563 [64] Tenorio-Lopes L, Henry MS, Marques D, et al. Neonatal maternal separation opposes the
- 564 facilitatory effect of castration on the respiratory response to hypercapnia of the adult male
- 565 rat: Evidence for the involvement of the medial amygdala. *J Neuroendocrinol* 2017; 29:
- 566 e12550.
- 567 [65] Troakes C, Ingram CD. Anxiety behaviour of the male rat on the elevated plus maze:
- 568 Associated regional increase in c-fos mRNA expression and modulation by early maternal
- 569 separation. *Stress* 2009; 12: 362–369.

- 570 [66] Trujillo V, Durando PE, Suárez MM. Maternal separation in early life modifies anxious
571 behavior and Fos and glucocorticoid receptor expression in limbic neurons after chronic
572 stress in rats: Effects of tianeptine. *Stress* 2016; 19: 91–103.
- 573 [67] van Hasselt FN, de Visser L, Tieskens JM, et al. Individual variations in maternal care early in
574 life correlate with later life decision-making and c-Fos expression in prefrontal subregions of
575 rats. *PLoS One* 2012; 7: e37820.
- 576 [68] Vivinetto AL, Suárez MM, Rivarola MA. Neurobiological effects of neonatal maternal
577 separation and post-weaning environmental enrichment. *Behav Brain Res* 2013; 240: 110–
578 118.
- 579 [69] Yajima H, Haijima A, Khairinisa MA, et al. Early-life stress induces cognitive disorder in middle-
580 aged mice. *Neurobiol Aging* 2018; 64: 139–146.
- 581 [70] Zhang X-J, Li Z, Chung EKY, et al. Activation of Extracellular Signal-Regulated Protein Kinase is
582 Associated with Colorectal Distension-Induced Spinal and Supraspinal Neuronal Response
583 and Neonatal Maternal Separation-Induced Visceral Hyperalgesia in Rats. *J Mol Neurosci*
584 2009; 37: 274–287.
- 585 [71] Zhao XP, Yu L, Chen ZY, et al. Effect of early-life stress on chronic functional visceral pain and
586 CRH R1 expression in the paraventricular nucleus in adult rats. *World Chinese J Dig* 2013; 21:
587 3344–3355.
- 588 [72] Shansky RM, Murphy AZ. Considering sex as a biological variable will require a global shift in
589 science culture. *Nat Neurosci* 2021; 24: 457–464.
- 590 [73] Bramham CR, Worley PF, Moore MJ, et al. The Immediate Early Gene Arc/Arg3.1: Regulation,
591 Mechanisms, and Function. *J Neurosci* 2008; 28: 11760–11767.
- 592 [74] Benekareddy M, Goodfellow NM, Lambe EK, et al. Enhanced function of prefrontal serotonin
593 5-HT2 receptors in a rat model of psychiatric vulnerability. *J Neurosci* 2010; 30: 12138–12150.
- 594 [75] McGregor NW, Dimatelis JJ, Van Zyl PJ, et al. A translational approach to the genetics of
595 anxiety disorders. *Behav Brain Res* 2018; 341: 91–97.

- 596 [76] Rincel M, Aubert P, Chevalier J, et al. Multi-hit early life adversity affects gut microbiota, brain
597 and behavior in a sex-dependent manner. *Brain Behav Immun* 2019; 80: 179–192.
- 598 [77] Solas M, Aisa B, Mugueta MC, et al. Interactions between age, stress and insulin on cognition:
599 Implications for alzheimer’s disease. *Neuropsychopharmacology* 2010; 35: 1664–1673.
- 600 [78] Beckmann AM, Wilce PA. Egr transcription factors in the nervous system. *Neurochem Int*
601 1997; 31: 477–510.
- 602 [79] Navailles S, Zimnisky R, Schmauss C. Expression of glucocorticoid receptor and early growth
603 response gene 1 during postnatal development of two inbred strains of mice exposed to early
604 life stress. *Dev Neurosci* 2010; 32: 139–148.
- 605 [80] Nestler EJ. δ fosB: A transcriptional regulator of stress and antidepressant responses. *Eur J*
606 *Pharmacol* 2015; 753: 66–72.
- 607 [81] Kim JY, Lee JH, Kim D, et al. Beneficial effects of highly palatable food on the behavioral and
608 neural adversities induced by early life stress experience in female rats. *Int J Biol Sci* 2015; 11:
609 1150–1159.
- 610 [82] Lippmann M, Bress A, Nemeroff CB, et al. Long-term behavioural and molecular alterations
611 associated with maternal separation in rats. *Eur J Neurosci* 2007; 25: 3091–3098.
- 612 [83] Wang H, Tao X, Huang ST, et al. Chronic stress is associated with pain precipitation and
613 elevation in DeltaFosB expression. *Front Pharmacol* 2016; 7: 138.
- 614 [84] Bonapersona V, Hoijsink H, Abbinck M, et al. Increasing the statistical power of animal
615 experiments with historical control data. *Nat Neurosci* 2021; 24: 470–477.
- 616 [85] Marrone DF, Schaner MJ, McNaughton BL, et al. Immediate-early gene expression at rest
617 recapitulates recent experience. *J Neurosci* 2008; 28: 1030–1033.
- 618 [86] De Kloet ER, Joëls M, Holsboer F. Stress and the brain: From adaptation to disease. *Nature*
619 *Reviews Neuroscience* 2005; 6: 463–475.
- 620 [87] Marrocco J, Gray JD, Kogan JF, et al. Early life stress restricts translational reactivity in CA3
621 neurons associated with altered stress responses in adulthood. *Front Behav Neurosci* 2019;

- 622 13: 157.
- 623 [88] Blaise JH, Koranda JL, Chow U, et al. Neonatal isolation stress alters bidirectional long-term
- 624 synaptic plasticity in amygdalo-hippocampal synapses in freely behaving adult rats. *Brain Res*
- 625 2008; 1193: 25–33.
- 626 [89] Majcher-Maślanka I, Solarz A, Wędzony K, et al. Previous Early-life Stress Modifies Acute
- 627 Corticosterone-induced Synaptic Plasticity in the Medial Prefrontal Cortex of Adolescent Rats.
- 628 *Neuroscience* 2018; 379: 316–333.
- 629 [90] Guzowski JF, Setlow B, Wagner EK, et al. Experience-dependent gene expression in the rat
- 630 hippocampus after spatial learning: A comparison of the immediate-early genes Arc, c-fos,
- 631 and zif268. *J Neurosci* 2001; 21: 5089–5098.
- 632 [91] Vialou V, Bagot RC, Cahill ME, et al. Prefrontal Cortical Circuit for Depression- and Anxiety-
- 633 Related Behaviors Mediated by Cholecystokinin: Role of Δ FosB. *J Neurosci* 2014; 34: 3878 LP
- 634 – 3887.
- 635 [92] Vialou V, Robison AJ, LaPlant QC, et al. Δ FosB in brain reward circuits mediates resilience to
- 636 stress and antidepressant responses. *Nat Neurosci* 2010; 13: 745–752.
- 637 [93] Li Y, Pehrson AL, Waller JA, et al. A critical evaluation of the activity-regulated cytoskeleton-
- 638 associated protein (Arc/Arg3.1) 's putative role in regulating dendritic plasticity, cognitive
- 639 processes, and mood in animal models of depression. *Front Neurosci* 2015; 9: 279.
- 640 [94] Marrocco J, Petty GH, Ríos MB, et al. A sexually dimorphic pre-stressed translational
- 641 signature in CA3 pyramidal neurons of BDNF Val66Met mice. *Nat Commun* 2017; 8: 808.

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644 **Supporting information captions:**

645 **S1.1. SYRCLE's study protocol.**

646 **S1.2. Search string.**

647 **S1.3. Extracted variables.**

648 **S1.4. Grouping of variables into functional units.**

649 **S2.1. Bias assessment.**

650 **S2.2. Systematic review.**

651 **S3. Supplementary references.**