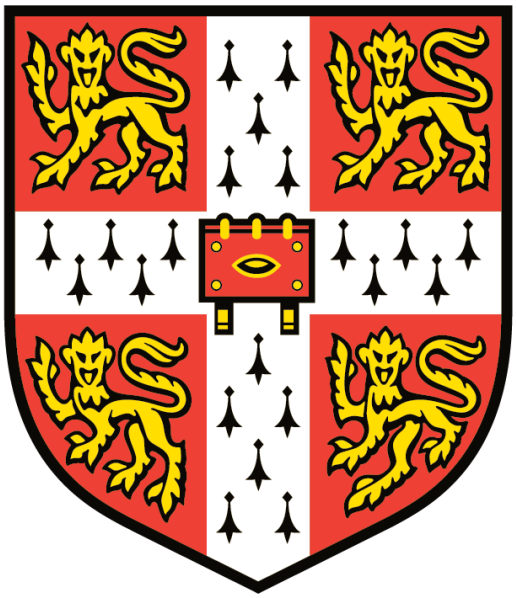
Behavioural, immunological, and neurobiological effects of early life stress in rats



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Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text.

It is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text.

It does not exceed the prescribed word limit (60,000 words) for the Faculty of Biology Degree Committee.

The work described in this thesis was carried out between October 2018 and January 2022.

It has only been previously referred to in one published article, Dutcher *et al.* (2020), and in my First Year Report. For that article, I set out the aims, designed the methods, conducted all data collection and analysis, conceptualised and created two of three figures, and wrote the initial draft of all sections. Where writings or visualisations within this thesis are similar to or the same as those found in these publications, they represent entirely my own work.

Ethan Dutcher

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Abstract

Early life stress (ELS), primarily encompassing childhood neglect and abuse, is associated with many adverse psychiatric and physical health outcomes in later life. What remains unclear, however, is precisely how these links are mediated. Answering this question is challenging, partly because there are many other exposures that may accompany childhood maltreatment or neglect, but also because there are many physical, social, and other life events that occur between childhood and adulthood which could interact with the effects of early life stress to together result in adulthood pathology.

Here, I conducted a large, controlled experiment in rats that sought to isolate key behavioural, immunological, and neurobiological effects into adulthood of early life stress itself. To do this, I used the repeated maternal separation (RMS) model of chronic early life stress, and I focused particularly on those effects of possible relevance to anxiety, depression, and inflammation-related physical disease.

In Chapter 3, I describe the long-term effects of RMS on commonly used measures of anxiety- and depression-like behaviour, as well as on comparatively sophisticated tasks capable of providing detailed insights into reward and punishment sensitivity, as well as attentional control. The probabilistic reversal learning task revealed long-lasting effects of RMS on the degree to which negative outcomes shaped animals' future decisions, as well as evidence suggesting that RMS animals were comparatively inefficient at directing their attention, even where they were equally accurate. Further, RMS animals exhibited a long-lasting sensitization to later-life stress on several behavioural metrics. These effects all persisted into late adulthood despite RMS having no effects on conventional measures of anxiety- or depression-like behaviour, even in early adulthood.

In Chapter 4, I present findings from my experiment and from a systematic review examining the short-term and long-term effects of RMS on cytokine levels in blood and non-blood tissue, as well as on microglial activation and density. I show that RMS causes short-term increases in pro-inflammatory signalling, but only causes long-term increases in pro-inflammatory signalling if animals are subjected to a later-life stress. Thus, I demonstrate that RMS causes a long-lasting sensitisation of the neuroimmune pathway that links stressor perception ultimately to pro-inflammatory cytokine release. However, these effects were largely limited to non-blood tissue such as brain tissue: in plasma, serum, or whole blood, studies generally found no effect of RMS on cytokine levels in the short- or long-term, even following later-life stress.

In Chapter 5, I present analyses of regional brain volumes determined from 9.4 Tesla structural magnetic resonance imaging scans at three timepoints following RMS. I show that RMS had no effect on the volume of any of six regions examined at post-natal day (PND) 20 or 62, but resulted in a larger amygdala during the scan at PND 285, which occurred after 9-13 days of adult stress. Given that the PND 62 and PND 285 scans both occurred in adulthood, this suggests that RMS may have interacted with later-life stress to increase amygdala volume.

In the General Discussion, I describe how these findings are concordant and together provide valuable insight into how early life stress can alter physiology and behaviour in such a way that may directly increase risk for mental and physical pathology.

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List of abbreviations

ACTH	Adrenocorticotropic hormone
BLA	Basolateral amygdala
CAD	Coronary artery disease
CNS	Central nervous system
CPA	Childhood physical abuse
CRH	Corticotropin releasing hormone
CRP	C-reactive protein
CSA	Childhood sexual abuse
CSF	Cerebrospinal fluid
CUS	Chronic unpredictable stress
DA	Dopamine
DRL	Deterministic reversal learning
DSM	Diagnostic and Statistical Manual of Mental Disorders
EDTA	Ethylenediaminetetraacetic acid
ELA	Early life adversity
ELISA	Enzyme-linked immune-sorbent assay
ELS	Early life stress
EMMs	Estimated marginal means
ENIGMA	Enhancing Neuro-Imaging Genetics Through Meta-Analysis
F	Female
FR	Fixed ratio
fMRI	Functional magnetic resonance imaging
G×G	Group × Gender
GAD	Generalized anxiety disorder
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GRO-KC	Growth-regulated oncogene - keratinocyte chemoattractant
HPA	Hypothalamic-pituitary-adrenal
IFN-g	Interferon gamma
IL	Interleukin

IP	Intraperitoneal
IQ	Intelligence quotient
IS	Intra-stress
ITI	Inter-trial interval
IV	Intravenous
LLOD/Q	Lower limit of detection or quantitation
M	Male
MCP-1	Monocyte chemoattractant protein 1
M-CSF	Macrophage colony-stimulating factor
MD	Maternal deprivation
MDD	Major depressive disorder
MIP-1a	Macrophage inflammatory protein 1 alpha
mOFC	Medial orbitofrontal cortex
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Maternal separation
MT	Magnetisation transfer
NAc	Nucleus accumbens
NPT	Novelty preference test
NRT	Novelty reactivity test
OFT	Open field test
PCR	Polymerase chain reaction
PD	Proton density
PFC	Prefrontal cortex
plPFC	Prelimbic prefrontal cortex
PND	Post-natal day
PR	Progressive ratio
PRL	Probabilistic reversal learning
PS	Pre-stress
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus

qPCR	Quantitative polymerase chain reaction
RANTES	Regulated upon activation, normal T cell expressed and secreted
RF	Radiofrequency
RMS	Repeated maternal separation
ROI	Region of interest
SC	Session count
SDS	Social defeat stress
SEMS	Single-episode maternal separation
sgACC	Subgenual anterior cingulate cortex
SPT	Sucrose preference test
STC	Sessions to criterion
T	Timepoint (in tables); Tesla (in text)
TBV	Total brain volume
TNF-a	Tumour necrosis factor alpha
TSST	Trier Social Stress Test
TTA	Touch training A
TTB	Touch training B
UK	United Kingdom
ULOD/Q	Upper limit of detection or quantitation
US	United States
USV	Ultrasonic vocalisation
VEGF	Vascular endothelial growth factor
vmPFC	Ventromedial prefrontal cortex

1 General introduction

1.1 Early life stress and the scope of the problem

Before diving into the unfortunate yet fascinating realm of early life stress, it is worth establishing some definitions. In the human literature, the terms early life adversity (ELA), childhood adversity, and adverse childhood experiences are used synonymously: all refer to the situation in which a child or adolescent has experienced abuse, neglect (broadly defined), or a significant loss (Maccari et al., 2014; Janusek et al., 2017; Boullier and Blair, 2018). Childhood maltreatment is a collective term that encompasses both child abuse and child neglect, and thus is also often used synonymously with the other broad terms (Teicher and Samson, 2013; Agnew-Blais and Danese, 2016). Early life stress (ELS), meanwhile, is a term used predominantly in the pre-clinical literature, presumably because the several means by which ELA is most commonly modelled in animals are known to be stressors (Van Bodegom et al., 2017), and to distinguish these paradigms from other known stressors which are usually applied in adulthood (Nestler and Hyman, 2010). Here, because virtually all causes of ELA in humans are known to be stressful (Miller et al., 2011), and because this thesis primarily concerns the use of animals to study the long-term effects of ELS, going forward I will generally just use the one term, ELS.

ELS is both highly prevalent and highly heterogeneous. In 2019, there were 650,000 confirmed victims of child maltreatment in the United States, representing a rate of 8.9 victims per 1000 children (U.S. Department of Health and Human Services, 2021). In 2021 in England, 219,190 children were referred to children's social care services and assessed by a social worker as having a primary need of either abuse or neglect, representing a rate of 18.1 per 1000 children (Children's Services Statistics Team, 2021). A category of interventions by the government called child protection plans were initiated most frequently for neglect, followed by emotional abuse and physical abuse, and were implemented at a rate of 4.1 per 1000 children (Children's Services Statistics Team, 2021). Similarly, in the US, among maltreatment detected in childhood, neglect comprises by far the largest portion, with 75% of victims being neglected, 18% being physically abused, and 9% being sexually abused (U.S. Department of Health and Human Services, 2021). Within neglect, the three broad categories used by the National Incidence Study are similarly common: 47% of neglected children experienced educational neglect, 38% suffered from physical neglect, and 25% experienced

psychological or emotional neglect (Sedlak et al., 2010). In these studies, educational neglect mostly refers to the missing of substantial portions of legally required schooling due to the permissive, negligent, or restrictive behaviour of caretakers. Physical neglect includes such acts as abandonment, refusal of custody, illegal transfer of custody, and inadequate provision of food, clothing, or shelter. Psychological or emotional neglect can refer to a failure to provide for the child's basic emotional needs, exposure of the child to maladaptive behaviours including violence in the home or community, or expecting the child to handle situations and tasks beyond their maturity or ability, all of which can sometimes be a function of caregiver substance abuse or mental illness (Sedlak et al., 2010; Teicher and Samson, 2013).

1.2 Clinical sequelae of ELS

ELS is associated with many adverse neuropsychiatric and physical health outcomes in later life. Here, I will discuss some of these associations in detail.

1.2.1 Depression and anxiety

ELS has now been linked by dozens of studies, including when summarized meta-analytically, to greater symptoms of depression and anxiety in later life, and to increased risk of multiple depressive and anxiety disorders (Teicher and Samson, 2013). For example, among a large, diverse group of children and adolescents measured at a summer camp program, a past history of neglect or physical, sexual, or emotional abuse were each individually already associated with higher self-rated depressive symptoms, but also with greater anxiety as judged by camp counsellors blinded to maltreatment history (Vachon et al., 2015). In another study, among a large sample of adults with at least one depression or anxiety disorder diagnosis, a self-reported history of psychological, physical, or sexual abuse were each associated with an increased risk of most individual depressive or anxiety disorders, including, in generally decreasing order of risk increase: dysthymia, MDD, generalised anxiety disorder (GAD), social phobia, and panic disorder (Spinhoven et al., 2010). In a non-disordered undergraduate population, self-reported childhood emotional abuse and emotional neglect were both associated with greater current combined symptoms of anxiety and depression, even after controlling for the also-significant influence of childhood sexual abuse (Wright et al., 2009). And in a large prospective birth cohort study with assessment of all subjects at age 32 years, number of adverse childhood experiences was highly predictive of past-year MDD (Danese et al., 2009). Altogether, per meta-analytic

evidence, ELS appears to increase the odds of developing most depressive and anxiety disorders by 2-3 fold, with perhaps fractionally higher conferred risk for depressive as opposed to anxiety disorders, with the exception of PTSD, for which risk appears to be increased 4-5 fold by ELS (Teicher and Samson, 2013).

In addition to likely increasing the risk of developing these disorders, ELS also appears to predispose to a more severe clinical course of at least some of them. A large meta-analysis concluded that among patients who suffer from depressive disorders, a history of ELS is associated with an increased number of depressive episodes, increased duration of the current depressive episode, and decreased responsiveness to treatment (Nanni et al., 2012). Similarly, a recent meta-analysis of bipolar disorder patients concluded that a history of childhood maltreatment is associated with earlier disorder onset, increased severity and number of depressive and manic episodes, and increased risk of: rapid cycling between mania and depression, suicide attempts, comorbid substance use disorders, and comorbid anxiety disorders including PTSD (Agnew-Blais and Danese, 2016).

1.2.2 Substance use disorders

In addition to depressive and anxiety disorders, ELS has also been repeatedly associated with increased risk of later development of substance use disorders. In one large case-control study in Australia of childhood sexual abuse (CSA), female CSA victims had over eight times the odds controls had of developing a substance use disorder, while male CSA victims had over three times the odds controls had (Cutajar et al., 2010). In another Australian study, among 6000 twins, a history of CSA was associated with alcohol dependence only in females but not males, while CSA was associated in both genders with opioid, sedative, and stimulant use and abuse, with the strongest associations being for opioid and sedative use (Nelson et al., 2006). In a large sample from the United States, specifically from navy recruits, the Australian pattern of greater vulnerability to CSA of females than males with respect to alcohol abuse outcomes was reversed, with male CSA victims having twofold greater odds of testing positive on an alcoholism screening test than non-victims, while female CSA victims had only 1.5 times the odds of non-victims, although the relationship was significant in both genders (Trent et al., 2007). Averaging across studies meta-analytically, ELS appears to cause a roughly twofold increase in the risk of alcohol use disorder, and a 2-3 fold increase in risk for other substance use disorders (Teicher and Samson, 2013).

1.2.3 Physical disease

The long-lasting detrimental effects of ELS are not limited to mental health outcomes. There is now a large body of evidence indicating that people exposed to ELS are at higher risk for disparate adverse physical health outcomes in later life. In a large study (n = 6,000), childhood physical abuse was associated with increased adulthood risk of arthritis, lung disease, and peptic ulcer disease, while childhood sexual abuse was associated with increased risk of cardiac disease, and childhood neglect was associated with an increased risk of diabetes and autoimmune disorders (Goodwin and Stein, 2004). In a meta-analysis, child abuse of any type was associated with large effect sizes (Cohen's d = 0.81-0.94) on risk of musculoskeletal problems including arthritis, and neurological problems including migraines, and with medium-to-large effect sizes (d = 0.63-0.71) on risk of cardiovascular problems including heart attack and stroke, respiratory problems including asthma and bronchitis, and gastrointestinal problems including colitis (Wegman and Stetler, 2009). Disease risk appeared greater in females than in males, although type of abuse was not adjusted for (Wegman and Stetler, 2009). In another meta-analysis, childhood sexual abuse was associated with, in order of decreasing effect size, greater prevalence of: gastrointestinal symptoms, gynaecologic symptoms, obesity, pain, poorer general health, and cardiopulmonary symptoms (Brown et al., 2010).

1.3 Possible mechanisms mediating ELS clinical sequelae

1.3.1 Psychological

One cognitive domain that has been repeatedly reported to be affected by ELS is attention (Raymond et al., 2021). Two distinct but likely related deficits have been identified. Firstly, ELS has been reported to result in a bias either toward or away from threat, with the directionality apparently depending on the age of the subject at the time of testing. In one study in 250 subjects aged 8-16 years, 60% of whom had been exposed to childhood maltreatment, maltreatment severity interacted with age to predict attentional bias toward or away from threat on a visual dot-probe task (Weissman et al., 2019). In children, maltreatment was positively associated with attentional bias toward a threat-associated stimulus, while in adolescents, higher maltreatment predicted attentional bias away from the threat-associated stimulus (Weissman et al., 2019). In line with the finding in adolescents, in another visual dot probe study in subjects mostly aged 10.5-12 years, ELS predicted

attentional bias away from threat (Humphreys et al., 2016). Similarly, in another study among subjects mostly aged 8-13, maltreated individuals, most of whom had a diagnosis of PTSD secondary to abuse, exhibited a bias of attention away from threat on a visual dot probe task (Pine et al., 2005). Attentional biases with respect to threat have repeatedly been associated with anxiety although not with depression (Mogg et al., 2000; Dalgleish et al., 2003; Armstrong and Olatunji, 2012). Greater directing of attention by threat-associated stimuli may thus be one mechanism by which ELS increases risk for anxiety disorders in later life.

Other studies have provided evidence for another attention related deficit, specifically in attentional control. ELS subjects appear to have more difficulty shifting attention to and maintaining attention on information relevant to the goal at hand, in the face of distractor stimuli, perhaps particularly but not exclusively in the context of threatening distractor stimuli. For example, in an emotional go/no-go task, children aged 5-10 who were raised in orphanages outside the US were found to have slower responding than controls on a simple go/no-go task featuring threat-associated stimuli, suggesting that maltreated individuals may have impaired attentional control (Malter Cohen et al., 2013). More sophisticated studies, specifically comparing performance between ELS and control subjects under conditions where distractor stimuli were present as opposed to when they weren't, have provided comparatively direct evidence of attentional control deficits in humans and non-human primates with ELS histories, and are discussed in detail in Chapter 3 (Crouch et al., 2012; Morin et al., 2019; Fields et al., 2021). Such deficits in turn have been exhaustively linked to anxiety (Eysenck et al., 2007; Derakshan and Eysenck, 2009; Shi et al., 2019), suggesting that impaired ability to focus attention and suppress distraction via top-down control may play a causal role in the link between ELS and later-life diagnosis of anxiety disorders.

Another psychological domain that ELS has been reported to disrupt is emotion regulation. Emotion regulation can be very broadly defined (Weissman et al., 2019), but many of the ELS-related deficits are specifically in how individuals respond behaviourally and cognitively to their initial emotional responses to situations (Herts et al., 2012). For example, among a sample of 250 children and adolescents, maltreated individuals were more likely than non-maltreated individuals to use expressive suppression as an emotion regulation strategy, as indicated by greater agreement with statements such as "I control my emotions by not expressing them", and this effect was consistent for both children and adolescents (Weissman et al., 2019). In the same study, maltreatment was also positively associated with the extent to which subjects reported responding to feeling sad by ruminating on the causes or

consequences of their sadness. Similarly, among 400 children aged 6-12 years, those who had been exposed to maltreatment were rated lower by blinded camp counsellors in their emotion regulation abilities, including their ability to exhibit socially appropriate emotional displays, empathy, and awareness of their emotional state (Kim and Cicchetti, 2010). In another study, children were present for a scripted interaction between their parent and a research assistant in which their interaction was initially friendly, then angry, then reconciliatory, with periods of assistant absence in between, and then each child's behaviour was classified by blinded researchers as either adaptive, undercontrolled (dysregulated), or overcontrolled (flat) (Maughan and Cicchetti, 2002). Maltreated children exhibited much higher rates of both undercontrolled and overcontrolled behavioural responses to the anger simulation. Impaired emotion regulation, in turn, has been casually implicated in depression. For example, emotion dysregulation, as reflected by a global score on a self-report scale examining multiple domains, was found to partially mediate the relationship between cumulative lifetime adversity and depressive symptomatology in a community sample of 750 adults (Abravanel and Sinha, 2015). Similarly, among 400 adolescents, structural equation modelling revealed that the relationship between stressful life events and depressive symptoms was mediated by maladaptive emotion regulation strategies such as self-blame, catastrophizing, and rumination (Stikkelbroek et al., 2016). Thus, effects of ELS on emotion regulation may be one mechanism by which ELS increases later-life risk for depression.

Finally, ELS has been robustly associated with later-life deficits in general intelligence and academic achievement (De Bellis and Zisk, 2014). In one prospective study of children randomly recruited from the community during preregistration for kindergarten, those who had experienced physical maltreatment prior to kindergarten matriculation went on to have poorer academic attainment in high school, particularly in the language arts, and were less likely to anticipate attending university (Lansford et al., 2002). In another prospective study, although girls who had experienced childhood sexual abuse did not differ from non-abused controls in receptive language ability at an initial assessment in childhood, thereafter abused girls acquired receptive language at a slower rate, with significant differences emerging from mid-adolescence, and they achieved a lower overall proficiency (Noll et al., 2010). Additionally, the highest level of education attained by 18 years after the initial assessment was lower among abused individuals (Noll et al., 2010). Similarly, in a large birth cohort study, a notification to child protective services of suspected maltreatment of any kind was associated with lower performance at age 14 years on a non-verbal test of fluid intelligence

and on a reading test, even after adjustment for a wide range of confounders, including family income, race, birth weight, gender, parental alcohol use, maternal age, maternal marital status, maternal education, and others (Mills et al., 2011).

Lower intelligence, in turn, is a risk factor for subsequent incident psychopathology, including depression, as well as for physical disease and mortality. In a large birth cohort study of New Zealand born males and females, lower average IQ across ages 7, 9, and 11 years predicted higher risk of meeting Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria for major depression and any anxiety disorder at any of ages 18, 21, 26, and 32, and also predicted greater severity of psychopathology, in the form higher psychiatric comorbidity and higher persistence of depression diagnosis across multiple timepoints (Koenen et al., 2009). All these results remained significant even after adjusting for a range of confounders, including childhood socioeconomic status, perinatal insults, birth weight, and childhood maltreatment. Similarly, lower intelligence quotient (IQ) at age of conscription into the Swedish military was associated with a higher risk of incident depression in the 27 subsequent years (Zammit et al., 2004), and in a birth cohort study of Danish males, childhood IQ was inversely associated with risk of any psychiatric illness in adulthood over 200,000 man years of follow-up (Batty et al., 2005). Regarding risk of disease in general, striking inverse relationships between IQ and mortality have been reported (Hemmingsson et al., 2006; Batty et al., 2007), including specifically from physical disease such as cardiovascular disease (Hemmingsson et al., 2006). While the mechanisms behind the inverse association between intelligence and physical health status are numerous and varied (Batty et al., 2007), they are beyond the scope of this discussion: what is clear here is that lower intelligence may partially mediate the broader relationship at hand between ELS and subsequent psychiatric and physical health risk.

1.3.2 Immunological

It is now well known that both acute and chronic stress leads to a range of consequences in the immune system (Miller and Raison, 2016). Psychosocial stress, especially in animal models, has repeatedly been shown to induce innate immune system activation, resulting both in the production of pro-inflammatory molecules usually associated with external threat from a pathogen, such as heat shock proteins, uric acid, and high mobility group box 1, as well as the production of a diverse set of pro-inflammatory cytokines and chemokines (Fleshner, 2013; Miller and Raison, 2016). In humans, even five to ten minutes of preparation and then

ten minutes of public speaking and mental arithmetic causes the activation of key inflammatory signalling pathways inside circulating blood mononuclear cells (Bierhaus et al., 2003). Further, ELS has even been linked in humans with elevated inflammation many years later, in adulthood. It has repeatedly been shown that people who have experienced considerable early life stress have exacerbated immune responses to psychosocial stress in adulthood, with an acute stressor resulting in a larger change in circulating interleukin (IL) 6 (IL-6) than in control subjects (Carpenter et al., 2010; Gouin et al., 2012b). Additionally, a recent large meta-analysis showed that people who were exposed to childhood trauma had significantly elevated circulating IL-6, tumour necrosis factor alpha (TNF-α), and C-reactive protein (CRP), even in the absence of any specific laboratory stressor (Baumeister et al., 2016).

Immune activation, in turn, has now been robustly linked with depression. A sizeable subgroup of patients especially with treatment resistant depression have elevated circulating cytokines (Maes, 1999; Miller and Raison, 2015; Köhler et al., 2017), and there is evidence that depressive symptoms in this group of people respond to targeted anti-inflammatory therapy (Raison et al., 2013). In depressed patients, even without a single-cell approach, whole blood RNA sequencing has demonstrated upregulated expression of Type I interferon signalling genes (Mostafavi et al., 2014), and genetic studies in depression have frequently implicated the immune system (Bufalino et al., 2013; Raison and Miller, 2013). With respect to the possibility that ELS-associated inflammation may in turn predispose to depression, it was recently shown that circulating IL-6 in children aged 9 years old predicts depression at age 18 years old (Khandaker et al., 2014). While this study does not establish causality, particularly because elevated stressor reactivity could potentially play a causal role in both findings, it does nevertheless raise further concern that inflammation may mediate some of the effects of ELS on later-life depression risk.

Finally, for many of the physical health conditions that ELS increases risk of, including peptic ulcer disease, many causes of arthritis, asthma, bronchitis, heart attack, stroke, colitis, and even migraines, immune system over-activity is a central component of their pathogenesis or pathophysiology, and many of these disorders are considered inflammatory conditions (Kumar et al., 2015). With respect to coronary artery disease (CAD), for example, activation of the inflammasome, a protein complex that is a key pro-inflammatory regulator of the innate immune response and which exhibits enhanced activity in response to psychosocial stress (Miller and Raison, 2016), has been implicated in the early stages of

atherosclerosis (Strowig et al., 2012). Further, in patients with advanced CAD, chronic stress has been postulated to worsen myocardial infarction outcomes by induction of stronger inflammatory and thrombotic reactions to atherosclerotic plaque rupture, resulting in more severe artery occlusion (Steptoe and Kivimäki, 2012). Altogether, the effects of ELS on later-life inflammation may play a causal role in the elevated risk of diverse physical health conditions.

1.3.3 Neurobiological

Most of the brain structures that have been reported to have disrupted structure or function in human ELS to date are part of either the conscious or unconscious portion of the brain's threat detection and response circuitry (Teicher et al., 2016). Overall, ELS appears to be associated with relative under-development of regions, pathways, and networks necessary for conscious perception of threating stimuli, in favour of over-development of pathways that facilitate arousal, endocrine, and autonomic responses to threat before or without conscious processing. These changes presumably facilitate physiological stress responses to the particular threat in question that are both faster and occur with lesser conscious distress, and thus are adaptive in the short-term (Teicher et al., 2016). Additionally, ELS has been associated with reduced reward reactivity, which particularly together with the potentiated threat responding, may facilitate reduced approach in favour of avoidance.

In analyses that have focused exclusively on a given type of child abuse, grey and white matter abnormalities have been identified specifically in cortical regions or white matter tracts that process most of the abuse-associated cues. For example, with respect to parental verbal abuse, where most of the abuse-related information is auditory, decreased integrity of the arcuate fasciculus, which connects Wernicke's area to Broca's area, has been reported (Choi et al., 2009). Meanwhile, witnessing parental verbal abuse was associated specifically with reduced cortical thickness in left and right visual association areas, while having no relationship with left or right V1 thickness (Tomoda et al., 2012). With respect to white matter abnormalities, witnessing domestic abuse was specifically associated with decreased integrity of the inferior longitudinal fasciculus, which carries information from the visual cortex to parts of the limbic system, including the hippocampus, and is important in conscious recognition of objects, persons, or faces (Choi et al., 2012). Although childhood sexual abuse has also been associated with reduced visual cortex volume (Tomoda et al., 2009), in another study among 50 medically healthy adult women, childhood sexual abuse

was associated specifically with reduced cortical thickness in the portion of the primary somatosensory cortex that receives input from the genitals (Heim et al., 2013).

The most robust finding from functional imaging in human ELS is an enhanced functional magnetic resonance imaging (fMRI) response of the amygdala to emotional faces (Teicher et al., 2016), and enhanced amygdala response to other stimuli has also been reported, such as in new mothers to crying sounds of their baby (Olsavsky et al., 2021), and to response inhibition (No-Go) trials in the context of emotional stimuli (van Rooij et al., 2020). Enhanced amygdala connectivity to numerous structures has been reported, particularly in task contexts involving some type of threat stimulus. For example, increased positive connectivity of the amygdala has been reported to the supplemental motor area, presumably to facilitate faster motor responding (Olsavsky et al., 2021). Increased positive connectivity has also been reported between the amygdala and the brainstem (van Rooij et al., 2020), including with a locus coeruleus seed (Steuwe et al., 2015), potentially mediating heightened arousal and autonomic effector activity in response to threat, given that the amygdala has indirect and direct projections to the locus coeruleus as well as to autonomic brainstem nuclei (Roozendaal et al., 2009; Ulrich-Lai and Herman, 2009; Waraczynski, 2016; Barman and Yates, 2017; Hennessey et al., 2018). Additionally, increased negative connectivity has been reported to: the ventromedial prefrontal cortex (vmPFC), specifically the subgenual anterior cingulate cortex (sgACC) and medial orbitofrontal cortex (mOFC), possibly reflecting the amygdala suppressing its own inhibition (Teicher et al., 2016; Peverill et al., 2019).

Another commonly reported finding in human ELS is reduced reward reactivity of the ventral striatum. For example, in an adult male sample, cumulative exposure to stressful life events from kindergarten through the conclusion of high school predicted lower monetary reward-induced ventral striatum fMRI activity (Hanson et al., 2016). Among a mixed-gender adolescent sample of mean age 13.5 years who were scanned at baseline and 2 years later, a history of emotional neglect was associated with a decrease from baseline to follow-up in the ventral striatal fMRI response to positive feedback, in a task where subjects were told their performance would determine the amount of monetary reward earned (Hanson et al., 2015). While neither of these analyses controlled for depressive symptomatology, other analyses have done this or have excluded subjects with psychopathology. For example, in a Japanese sample, 16 young adolescents with reactive attachment disorder who had all experienced severe childhood maltreatment were compared to controls, and those exposed to ELS were reported to have lower reward-associated ventral striatum fMRI responses, even after

controlling for depressive symptoms (Takiguchi et al., 2015). Further, in a sample of 160 healthy young adults from a birth cohort, parent-rated family adversity at age 3 months predicted reduced ventral striatal response to reward anticipation but not reward delivery in adulthood, even after controlling for subclinical psychiatric symptoms (Boecker et al., 2014).

Altogether, it seems there are at least two broad possible neurobiological sequelae of ELS in humans that could increase risk for psychopathology. Firstly, it seems that there may be a shift towards more automatic, unconscious responding to threat. In cases of abuse, there is relative under-development of cortical regions and subcortical pathways responsible particularly for conscious processing of threat stimuli. Simultaneously, there is enhanced amygdala activity in response to threat, including enhanced amygdala connectivity to regions involved in behavioural and physiological threat responding, including motor and brainstem regions. Given these findings, there may also be increased efferent amygdala stimulation of the hypothalamic paraventricular nucleus (PVN) in response to threat-associated cues (Sanders and Anticevic, 2007). Additionally, there may be decreased prefrontal ability to inhibit all of these responses. In individuals with these neurobiological alterations, in the face of the ongoing daily exposure to potential threat, including during the numerous social interactions many people must engage in each day, neither sympathetic nervous overactivity with consequent immune overactivation, nor neuroendocrine dysregulation, are unexpected. Both of these consequences, as well as potential cognitive biases resulting from altered processing of threat, could well contribute to the increased risk of depression in ELS subjects, given how each of these disturbances has been causally implicated in at least some cases of depression (Otte et al., 2016). Anxiety, meanwhile, may potentially be fostered by virtue of the influence that amygdala hyperresponsiveness has on vigilance as well as on cognition directly (Newman et al., 2013), via both brainstem mechanisms and altered connectivity with the vmPFC (Myers-Schulz and Koenigs, 2012). Secondly, the reduced ventral striatal responsiveness to anticipated reward that may follow ELS could increase risk for both depression and certain types of substance use disorder (Balodis and Potenza, 2015; Treadway et al., 2019). Indeed, among subjects exposed to ELS, low ventral striatal reactivity predicts both greater anhedonia and, via greater anhedonia, increased use of alcohol specifically as a coping mechanism and increased risk of problematic alcohol use (Corral-Frías et al., 2015). Finally, either enhanced sensitivity to threat or diminished sensitivity to reward, much less both simultaneously, could shift cognitive and behavioural responding towards avoidance over approach (Teicher et al., 2016). While this may be adaptive at the time of ELS exposure,

in later life it may predispose to or perpetuate both anxiety and depression (Chawla and Ostafin, 2007; Trew, 2011).

1.4 Interrogating ELS mechanisms using rodents

1.4.1 Value of pre-clinical experiments

It is important to understand the mechanisms mediating the relationships between ELS and increases in later-life disease risk and severity, so that interventions can be targeted at these mechanisms in the prevention or even treatment of these conditions. However, this is extremely challenging to do in human studies alone, because neglect and abuse are often associated with many other exposures or predispositions which could also play a causal role in these outcomes. For example, household income, race, number and nature of caregivers, parental age, and maternal antenatal stress and inflammation are all factors which could be associated in some samples with both ELS and with poorer health outcomes in later life, and which can be difficult to accurately measure and control for (Koenen et al., 2009; Mills et al., 2011; Teicher et al., 2016; Morin et al., 2020). Additionally, it is possible that ELS can interact with later-life circumstances that are difficult to measure. For example, by altering how individuals respond to their emotions or by altering approach-avoidance evaluations, ELS could alter the way that subjects behave throughout their schooling career in such a way that increases the likelihood of stressful or traumatic exposures and decreases the likelihood of positive, beneficial experiences and relationships (Kirkham and Levita, 2020; Fernández-Teruel, 2021). Further, it is possible that that are certain behavioural tendencies or psychological traits that both increase risk of being exposed to neglect or abuse and that increase risk of adverse later-life health outcomes (Morin et al., 2020). Additional limitations of existing human studies include the fact that a high proportion of them: have small sample sizes, increasing the risk of spurious false positives; are cross-sectional rather than longitudinal, making causality very difficult to infer; and rely on retrospective self-report measures of maltreatment, which is more sensitive than using notifications to child protective authorities but also more vulnerable to false positives (Teicher et al., 2016).

Rodent studies, however, circumvent all these challenges. They are precisely controlled, so that the effects of ELS exposure alone can be examined. Sample sizes can easily be scaled to achieve sufficient statistical power. Prospective studies can be conducted to yield information regarding the timing of the emergence of ELS effects and to provide better windows into causality. The exposure status of subjects is known with certainty.

Additionally, tissues such as the brain can easily be collected for molecular and cellular interrogation of mechanisms, which is only rarely possible in humans, and which has serious limitations even where it is possible. Study at this molecular and cellular level is important, given that this is the level at pharmacological agents potentially useful in prevention or treatment of ELS consequences must act. The elucidation of the molecular consequences of ELS may thus result in the development of novel, targeted pharmacological agents, or the repurposing of existing agents for use following ELS. Additionally, once a rodent paradigm has been characterised with respect to outcomes of interest, be they behavioural, immunological, or neurobiological, further rodent studies offer the ability to easily test interventions, be they pharmacological or non-pharmacological, for beneficial effects on those outcomes.

1.4.2 Validity of pre-clinical experiments

Nevertheless, rodent studies have limitations of their own, and the central challenge is that rodent paradigms can only ever approximate a particular human condition, for the simple fact that rodents are not human. The accuracy of that approximation can range from poor to good, depending on what precisely is being modelled and how, and this accuracy is what is referred to as the validity of the model (Geyer and Markou, 1995). In this context, the human condition that is being modelled can refer either to a diagnosable mental illness, such as major depression, or to the state following a particular exposure, such as ELS, even if that state does not involve a diagnosable mental illness (Tractenberg et al., 2016; Vannan et al., 2018). In order to draw attention to several important facets of its assessment, validity has been subdivided into etiological validity, face validity, construct validity, and predictive validity (McArthur and Borsini, 2006; Nestler and Hyman, 2010; Powell et al., 2012). Etiological validity refers to the degree to which the manipulation used to induce the model mirrors the causes of the condition in humans, which can obviously only be evaluated to the extent that those causes are known. For example, some rodent models of depression depend on the application of chronic stress, which adds to the etiological validity of these models because chronic stress is a well-established etiological factor in the development of many cases of depression (Otte et al., 2016). Face validity refers to the degree to which, superficially ("on the face of it" or "at face value"), the behavioural changes induced by the model appear to mirror the behavioural features of the human condition. For example, if a rodent model of depression resulted in reduced effort expended to achieve sucrose reward on a progressive ratio task, this would add to its face validity given that anhedonia is a central

symptom of depression in humans and that depression has been associated with reduced effort expended for reward on similar tasks (Der-Avakian and Markou, 2012; American Psychiatric Association, 2013). Construct validity refers to the degree to which the biological changes induced by the model appear to mirror those in the human condition. For example, if a rodent model of depression caused hippocampal atrophy, this would add to its construct validity given that the same change has been extensively associated with human depression (Schmaal et al., 2016). Predictive validity refers to the degree to which, after model induction, additional behavioural or biological changes induced by subsequent manipulations are predictive of changes in analogous parameters in humans in response to the same intervention. For example, in a rat model of depression, if serotonergic antidepressants caused recovery of model-induced deficits on several behavioural tests of anhedonia over 3-4 weeks, but not within hours, the model and corresponding test would be said to together have predictive validity, given that this time course is typical of antidepressant action in humans (Nestler and Hyman, 2010).

1.4.3 Repeated maternal separation

1.4.3.1 Common and variable protocol features

Systematic and comprehensive reviews have demonstrated that the most widely used animal model for investigating the relationships between ELS and its psychiatric and physical consequences is repeated maternal separation (RMS) (Van Bodegom et al., 2017; Orso et al., 2019). This procedure involves repeatedly separating rat or mouse pups from their mother each day, most commonly for 3-6 hours per day and beginning on either post-natal day (PND) 1 or 2 and continuing through either PND 14 or 21. During these limited periods of separation from their mother, around 35-40% of studies also separate pups from one another; this combined separation is sometimes referred to as early deprivation (Pryce and Feldon, 2003). Other aspects of the protocol are less consistent across studies, such as animal gender, species, and strain, whether pups are kept in the home cage or a novel cage during separation, whether pups are warmed or not warmed during separation, whether separation occurs during the light or dark cycle, and the extent to which control animals are handled or not handled during early life (Nylander and Roman, 2013; Tractenberg et al., 2016).

1.4.3.2 Validity of RMS

RMS arguably has good etiological validity. In humans, neglect is by far the most common cause of documented childhood maltreatment (U.S. Department of Health and

Human Services, 2021), and refers to the situation in which children's basic emotional, physical, or educational needs are unmet (Sedlak et al., 2010). Similarly, in RMS, for several hours each day, animals' physical and emotional needs go unmet. Pups are deprived of the supply of milk and close thermoregulation that they normally receive from their mother, which she ordinarily provides in response to their signalling (Blumberg et al., 1992; Tractenberg et al., 2016). This signalling includes ultrasonic vocalization calls that indicate distress, and thus RMS involves at least one type of emotional deprivation, in that pups' distress goes unanswered by their mother (Burenkova et al., 2020). Adding a final element to the etiological validity of RMS is its timing: it occurs in a period of early life in rodents considered to be analogous to early childhood in humans (Semple et al., 2013).

RMS is considered to have construct validity (McArthur and Borsini, 2006) as an analogue of human ELS because it has repeatedly been shown to be stressful for pups, as reflected by the ability of separation episodes to acutely increase corticosterone (Levine et al., 1991; Schmidt et al., 2004; Schmidt et al., 2006; Enthoven et al., 2008; Daskalakis et al., 2011).

The face validity of RMS depends on what specifically one is trying to model. For example, with respect to depressive symptoms, many investigators have found the RMS alone does not cause a long-lasting anhedonia (Shalev and Kafkafi, 2002; Zhang et al., 2013; Harrison et al., 2014; Hill et al., 2014; Chocyk et al., 2015; Feldcamp et al., 2016; Gracia-Rubio et al., 2016; Mrdalj et al., 2016; Borges-Aguiar et al., 2018; Wei et al., 2018; Stuart et al., 2019). Yet, in humans, ELS is significantly associated with depression symptoms, diagnosis, and severity in later-life (Danese et al., 2009; Wright et al., 2009; Spinhoven et al., 2010). Thus, if the goal is to model depression in which ELS is a causal factor, RMS with measurement in adulthood but without further stress does not have face validity, while RMS followed by chronic stressors may or may not (Zhang et al., 2013; Shu et al., 2015; Mrdalj et al., 2016; Wei et al., 2018; Huang et al., 2021). However, if the goal is to model ELS alone, for example in an attempt to separate the effects of ELS that depend on exposure to further stressors or other events from those that do not, there is little evidence either in support of or against the hypothesis that RMS has face validity for this purpose. This is because the analogous situation in humans is essentially not possible to study, given that adult humans routinely encounter stressors (Gouin et al., 2012a). Thus, where findings are absent in RMS in adulthood without further stress, but seemingly present in human ELS, this may usefully suggest that those consequences depend on further exposures, and that analogues of those

exposures may need to be included in preclinical study protocols if those outcomes are desired. Equally useful, where there is overlap in the significant findings between RMS and human ELS, this adds to the face validity for RMS as a model for human ELS, and also provides evidence that these outcomes may not depend on further exposures.

While predictive validity is often discussed in relation to the efficacy of pharmacological agents in reversing detrimental features of rodent models, in fact it refers more broadly to whether the effects of any additional intervention in the model mirror the effects of that intervention in humans (Geyer and Markou, 1995; McArthur and Borsini, 2006; Powell et al., 2012). Thus, whether the effects of later-life stress are common to both RMS and human ELS provides insight into the predictive validity of RMS. Regarding the hypothalamic-pituitary-adrenal (HPA) axis, laboratory stressors in later life appear to result in relatively blunted cortisol responses in humans exposed to ELS (Carpenter et al., 2007; Elzinga et al., 2008; Carpenter et al., 2009), but potentiated responses in RMS, per a comprehensive review (Van Bodegom et al., 2017). However, most of the RMS studies have occurred in the absence of later-life stress, which is unusual in humans, in whom ongoing corticolimbic stress system activation, which is likely to be higher in humans with ELS exposure, is known to blunt HPA axis reactivity (Roos et al., 2018; Lam et al., 2019; Schmalbach et al., 2020; Zhang et al., 2020). Thus, RMS specifically with further chronic stress may have better predictive validity for responses to acute stress than RMS without further chronic stress, at least with respect to the neuroimmune axis.

1.4.3.3 The role of RMS protocol variability

Systematic (Tractenberg et al., 2016; Dutcher et al., 2020) and comprehensive (Van Bodegom et al., 2017) reviews have revealed highly variable behavioural, immunological, and neurobiological outcomes following RMS. However, when findings are stratified depending on key aspects of study methodology, patterns emerge.

Dutcher et al. (2020) revealed that with respect to immune outcomes, three factors are highly influential in determining whether or not RMS is likely to be shown to have a significant effect on cytokine levels: (1) whether measurement occurs during or shortly following RMS as opposed to later in life, e.g. in adulthood, (2) if measurement occurs in later life, whether it occurs in the context of a second stressor or not, and (3) whether the measurement was of blood or non-blood cytokine levels. Once these factors were considered, consistency emerged from seemingly inconsistent findings. It was revealed that when

measurements are conducted in non-blood tissue, RMS animals have higher pro-inflammatory cytokine levels than controls, but only when measurement is in the context of a recent stressor, be it RMS itself or a second stressor in later-life. Simultaneously, when measurement was conducted in blood, the timing or stress context of that measurement had no impact: there were consistent findings between studies of no effect of RMS in the short-term and long-term with further stress. This consistency was observed despite widely variable RMS methods, including in the species (57% rat, 43% mouse) and gender (59% male, 26% male and female, 9% unclear, 7% female) used, and widely variable second stress methods, with 28 different stressors or stressor combinations. Taking tumour necrosis factor alpha (TNF-a) as an example case, among nine studies that all reported increased non-blood TNF-a in adulthood in the context of later-life stress: three different control paradigms were used involving levels of handling ranging from never-handled through to daily handling, daily separation duration ranged from three to six hours, five different lengths of RMS in days were used, five different strains were used across two species, positive findings were identified individually in each gender in at least one case, pups were separated from one another in two cases, ambient temperature during RMS ranged from 21 to 37 ℃, and eight different later-life stressors were used. Thus, with respect to immune outcomes, inter-study variability in aspects of the RMS protocol appeared to be of little significance relative to variability in outcome measurement.

With respect to neuroendocrine outcomes, it is clear that RMS increases plasma corticosterone in the short-term (Levine et al., 1991; Schmidt et al., 2004; Schmidt et al., 2006; Enthoven et al., 2008; Daskalakis et al., 2011; Horii-Hayashi et al., 2013). In the long-term, again, where measurement timing and stress context are taken into account, there is a high degree of consistency. When measured in adulthood following diverse acute stressors, 62% of 13 findings in one review showed an increase in plasma corticosterone, including from multiple rodent strains and species and varying RMS protocols, while 38% found no significant effect (Van Bodegom et al., 2017). When measured in adulthood without acute stress, 63% of 16 findings reported no significant effect, while 31% reported an increase, again with no clear dependence on strain or species. Thus, for the neuroendocrine axis, as for the neuroimmune axis, RMS appears to result in a long-lasting sensitization for later-life stress, and aspects of measurement appeared more influential than aspects of the RMS protocol.

With respect to behavioural outcomes, while systematic reviews of RMS have been conducted (Tractenberg et al., 2016; Bonapersona et al., 2019), to date, none have stratified results by time since RMS and later-life stress context in their analyses. Unsurprisingly, they reveal highly inconsistent findings and do not draw firm conclusions. However, in my own search of the literature, I identified five articles examining the short-term effects of RMS on the sucrose preference test (SPT), of which four (80%) reported statistically significant anhedonia in at least one gender, while one reported no effect. Among the individual experiments with positive findings, daily separation duration ranged from two to eight hours, control group handling ranged from unhandled to handled once weekly as part of normal animal facility rearing, significant effects were present in each gender in at least two experiments, RMS was conducted during the light cycle in most cases but the dark cycle in one case, no external heat was provided during RMS in one case, and five strains were used across two species. Regarding the long-term effects of RMS on the SPT, I found 14 studies, of which 11 (79%) reported no long-term effect, with only 2 (14%) reporting a long-lasting anhedonia. Thus, for behavioural outcomes, as for immunological and neuroendocrine outcomes, measurement factors appear far more consequential than the particulars of the RMS protocol. Findings in the context of later-life chronic stressors that could be expected to induce anhedonia are comparatively mixed. For example, following 3-4 weeks of chronic unpredictable stress, several experiments have reported no effect of RMS on sucrose preference in females (Wei et al., 2018; Huang et al., 2021) or males (Mrdalj et al., 2016; Wei et al., 2018), but decreased (Zhang et al., 2013) and increased (Shu et al., 2015) preference has also been reported in males.

Altogether, in the short-term, RMS appears to cause: pro-inflammatory signalling that is detectable in non-blood tissue, hypothalamic-pituitary-adrenal activation, and anhedonia. In the long-term without further stress, the majority of findings suggest that RMS has no effect on any of these three outcomes. When further acute or chronic stress is applied, latent sensitizing effects of RMS are revealed on the neuroimmune and neuroendocrine systems. To date there is little evidence for behavioural sensitization with respect to anhedonia. Where positive findings have been reported on these outcomes, features of the RMS protocols used vary widely, suggesting that it is the common features (i.e. separation from the dam for at least two hours a day for at least 13 days, always at least encompassing PND 5-14) that are responsible for these outcomes.

1.4.4 Alternative ELS paradigms

There are three other ELS paradigms that have found at least moderate use: maternal deprivation, early social deprivation, and limited nesting.

Maternal deprivation (MD) consists of a single, 24-hour episode of maternal separation, representing an extreme but acute early life stressor. Investigators have found utility in the use of MD for studying neuroendocrine function (Schmidt et al., 2011), and some believe it to be useful in studying the relationship between early life stress and elements of schizophrenia (Ellenbroek and Riva, 2003). Although there have been reports of depressive-like behaviour resulting from this model (Marco et al., 2009), these appear largely confined to tests like the forced swim test, which has limited validity for studying depression (Nestler and Hyman, 2010; Liu et al., 2018), and there are many negative findings regarding the presence of other long-term behavioural effects (Schmidt et al., 2011).

In early social deprivation, also known as isolation rearing, animals are isolation housed from the time of weaning or shortly after (Burke et al., 2017). While the directionality of effects has not always been consistent, many studies have reported effects of early social deprivation on locomotor activity and response to subsequent social interaction, with reports particularly of increased anxiety or avoidance in social situations and increased aggression (McArthur and Borsini, 2006; Burke et al., 2017). Long-term measurements of supposed depression-like behaviour, particularly using the forced swim test, have often been negative (Andersen, 2015; Burke et al., 2017).

Limited nesting, also known as limited bedding or limited nesting/bedding, has become commonly used only recently (Van Bodegom et al., 2017). In this paradigm, only a small amount of nesting and bedding material are provided in the home cage for the dam, resulting in most of the cage floor consisting of a metal grid (Rice et al., 2008). This represents a chronic stress for the dam and results in disrupted maternal care, representing in turn a chronic stress for the pups (Ivy et al., 2008; Rice et al., 2008). This paradigm results in very different developmental trajectories in pup distress to RMS, as measured by ultrasonic vocalizations, and seems to cause greater distress in females, while RMS may be more stressful for males (Granata et al., 2021).

While all these paradigms represent important tools in the study of ELS, they are each much less studied than RMS, and their relation to RMS in terms of their endocrine, neurocognitive, and immunological consequences is poorly characterised at present. As this

thesis is intended to advance knowledge regarding the use of the promising RMS paradigm for the study of the long-lasting consequences of ELS, I will focus discussions throughout this thesis on that model.

1.4.4.1 The role of later-life stressors

It has become increasingly clear, particularly over the past decade, that while RMS has diverse short-term effects, including behavioural (Veenema et al., 2008; Maniam and Morris, 2010a, 2010b), immunological (Dutcher et al., 2020), and neuroendocrine (Daskalakis et al., 2013), measurements in adulthood very often yield negative results, unless they were conducted in the context of later-life stress (Van Bodegom et al., 2017; Dutcher et al., 2020). This realisation has led to the conduct of many so-called "two-hit" or "second-hit" studies, which have been invaluable in elucidating the long-lasting effects of RMS. These studies involve both an early life stressor, in the form of RMS, and some form of later-life stressor, be it a psychosocial stressor such as the chronic unpredictable stress paradigm (Zhang et al., 2013; Shu et al., 2015; Minami et al., 2017; Wei et al., 2018), or mixed physiological-psychosocial stressors such as lipopolysaccharide (Zajdel et al., 2019), polyinosinic:polycytidylic acid, also known as poly(I:C) (Viola et al., 2019), chronic corticosterone (Hill et al., 2014), and food restriction (Ganguly et al., 2018).

The type of second stress required for a long-lasting effect of RMS to be unmasked may depend on the type of measurement in question. For immunological measurements, where even brief psychological stress can elicit a potent pro-inflammatory immune response in both humans (Bierhaus et al., 2003; Pace et al., 2006; Carpenter et al., 2010; Janusek et al., 2017) and rodents (Deak et al., 2005; Maslanik et al., 2013; Weber et al., 2015; Roque et al., 2016), relatively brief stressors may be sufficient to reveal an RMS-induced neuroimmune sensitisation (Tang et al., 2017; Ganguly et al., 2019), although greater stressor duration and intensity will likely increase sensitivity. For anhedonia, however, which usually requires 2-4 weeks of chronic stress to develop in rodents (Stedenfeld et al., 2011; Erburu et al., 2015; Meng et al., 2016; Papp et al., 2016; Gao et al., 2017; Gottschalk et al., 2018), it may be that a chronic stressor of equal intensity to chronic unpredictable stress, social defeat stress, or chronic footshock stress would be required to reveal a greater susceptibility to stress-induced anhedonia in RMS animals, if such a susceptibility exists.

1.4.5 Behavioural tests

Below, I provide some background information regarding tasks used to measure sensitivity to positive and negative feedback and anhedonia in rodents, including information regarding their translational relevance.

1.4.5.1 Probabilistic reversal learning

The probabilistic reversal learning (PRL) task is a computerised task that can be implemented in humans or animals and that is capable of simultaneously measuring many different aspects of behaviour and cognition. There are two broad versions of the task: a spatial version, and a visual version. In both versions, on each trial, subjects are presented with two stimuli and must select one. At any given time, one stimulus is the "correct" stimulus and one is the "incorrect" stimulus. In the spatial PRL task, the stimuli are visually identical and differ only in their spatial location (e.g. left vs right). In the visual version, the stimuli are visually distinct (e.g. circle vs square), and their location is randomised or balanced across the possible spatial locations across trials. The correct stimulus typically has a 70% or 80% probability of reward, while the incorrect stimulus has a 30% or 20% probability of non-reward or other punishment. After a certain number of sequential responses on the correct target (or, in some versions of the task, a certain number of trials), a "reversal" occurs: the previously-correct target becomes the incorrect target, and vice versa. In human versions of the task, participants typically accumulate either virtual money or points, but often are not actually provided with tangible reward (e.g. physical money) based on their performance. Animal versions of the task are run within an operant chamber using either a touchscreen or levers, typically use a sucrose pellet reward.

The PRL task is often used particularly for the purpose of providing insight into subjects' sensitivity to punishment and reward (Rygula et al., 2015; Phillips et al., 2018; Mukherjee et al., 2020; Roberts et al., 2020). In the context of this task, "sensitivity" is defined as the influence that reward or punishment have on an animal's future decision making. Thus, reward sensitivity refers to the degree to which receiving a reward after taking an action increases the future likelihood of taking that same action again. Punishment sensitivity refers to the degree to which not receiving a reward after taking an action decreases the future likelihood of taking that same action again. The terms "negative outcome", "negative feedback", and "punishment" are used interchangeably to refer to the undesirable potential

outcome of each decision, i.e. signalled non-reward, while the terms “positive outcome” and “positive feedback” refer to the desirable outcome, i.e. reward.

In addition to punishment and reward sensitivity, the PRL task simultaneously measures several other distinct aspects of behaviour. Because subjects are free to complete more trials than all but the most motivated can complete within a given session, trial count provides an index of motivation for reward. Because, in order to maximise reward, subjects must repeatedly learn and unlearn which of the two stimuli yields the higher probability of reward, and because reversals follow eight consecutive responses on the same target, both reversal count and perseverative responses per reversal provide indices of behavioural flexibility (Izquierdo et al., 2017; Roberts et al., 2020). Because, after having made a selection resulting in reward, animals must physically move to the sucrose pellet magazine, the latency between response selection and reward collection on rewarded trials provides an index of locomotor functioning. And because decision latencies are measured, and can be compared to task performance, one can make inferences regarding decisional efficiency, which is reduced in cases of impaired attentional control (Eysenck and Derakshan, 2011; Berggren and Derakshan, 2013).

1.4.5.2 Sucrose preference

In the sucrose preference test, animals are first habituated to the taste of sucrose solution and to the test chambers, and then ultimately are offered two drink bottles: one containing water, and one containing a sweet solution of sucrose in water (Liu et al., 2018). Normal animals display a strong preference for the sucrose solution, while, per systematic reviews, animals that have been subjected to recent or ongoing chronic stress commonly exhibit a preference closer to 50:50 (Vasconcelos et al., 2015; Antoniuk et al., 2019). Indeed, a recent systematic review affirmed the responsiveness of the SPT to chronic stress, finding over seven dozen experiments using the chronic unpredictable stress paradigm that have reported a depressive-like effect on the test (Antoniuk et al., 2019). The meta-analytic weighted estimate of Cohen’s d for the effect of CUS on sucrose preference was below -1.70 for all five strains across both species examined (rats and mice), representing an extremely large effect (Cohen, 1988; Lakens, 2013). Because preference for the sucrose solution is necessarily driven by the reinforcing, hedonic properties of sucrose, and because anhedonia, defined as decreased pleasure from, interest in, or engagement in previously-enjoyable activities (Der-Avakian and Markou, 2012), is a core symptom of depression (American Psychiatric Association, 2013), reduced sucrose preference is widely considered to represent anhedonia and thus “depression-

like behaviour" (Nestler and Hyman, 2010; Andersen, 2015; Liu et al., 2018). However, the validity of the sucrose preference test has been called into question by some authors (Matthews et al., 1995; Stuart et al., 2019). Two main arguments have been put forth.

Firstly, it has been claimed that supposedly analogous studies in humans with depression have not borne out a similar deficit. However, a close examination reveals that the measurements used in these studies cannot be considered analogous to the sucrose preference test. In the human paradigm in question, sometimes called the sweet taste test, depressed and control subjects hold sucrose solutions of varying concentrations in their mouth for a brief period and then spit the solution out (Amsterdam et al., 1987; Berlin et al., 1998; Swiecicki et al., 2009; Dichter et al., 2010). Participants then provide a subjective rating, typically on a visual scale, of their "hedonic response", i.e. how "pleasant" the taste was or how much they "liked" or "disliked" the taste. Sometimes "intensity" is also measured, for which subjects are asked to rate how "sweet" the taste was or how "weak" or "strong" it was.

These studies have generally reported that depressed and MDD patients do not differ in their "hedonic response" to the various sucrose solutions (Berlin et al., 1998; Swiecicki et al., 2009; Dichter et al., 2010) or in perceived intensity (Swiecicki et al., 2009; Dichter et al., 2010). However, these studies have had small sample sizes, at only 12-20 depressed subjects, and there have been other reports that depressed patients perceive sucrose solutions as having a "weaker" taste than controls, particularly at higher concentrations (Amsterdam et al., 1987), and that a higher concentration of sucrose solution is required before they report detecting a sweet taste (Berlin et al., 1998), although other investigators have not replicated this finding (Swiecicki et al., 2009). Additionally, concerns have been raised that due to high inter-individual variability in taste sensitivity in the general population, the statistical power of these studies to detect group differences may be low (Treadway and Zald, 2011).

Regardless of the true nature of differences between depressed and control subjects in their subjective assessment of the taste of sweet solutions, these findings have no bearing on the question of the validity of the SPT, because the SPT differs from the sweet taste test in several fundamental ways. In the sweet taste test, depressed and control subjects taste an essentially identical volume of sucrose for an identical duration, and that volume and duration is under the control of the experimenters, not the participants. There is no consumption, and the act of tasting the solution within the experimental setup is not a voluntary, goal-directed action driven by the reinforcing properties of the solution. In contrast, sucrose consumption on the SPT is not inevitable: it is voluntary, goal-directed, and reinforcer-driven. In order to

have a sucrose preference above 50%, animals must learn which spatial location contains the sweet solution, repeatedly move to that bottle preferentially, and then repeatedly lick at that bottle throughout the 1-24 hour test period. They are free to do these things as much as they like during the test period to obtain however much reward they wish. For this reason, contrary to some descriptions of the SPT as simply capturing "consummatory anhedonia" or decreased "liking", defined as decreased pleasure experienced in response to a rewarding stimulus, SPT performance must necessarily also capture "motivational anhedonia" or decreased "wanting", defined as reduced willingness or motivation to take action to obtain a reward (Berridge, 2007; Treadway and Zald, 2011).

To my knowledge, no study to date has been conducted in humans with depression where drive to obtain a food reward has been directly measured in a laboratory setting. However, a large subgroup of depressed subjects report appetite loss, which necessarily involves less action taken to consume food, and thus potentially reflects so-called motivational anhedonia. The median reported prevalence of appetite loss in depressed subjects across multiple studies is 48% in adults and 58% in adolescents, with 32-33% of both populations also reporting depression-associated weight loss (Maxwell and Cole, 2009). Indeed, among a large sample of MDD patients, anhedonia has been shown to be inversely associated with appetite and weight gain (Buckner et al., 2008). Together with the high prevalence of self-reported anhedonia in depression and the extensive evidence that depressed patients are less willing to take action to obtain monetary reward (Hughes et al., 1985; Treadway et al., 2009; Treadway et al., 2012; Yang et al., 2014; Hershenberg et al., 2016), the arguments that the SPT has face, construct, etiological, and predictive validity are strong (Nestler and Hyman, 2010; Powell et al., 2012).

The other concern that has been raised about the sucrose preference test, and by extension tests such as the progressive ratio test and trial count on probabilistic reversal learning, is that some of the interventions classically used to induce reduced drive to consume sucrose, such as chronic unpredictable stress, also often cause weight loss, which may in turn lead to lower bodily energy demands and thus lower motivation for sucrose consumption (Matthews et al., 1995). However, it is possible that motivational anhedonia is the proximate cause of both deficits. Weight loss in animals subjected to chronic stress seems to be driven at least in part by reduced food intake (Martí et al., 1994; Martí et al., 1999; Harris et al., 2002; Dagytė et al., 2010; Varga et al., 2011; Jeong et al., 2013; Merali et al., 2013; Pastor-Ciurana et al., 2014; Harris, 2015), or in other words, reduced volitional actions directed at consuming

reinforcing substances. Thus, although it has been pointed out that normalisation of sucrose consumption to body weight can nullify otherwise-apparent sucrose consumption differences between groups, in doing so, one may in essence be dividing the anhedonia signal by itself.

1.4.5.3 Progressive ratio

In progressive ratio (PR) schedules of reinforcement, animal or human subjects are able to repeatedly respond on a button, lever, or touchscreen stimulus in order to earn a reward (Hailwood et al., 2018). Each time a reward is earned, the number of responses required to earn another reward increases, i.e. the ratio of effort to reward increases. The session is terminated if the session time limit is reached or if subjects fail to make any response for a given period of time. The final number of responses successfully completed to earn one reward is known as the "breakpoint" and serves as an index of motivation for appetitive reward (Salamone et al., 2016).

Although it has only been conducted in humans with depression using monetary rather than food reward, unlike the SPT, the progression ratio (PR) task has been studied as an index of motivational anhedonia in human depression in two articles. In the earlier study, involving only 6 inpatient participants, found that the 3 patients who responded to treatment showed concurrent increases from baseline in their willingness to work for reward on the PR task, while the 3 non-responders did not (Hughes et al., 1985). The more recent study involved almost 100 total participants, half with an active depressive episode as part of either a unipolar or bipolar depressive disorder, and found that both types of depressed individuals had lower breakpoints on PR than controls (Hershenberg et al., 2016).

1.4.6 Immune phenotyping

The immune system is extremely multifaceted and complex, consisting of dozens of distinct cell types and hundreds of identified signalling proteins (Opp, 2016). Cytokines are proteins that are secreted by immune or non-immune cells which act to facilitate immune system functioning, typically by communicating information to immune cells (Murphy and Weaver, 2016). Chemokines are similar to cytokines in that they are proteins that act as signalling molecules, but their primary function is as a chemoattractant, i.e. a molecule that signals immune cells to migrate to a particular tissue location. The most common way at present to assess the effects of interventions on the immune system, or the relationship between variables such as stress or behaviour and the immune system, is to quantify cytokines. In humans, cytokines are typically quantified in the blood or cerebrospinal fluid

(CSF), while in animals, they are commonly quantified in both blood and non-blood tissue because sampling of such tissue is routinely possible (Dutcher et al., 2020). In blood, protein levels are usually quantified, while in non-blood tissue, both protein and mRNA quantitation are often used (Dutcher et al., 2020).

Where protein levels are quantified, this is commonly performed using enzyme-linked immune-sorbent assays (ELISAs). In the "sandwich" version of the ELISA, the tissue of interest is homogenised, centrifuged, and then the supernatant is added to a plate well that has been coated with a capture antibody specific to the protein of interest (Abcam, 2021). Then, another specific antibody, the detection antibody is added. Either the detection antibody or a further "secondary" antibody is associated at manufacture with a unique enzyme, so that when an additional reagent is added, the enzyme initiates a chemical reaction, converting the substance to one with colour or fluorescence, which can then be measured with a spectrometer. More recently, new but related technologies have been developed, such as the MAGPIX system by Luminex. This system can be thought of as a sophisticated fluorescence-based sandwich ELISA in which, rather than capture antibodies coating a plate well, they coat microscopic magnetic beads (Luminex Corporation, 2021; R&D Systems, Inc., 2021). The detection antibody is linked to streptavidin-phycoerythrin, and thus fluoresces upon excitation with a laser, allowing quantification. Unlike an ELISA, which can usually only measure a single analyte in a given well, the MAGPIX system is capable of measuring up to 50 analytes at once in a single well (called "multiplexing"), because many different types of beads can be used, each with differing fluorescence properties when excited by a second laser. Thus, when the detection signal is measured from a given bead by the first laser, the second laser is used to identify the bead type, and thus identify the analyte that the bead's capture antibodies are specific to.

1.4.7 Brain magnetic resonance imaging

The final category of measurement techniques I will discuss is magnetic resonance imaging (MRI). The basic principles of MRI are as follows (Plewes and Kucharczyk, 2012; Jenkinson and Chappell, 2018). Atomic nuclei have a property called spin, which describes a rotation about an axis, and along that axis each nucleus has an extremely weak magnetic field. In an MRI scanner, a large coil made of superconducting wire creates a strong magnetic field in a given direction within and parallel to the scanner bore, which causes the spin axis of atomic nuclei to rotate ("precess") very tightly around the axis of the strong magnetic field.

Then, a separate wire coil, called the transmission coil, emits a radiofrequency (RF) pulse at precisely the frequency at which the hydrogen nuclei (i.e. protons) spins are precessing about the axis of the strong magnetic field. This RF pulse causes hydrogen nuclei to become "excited", causing the precession vector to reverse direction, such that with a sufficiently strong RF pulse, atomic nuclei that were precessing around a vector from the feet to the head of a human subject (for example) will then precess around a vector from the head to the feet. One key effect of this is that the longitudinal magnetization, representing the measurable summed magnetic field created by all the hydrogen nuclei spinning in almost the same direction, is reduced by the RF pulse. The amount of time it takes to return to normal (referred to as T1) in a given tissue location under the influence of the strong magnetic field depends on how quickly the protons lose that gained energy via interaction with neighbouring molecules, which depends on the tissue-specific molecular composition. Secondly, initially, all excited hydrogen nuclei will have the same phase, i.e. their spin axes will point in the same direction. However, interaction with nearby molecules causes dephasing, and the time it takes for this to complete is measured and referred to as T2. T1 and T2 are influenced by distinct molecular properties of tissue, and thus a scanning protocol designed to emphasise one piece of information or the other will provide unique tissue contrasts.

Precise square or rectangular locations (voxels) can be imaged through the use of additional coils called gradient coils. Three gradient coils cause the strength of the strong magnetic field to vary slightly along each of three perpendicular axes. Because the precession frequency varies with the strength of the strong magnetic field, and because measured signals from hydrogen atoms have a frequency that is the same as their precession frequency, Fourier transforms can be used to isolate the signal from specific spatial locations by isolating specific frequencies. Alternatively, location information can be encoded by systematically varying the phase of precession across a plane, creating a phase gradient rather than a frequency gradient.

In addition to the standard T1 and T2 acquisition sequences, other contrasts can be obtained. One of the mechanisms that is thought to contribute to T1 (longitudinal) and T2 (transverse) relaxation is interaction between the magnetic dipoles of different nuclei, and a specific subset of these are interactions between the protons in water molecules and the protons in macromolecules. (Wolff and Balaban, 1994). The signal from protons contained within macromolecules contributes negligibly to standard structural sequences because the T2 relaxation time of those protons is relatively short compared to those of protons in water

molecules (Henkelman et al., 2001). However, using an off-resonance RF pulse, the spins of macromolecular protons can be preferentially targeted such that they then exhibit very large impact on the spins of neighbouring water protons through dipole-dipole interactions, i.e. the effects of their magnetization are “transferred” (Henkelman et al., 2001). Images obtained using this technique are thus weighted by macromolecular content and provide another unique tissue contrast, called magnetization transfer (MT) (Wolff and Balaban, 1994; Henkelman et al., 2001). An additional contrast, called proton density (PD), simply minimises the influence of T1 and T2 relaxation on the signal, and thereby provides an image that largely reflects just the density of hydrogen nuclei rather than their interactions with neighbouring molecules (Feger and Drew, 2021). In my experience, concerning MRI brain scans in rats, the MT sequence provides the strongest contrast between grey matter, white matter, and CSF, among the MT, T1, and PD sequences.

Once images are acquired, they can then be analysed. For volumetric analysis, i.e. quantitation of the volumes of brain regions, a series of steps are required (Jenkinson and Chappell, 2018). Here, a simplified overview of this processes is provided. First, a study template image is generated, essentially by overlaying each subject’s scans with one another such that the brains are aligned across all of them, and then taking an average of all of the images, and iteratively repeating this process. Then, a spatial transform is identified for each scan by moving, enlarging or shrinking, and distorting the raw image so that image features such as visible boundaries between structures best align with the study-average template image. The next steps involve the use of a brain atlas, which consists of a collection of “masks”, with one for each region of interest (ROI), where voxels have a value of one if they refer to the region and zero if they do not. An atlas always comes with an accompanying brain scan image. A transformation is identified to make the atlas’s brain scan image fit to the study template, and this transformation is then used to move the atlas itself into alignment with the study template. Once good alignment between the atlas and the study template has been achieved, specific ROI masks can be moved into alignment with the raw scans for each subject, by reversing the transformations identified earlier between these raw scans and the study template. Once ROI masks are in subject space, their volume can then finally be quantified.

1.5 Thesis objectives and overview

The overarching aim of this thesis, encapsulating the chapter-specific aims, is to augment the scientific understanding of what RMS does to behaviour, the immune system, and the brain.

In Chapter 2, I describe general methods that are relevant to an experiment involving a large cohort of RMS animals that were studied in Chapters 3, 4, and 5.

In Chapter 3, I aim to describe the long-lasting behavioural effects of RMS on novel measurements of possible relevance to anxiety and depressive disorders. I hypothesised that RMS would increase sensitivity to negative feedback and slow responding on the probabilistic reversal learning task. I predicted that a novel subchronic stressor in adulthood would exacerbate pre-existing effects of RMS on this task.

In Chapter 4, I aim to describe the short- and long-term effects of RMS on inflammatory signalling in the blood and in non-blood tissue. I address these questions first using measurements in my own animals, and then more robustly and broadly by conducting a systematic review of the literature. I hypothesised that RMS would increase pro-inflammatory signalling in the blood and non-blood tissue in the presence of recent stress, but not in the absence of it.

In Chapter 5, I aim to describe the short- and long-term effects of RMS on regional brain volumes as measured using magnetic resonance imaging. I hypothesised that RMS would cause enlargement of the amygdala from the time of RMS through to late adulthood.

In Chapter 6, the General Discussion, I summarize my findings discuss their implications for our understanding of the consequences of human ELS.

2 General methods

The methods described below are pertinent to all subsequent experimental chapters.

2.1 Subjects

Fourteen pregnant female Lister-Hooded rats were purchased from Envigo (Blackthorn, UK). Nest material was provided to each dam and litters were delivered by spontaneous partum on gestational days 22-24. Within three days after birth, litter size was adjusted to between four and six pups, with each litter generally consisting of two female and two male pups. At this time, where two litters were born within 24 hours of each other, pups were mixed between litters to mitigate genetic and epigenetic influences on my results, given that maternal separation occurred on a litter-by-litter basis. After litter size was adjusted, litters were allocated to either the maternal separation (n = 30 pups: 14 female, 16 male) or control (n = 28 pups: 14 female, 14 male) condition.

PND 0 was defined as the day of delivery. Food and water were available ad libitum until food restriction from PND 73-78 onwards for appetitive behavioural testing. Food restriction involved once-daily administration of an amount of chow calculated to maintain each animal above 85% of its individually predicted free-feeding weight based on baseline weight, gender, and current age. Temperature in the home cages was maintained at 23 ± 0.2 °C, and relative humidity was maintained at 60 ± 5 %. Animals were continuously kept on a 12-hour reverse light/dark cycle (lights on from 2100 to 0900). Experiments were conducted in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (Project Licence PA9FBFA9F) and approved by the local ethics committee.

2.2 Maternal separation

For two weeks from PND 5 through PND 19 inclusive, pups from MS litters were separated from their dam for six hours a day, beginning between 1100 and 1230 each day. During the separation periods, the dams remained in their home cages while the pups were taken to a different room and placed together in a small cage inside a ventilated cabinet. One centimetre of bedding was provided and temperature at the surface of the bedding was kept between 30 °C and 35 °C through warming of the air and use of a heat pad. Control pups were subject only to normal animal facility rearing, including once-weekly cage changes. At PND 21, all pups were weaned and housed in same-sex pairs.

This protocol was chosen because of prior institutional experience with it (Matthews et al., 1996b; Matthews et al., 1996a; Matthews et al., 1999; Matthews et al., 2001), together with a lack of evidence that other protocols would be more appropriate for studying the long-lasting effects of chronic stress on our main outcomes of interest, as described in the General Introduction. However, there is a very high degree of consistency in the RMS literature regarding the control group used, which is animal facility rearing in 85% of cases (Tractenberg et al., 2016). We used this control condition rather than the brief daily separations used in the experiments conducted previously at the University of Cambridge to enhance comparability with the bulk of the literature, given that brief separations have been shown to drastically increase social and exploratory behaviour and alter immune functioning (Kruschinski et al., 2008; Mrdalj et al., 2016).

2.3 Adult stress

All animals underwent a subchronic footshock stress in late adulthood, beginning at 8.5-10 months of age. Across a 19 calendar day period (referred to as stress days 0-18), on 14 to 16 days, animals were placed inside operant chambers for 30 minutes each day, where they received either 1 or 2 footshocks. All animals received exactly 20 shocks in total. The number of shocks delivered across the first 8 days for all animals was as follows: 2, 1, 2, 0, 1, 2, 0, 1. The distribution of shock delivery across the remaining 12 days varied between animals but was balanced across group and gender, and animals always received 2 shocks on the day immediately before: each animal's MRI scan day (any of days 9-13), the sucrose preference test day (any of days 16-17), and the sacrifice day (day 19). Animals never received shock sessions on their sacrifice day (day 19). The timing of the footshocks within each session was random, except that shocks were never delivered during the first five minutes or last ten minutes of the session. Shock intensity was 0.5 mA and duration was 0.5 seconds. The chambers used were conventional operant chambers (Med Associates, St Albans, VT) equipped only with a grid floor, a house light, high-contrast distinctive wallpaper behind the transparent plexiglass surfaces, and a small overhead camera. The grid floor was connected to a scrambled shock generator (Med Associates).

This adult stressor design was chosen in part because it fit within the parameters of the local ethical approval, and because acute foot shock has been shown to increase circulating pro-inflammatory cytokines (Deak et al., 2005; Maslanik et al., 2012; Maslanik et al., 2013). The decision to subject all animals to the adult stress was made to maximize power to detect

differences between RMS and control animals in the context of later-life stress (in the context of a resource-related limitations on sample size), at the cost of the ability to definitively ascribe any observed differences to an interaction between RMS and the stressor itself, and of the ability to know with certainty that the second stressor had an effect on our outcomes.

2.4 Data analysis

All data processing, graphing, and statistical analysis was performed using R v3.6.3 (R Core Team, 2018). Data manipulation depended on the following packages: *dplyr (Wickham et al., 2021)*, *tidyr (Wickham, 2021)*, *stringr (Wickham, 2019)*, *magrittr (Bache and Wickham, 2020)*, and *lubridate* (Grolemund and Wickham, 2011). A bespoke function was written by the present author for conducting permutation testing and nonparametric bootstrapping that additionally depended on the *sample* function from base R, as well as the *furrr (Vaughan and Dancho, 2021)*, *purrr (Henry and Wickham, 2020)*, and *future (Bengtsson, 2020)* packages for parallelisation.

2.4.1 Modelling

All analyses in Chapters 3, 4, and 5 initially involved fitting either a linear or logistic regression model. In situations involving repeated measures, a mixed-effects model was fitted, but otherwise a fixed-effects model was fitted. Categorical predictors were handled using sum-to-zero coding.

Where mixed-effects models were used, model fitting used restricted maximum likelihood estimation (Bates et al., 2015), except where logistic regression was performed, in which case maximum likelihood estimation was used. Model fitting was performed using the *lme4* package, v1.1-26 (Bates et al., 2015), and models included a random intercept for each subject with no further random effects, except where specified. The degrees of freedom were approximated using the Kenward-Roger method (Luke, 2017; Fox and Weisberg, 2019; Lenth, 2021).

After model fitting, model assumptions were checked using diagnostic plots. Specifically, normality of residuals was evaluated using quantile-quantile plots while homogeneity of variance and linearity were evaluated using plots of residuals vs fitted values. For linear mixed-effects models, diagnostic plots were generated using the *redres* package v0.0.0.9 (Goode et al., 2021), whereas for logistic mixed-effects models, diagnostic plots were generated using the *simulateResiduals* function in the *dharma* package v0.4.3 (Hartig, 2021),

which also confirmed no over- or under-dispersion of the model residuals. A data point was considered to be influential, i.e. to unduly influence the model, when it had a Cook's distance of ≥ 1. When any model assumption appeared to be violated, or there was at least one influential point, nonparametric methods were used: specifically, permutation testing was used for hypothesis testing, and nonparametric bootstrapping was used for descriptive statistics.

2.4.2 Hypothesis testing

Parametric test statistics were calculated from models by running ANOVAs or ANCOVAs using the *car::Anova* function, v3.0-10 (Fox and Weisberg, 2019) or, for post-hoc comparisons, the *emmeans* or *emtrends* functions from the package *emmeans*, v1.6.0 (Lenth, 2021). Test statistics were Type II F-statistics in the case of linear models, Type II Wald χ^2 statistics in the case of logistic regression models, and two-sample t-statistics in the case of post-hoc comparisons.

In permutation tests, the data for predictors meeting the assumption of exchangeability are shuffled (permuted) many times, and then a test statistic of interest is calculated for each permuted dataset, thereby generating a reference distribution of the test statistic under the null hypothesis (Ludbrook and Dudley, 1998; LaFleur and Greevy, 2009; Buskirk et al., 2013). For calculation of the significance level, the permutation null distribution is used in place of the statistic's theoretical null distribution, circumventing the need for assumptions such as normality of residuals to be met, and increasing robustness to outliers (Buskirk et al., 2013). Here, 10,000 permutations (complete, random samples without replacement) of the predictor data were generated for each response variable. The only predictors permuted were the labels for group and gender, and these were permuted jointly across animals, thereby holding the number of animals belonging to the four conditions constant across all permutations. In cases of repeated measures, observations from a given animal were kept together and in their true sequence over time. Then, for each permuted dataset, a model was fit and test statistics (F- or t-statistics as appropriate) were extracted in the same way as for parametric hypothesis testing. The p-value was calculated as the proportion of permutation-derived test statistics that were equal to or larger than the test statistic derived from the unpermuted data.

2.4.3 Descriptive statistics

Estimated marginal means (EMMs) were generated using the *emmeans* R package (Lenth, 2021), and except in the case of nonparametric bootstrapping, standard errors were also derived using the same package. EMMs represent model-derived estimates of the response variable mean for each level of a specific factor or factors of interest (e.g. group). Where another factor was used in the model (e.g. gender), and EMMs were not calculated jointly for both factors (i.e. for all combinations of the factors' levels), EMMs represent the estimated mean of the response variable for each level of the factor of interest, averaged across the levels of the other factor, weighting those levels equally so that the means are not unduly influenced by a higher sample size for one subgroup. Where a continuous covariate was included in the model, the EMMs represent the estimated means for each level of the factor of interest, at a certain level or levels of the covariate. Where EMMs were not reported for each level of the covariate (e.g. at every real-world value of PRL session number, as in regression lines), the EMMs represent the estimated mean at the mean level of that covariate (e.g. session number four where there were seven sessions). EMMs are useful for several reasons: they can adjust for continuous predictors (e.g. baseline value or session number), they can account for unbalanced designs in adjusting for factors, they can leverage repeated measures in estimating group means at specified levels of within-subject variables (e.g. session number), and they account for any modelled random effects.

To obtain EMMs and associated standard errors via nonparametric bootstrapping, data were first resampled within each group-gender condition 10,000 times with replacement, using original sample sizes, and the appropriate model was fitted using each resampled dataset. From each model, any appropriate EMMs were calculated. To obtain the final estimate of a given EMM, the mean of the 10,000 sample-derived EMMs was calculated, and the standard error was calculated by determining the quantiles corresponding to the probabilities 0.1587 and 0.8413 (z-score -1 and +1), after slight expansion of these probabilities to adjust for the moderately small sample sizes (Hesterberg, 2014, 2015; Helwig, 2017), and dividing the difference in these quantiles by two.

2.4.4 Reporting

Test statistics and p-values were provided in the main text for all significant ($p < 0.05$) and trend ($p < 0.1$) effects involving group (i.e. main effects or post-hoc effects of group, or interaction effects involving group), except where a higher-order effect (interaction term) was

significant, in which case lower-order effects (interaction or main effect terms) were not reported in the main text. In reporting permutation test results, test statistics derived from the unpermuted data were reported together with the p-value derived from the permutation distribution. Test statistics and p-values not reported in the main text can be found in Appendix 7.1, including those for effects not related to the hypotheses under investigation by the present study, such as main effects of gender or time.

Except for histograms, all visualizations represent EMMs ± the standard error of the mean (SEM). In general, visualizations did not depict the influence of predictors other than group unless statistical analyses revealed significant interactions of those variables with group. Thus, because group × gender interactions were most commonly non-significant, graphs were generally not split by gender. There were some exceptions relating to the PRL analyses: for win-stay and lose-shift proportions, where if there was an interaction for at least one of these proportions, all were visualised segregated by gender, as was trial count. Visualizations were produced using the packages *ggplot2* (Wickham, 2016), *corrplot* (Wei and Simko, 2017), and *patchwork* (Pedersen, 2020).

2.5 Experimental timeline

A timeline is provided in Figure 1 that describes the sequence and timing of all procedures that animals underwent. Further detail is provided in subsequent methods sections.

Procedure or test	Start range (PND)	End range (PND)
Repeated maternal separation	5	19
1st blood collection & 1st MRI scan	20	N/A
2nd blood collection	53-55	N/A
2nd MRI scan	61-62	N/A
Elevated plus maze	66-69	N/A
Novelty reactivity test	70-73	N/A
Novelty preference test	74-76	N/A
Progressive ratio task (including training)	80-84	115-150
Sucrose preference test (pre-stress)	143-161	147-165
Probabilistic reversal learning (including training)	241-277	N/A
Adulthood stress start	260-304; stress day 0	N/A
3rd MRI scan	271-309; stress days 9-13	N/A
Sucrose preference test (intra-stress)	276-321; stress days 16-17	N/A
Sacrifice (inc. 3rd blood collection)	279-323; stress day 19	N/A

Figure 1. Experimental timeline. The range of post-natal days (PND) for the start and end of each procedure underwent by repeated maternal separation (RMS) and control animals are provided.

3 Repeated maternal separation has long-lasting behavioural consequences

3.1 Introduction

ELS in humans is associated with increased later-life symptoms and risk of diagnosis of depression, anxiety, and substance use disorders (Nelson et al., 2006; Trent et al., 2007; Danese et al., 2009; Wright et al., 2009; Cutajar et al., 2010; Spinhoven et al., 2010). Additionally, there is good evidence from meta-analyses that when these disorders do develop, their severity is considerably greater in individuals with a background of ELS (Nanni et al., 2012; Agnew-Blais and Danese, 2016).

While ELS is associated with increased risk of these adverse outcomes, none occurs deterministically following any given type of ELS, per meta-analytic evidence (Teicher and Samson, 2013). The associations between ELS and risk of these disorders must therefore either be causally mediated by other exposures that simply frequently co-occur with ELS (e.g. low socioeconomic status or later-life stressor exposure), or must depend on interactions between the effects of ELS and other factors, potentially including individuals' evolving environmental circumstances throughout childhood, adolescence, and adulthood (Fishbein et al., 2009; Wright et al., 2009; Teicher and Samson, 2016). Years or decades often pass between ELS exposure and the onset of clear mental or physical pathology, and during that time each exposed individual navigates an enormous number of social, financial, academic, and occupational decisions as they go about their daily lives (O'Rand et al., 2009; Wright et al., 2009). If ELS biases decision making in one or more of these contexts, over time this could potentially lead to engrained patterns of cognition, emotional responding, or behaviour that could eventually manifest as mental illness (Pine et al., 2005; Leppänen, 2006; Harmer et al., 2009; Gotlib and Joormann, 2010; Everaert et al., 2012; Humphreys et al., 2016; Raymond et al., 2021). If ELS slows decision making in certain contexts, this might indirectly or directly constrain occupational achievement and increase the risk of experiencing adulthood stress (DePrince et al., 2009; Fields et al., 2021).

Here, to probe these possibilities, I used the probabilistic reversal learning (PRL) task to conduct novel measurements of the long-lasting effects of RMS on sensitivity to positive and negative feedback, as well as reaction time in a decisional context with negatively-valenced

stimuli. Because ELS appears to result in an attentional bias away from threat-associated stimuli, at least in adolescence (Pine et al., 2005; Humphreys et al., 2016; Weissman et al., 2019), I predicted that ELS would increase negative feedback sensitivity on the probabilistic reversal learning (PRL) task. Additionally, because response times have been reported to be increased in human and non-human primate ELS on various tasks (Malter Cohen et al., 2013; Morin et al., 2019; Fields et al., 2021), particularly in the context of decisional tasks involving negatively valenced stimuli, I hypothesised that they would be prolonged in the PRL task.

Although there have been a few discrepant reports (Rüedi-Bettschen et al., 2005; Kosten et al., 2006; Shu et al., 2015; Huang et al., 2021), well over a dozen studies have reported that RMS does not cause a long-lasting anhedonia, whether on the sucrose preference test (SPT) (Shalev and Kafkafi, 2002; Zhang et al., 2013; Harrison et al., 2014; Hill et al., 2014; Chocyk et al., 2015; Feldcamp et al., 2016; Gracia-Rubio et al., 2016; Mrdalj et al., 2016; Borges-Aguiar et al., 2018; Wei et al., 2018; Stuart et al., 2019) or progressive ratio (PR) task (Shalev and Kafkafi, 2002; Zhang et al., 2005; Cruz et al., 2008; Stuart et al., 2019; Thornton et al., 2021). Although there was therefore little reason to anticipate a long-lasting anhedonia, I nevertheless conducted the SPT and PR task, so that I would be confident of detecting anhedonia if it was present and, if so, consider whether it might be contributing to any significant findings on the PRL task.

I also conducted the elevated plus maze (EPM) as an index of anxiety-like behaviour (Cryan and Sweeney, 2011). I did not have strong hypotheses regarding the anticipated long-term effect of RMS on this test, given that a recent meta-analysis revealed that increased and decreased anxiety-like behaviour following RMS have both been reported by large numbers of investigators (Tractenberg et al., 2016). Nevertheless, I conducted this test so that my interpretation of the results on PRL could be put in the context of any between-group differences in anxiety-like behaviour.

As exploratory measurements, I also conducted the novelty reactivity test (NRT) and novelty preference test (NPT). The NRT is worth conducting in the context of the relationship between ELS and later-life risk for substance use disorders, given that high novelty reactivity has been shown to predict intravenous self-administration in rats of both amphetamine (Piazza et al., 1989) and cocaine (Belin et al., 2008). As with the NRT, the NPT is useful in interrogating the effects of ELS on addiction vulnerability factors, given that high

novelty preference has been shown to predict compulsive "addiction-like behaviour", defined as persistent drug seeking or taking despite punishment (Belin et al., 2011).

Finally, I used a novel subchronic stressor to explore whether RMS could interact with such a stressor to affect performance on the PRL task, including sensitivity to positive and negative feedback. As before, I also conducted the SPT to specifically index motivation for reward, to contextualize the PRL findings. I did not expect that RMS would predispose to anhedonia in response to the novel stressor used, which was much less intense than shock-based stressors that have been previously used to induce anhedonia, with a lower number of shocks at lower voltage and lower duration (Kim et al., 2017). However, I expected that any pre-existing effects of RMS on the PRL task would be potentiated following stress, particularly given that non-RMS adulthood stressors can themselves affect PRL parameters including sensitivity to positive and negative feedback (Bergamini et al., 2016).

3.2 Methods

3.2.1 Arena-based behavioural testing

Three behavioural tasks used involved monitoring animal performance in an arena or maze. For each of these tasks, the appropriate plexiglass arena was placed above a 1.1 m × 1.1 m infrared-illuminated platform. Rat movements were recorded from above using an infrared camera (FLIR Systems, Wilsonville, OR, USA), and analysed using VideoTrack software (ViewPoint Behaviour Technology, Lyon, France). Rats were tested on each of these tasks only once. Testing always occurred during the dark phase of the light cycle, which occurred between 0900 and 2100.

3.2.1.1 Elevated plus maze

Rats were tested on the elevated plus maze (EPM) between PND 66-69. This task exploits rats' natural fear of open and well-lit places in order to obtain a measure of anxiety (Pellow et al., 1985; Tucker and McCabe, 2017), calculated as the ratio of time spent in the exposed, brightly lit arms to the time spent in the closed, darker arms. The maze consisted of a plus-shaped platform with four arms (45 cm length × 10 cm width) elevated 80 cm above the infrared-illuminated base. The closed arms were surrounded by a 45 cm tall opaque wall, while the open arms were surrounded by only a 1 cm tall transparent lip. Rats were placed individually in the centre of the maze such that each animal initially faced a closed arm.

Illumination was kept at 10-15 lux within the closed arms and 55-65 lux within the open arms.

3.2.1.2 Novelty reactivity

Novelty-induced locomotor reactivity was assessed between PND 70-73. In this task, rats were placed in a 50 cm × 50 cm × 50 cm arena with grey walls and a white floor, and their movements were recorded and tracked. Distance moved across the whole 2-hour session was determined. The arenas were brightly lit at 500-600 lux.

3.2.1.3 Novelty preference

The novelty preference test was performed between PND 74-76. The arena for this task contained two large rectangular chambers (20 cm × 50 cm × 50 cm) divided by a small rectangular alleyway (10 cm × 50 cm × 50 cm). The two large chambers differed in colour (white or black) and texture (smooth or dotted with small round pits). Rats were placed for 5 minutes in the central alleyway and allowed to habituate, before a door was opened to one of the other two chambers for 25 minutes. Finally, doors were opened to both chambers for 15 minutes, so that the animal could freely explore all three chambers. Novelty preference was defined as the time spent in the novel chamber (the second available chamber) as a percentage of the combined time spent in the familiar chamber (the first available chamber) and the novel chamber (Belin et al., 2011). Illumination within all three chambers was kept very low at < 2 lux.

3.2.2 Sucrose preference test

The sucrose preference test, a putative measure of anhedonia that, per systematic reviews of different stress procedures, is sensitive in the short-term to chronic high-intensity stress in animals (Vasconcelos et al., 2015; Antoniuk et al., 2019), was conducted in adulthood both before and during the adult stress. Testing before adult stress ("pre-stress") was conducted at age 4.5-5.5 months and measured preference for 0.5%, 1%, and 2% sucrose over water. Testing during adult stress was conducted at age 9-10.5 months and measured preference for a 1% sucrose solution over water. Sucrose solutions were prepared fresh immediately before provision to animals, using food-grade sucrose (MP Biomedicals, Solon, USA).

At each of the two testing timepoints, animals initially underwent three habituation steps. First, animals were provided with one bottle of 1% sucrose solution in their home cage for 24 hours, in addition to the water normally available via the cage rack water delivery system.

After the 24 hours had concluded, animals were placed individually into large cages with clean bedding. One bottle containing 1% sucrose solution and one containing water were then provided. After 45 minutes, the bottles were removed and animals were placed back into their home cages. The next day, each animal was placed into the same cage as before for 45 minutes, but this time the location of the sucrose bottle and water bottle were swapped.

The following day, to conduct the test itself, each animal was again placed into the same cage, but was left there for four hours. The location of each bottle was reversed compared to the previous day, and these locations were again reversed two hours into the test session. At the pre-stress timepoint, tests of preference for the 0.5%, 1%, and 2% sucrose solutions were separated by a 44-hour washout period in the home cage. For a given animal, all sessions (habituation or test) in the test cage commenced at the same time of day, and the daily food ration was always provided approximately 20 hours prior to that time.

The weight of the sucrose and water bottles was weighed before and after each test. For each bottle, the weight after was subtracted from the weight before, giving a measure of absolute sucrose solution and water consumption. Sucrose preference over the four hours was defined as the amount of sucrose solution consumed as a percentage of the total amount of sucrose solution and water consumed.

3.2.3 Operant behavioural testing

3.2.3.1 Apparatus

Experiments were performed using eight identical operant chambers (Med Associates, St. Albans, VT, USA). Chambers were contained within fan-ventilated, light- and sound-attenuating boxes. One wall of each chamber consisted of a 15-inch LCD touchscreen capable of infrared touch detection (Nexio Co. Ltd, Incheon, Korea), while the opposite wall contained a pellet receptacle with a head-entry detector and accompanying light. An electronic pellet dispenser delivered small 50% sucrose pellets (TestDiet, St. Louis, MO, USA) into the pellet receptacle as rewards for completing trials. Chambers were controlled using the K-Limbic software (Conclusive Marketing Ltd., High Wych, UK).

3.2.3.2 Progressive ratio schedules of reinforcement

The progressive ratio task was used to assess motivation for food reward. During each training or test session, animals were able to touch a single white square stimulus on the touchscreen a certain number of times to earn a pellet reward. The stimulus was always

located in the middle of the screen by width, and roughly 2 cm above the metal bar floor. Where a stimulus touch or omission resulted in pellet delivery, a 1 s tone (2.9 kHz) was generated, the magazine light was turned on, and the stimulus was removed from the screen. Upon head entry into the magazine, the magazine light was turned off, and a 5 s inter-trial interval was initiated, after which the stimulus was returned to the screen. Where a stimulus touch did not result in reward delivery, a 20 ms tone was generated and the stimulus was removed from the screen for 0.5 s. The house light remained off at all times. Animals underwent a maximum of one training or test session per day, and all training and testing occurred between PND 80-150 (~2.5-5 months of age).

Rats were first exposed to a single pre-training session, intended to familiarise animals with the chambers and reward delivery. The stimulus was presented for up to 30 s at a time. If animals touched the stimulus once, or the 30 s period elapsed without any stimulus touch (an omission), reward was delivered as described above. The session was terminated after 100 rewards were delivered or 45 min elapsed.

Rats were then trained on a fixed ratio 1 (FR1) schedule of reinforcement, where each press on the stimulus was rewarded with a pellet. Animals were given repeated FR1 sessions until it was clear that they had acquired the task, demonstrated by their earning 100 pellets in a given session (Hailwood et al., 2018). Animals were then trained on a fixed ratio 5 (FR5) schedule, where 5 stimulus touches were necessary to earn a reward. When animals earned 100 pellets in two FR5 sessions, or over 60 pellets on five sessions, they were moved to the progressive ratio task.

Animals completed nine sessions on each of three motivationally demanding progressive ratio schedules: progressive ratio 4 (PR4), progressive ratio 8 (PR8), and progressive ratio 16 (PR16). In progressive ratio schedules, the number of stimulus responses necessary to earn a reward increases by the specified number each time a reward is earned. For example, in PR4, the number of responses necessary to earn a reward increases as follows: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, and so on. Each session lasted for 45 minutes unless 100 pellets were earned or unless a time-out elapsed during which the animal ceased touching the stimulus for 180 seconds.

Measures of interest included the breakpoint, defined as the number of stimulus touches made during the last successfully completed trial, as well as the average time between the first stimulus presentation and response on each trial (latency to respond), and the average

time between reward delivery signalling and collection (latency to collect). The number of sessions each animal required to progress through the FR1 and FR5 training stages was also recorded. The total number of rewards earned represents a linear transformation of the breakpoint, and the total number of lever presses is highly correlated with the breakpoint, so neither was analysed here.

3.2.3.3 Probabilistic reversal learning

The probabilistic reversal learning task was used to measure multiple aspects of reward- and punishment-associated behaviour. In the first stage of training (touch training A), at the start of each trial, a visual stimulus was randomly presented on either the left or right side of the touchscreen. The visual stimulus consisted of a white square overlayed with a thick black 'X'. If the stimulus was touched by the animal, it was rewarded: a 0.5 s tone was generated, the pellet receptacle light was turned on, and a sucrose pellet was delivered. The receptacle light remained on until the animal made a head entry into the receptacle, at which point the light was turned off, concluding the trial and initiating a 5 s inter-trial interval (ITI).

The second stage of training (touch training B) was identical to the first, except that background touches were punished. If an animal touched an area of the touchscreen where no stimulus was being presented, the house light was turned on for 5 s, and then the receptacle light was turned on until the animal made a head entry, at which point the light was turned off, concluding the trial and initiating a 5 s ITI.

The third stage of training consisted of a deterministic reversal learning (DRL) task. In this task, two visually identical stimuli were always presented simultaneously, with one stimulus each on the left and right sides of the touchscreen. At any given time, one of these stimuli was the 'correct' stimulus and one was the 'incorrect' stimulus. During the DRL task, a touch on the correct stimulus resulted in reward (described above) on 100% of trials, while a touch on the incorrect stimulus resulted in punishment (described above) on 100% of trials. If an animal touched the correct stimulus eight trials in a row (suggesting that it had successfully identified the correct stimulus), the correct and incorrect stimuli were reversed, such that the side that was previously rewarded during 100% of trials was now punished during 100% of trials.

The PRL task was identical to the DRL task, except that the correct stimulus resulted in reward randomly on 80% of trials and punishment on 20% of trials, while the incorrect stimulus resulted in reward randomly on 20% of trials and punishment on 80% of trials.

For each animal, only one chamber session was conducted per day. Sessions concluded after either 40 minutes had passed, or 200 trials were completed. Animals commenced touch training A approximately 3-4 weeks in advance of the day they were scheduled to commence the adult stress. Animals were progressed to touch training B once they had completed one session of the touch training A in which they earned at least 100 rewards. Animals were progressed to the DRL task once they had completed two consecutive sessions of touch training B in which they earned at least 100 rewards on both occasions. Animals were progressed to the PRL task once they had completed two consecutive sessions of the DRL task in which they achieved at least 4 reversals on both occasions. Once they began touch training, animals completed one session daily of the appropriate task up to and including the first day of the adult stress, when they were tested in the morning and commenced on the stress in the afternoon. After adult stress commencement, they were tested on stress days 3 and 6, and also day 11 where this did not conflict with an MRI scan. Almost all animals completed at least seven sessions of the PRL task before the adult stress began, but many did not complete more sessions than this, so pre-stress analysis was limited to the first seven sessions. Animals were ~8-9 months old at commencement of touch training, and ~8.5-9.5 months old at conclusion of the seven initial pre-stress sessions of the PRL task.

Several behavioural indices were extracted from the raw data for each session. The trial count was recorded, and to describe the animal's proficiency at the task among completed trials, the proportion of responses on the correct stimulus was calculated. The average latency between stimulus presentation and stimulus selection was calculated (latency to respond), and the average latency between reward signalling onset and reward collection was calculated (latency to collect). Prior to calculation of these means, to minimise the impact of extreme intra-session outliers, latency data were winsorized within each session using a conservative threshold of 3.5 median absolute deviations (Iglewicz and Hoaglin, 1993; Leys et al., 2013; Alfons, 2019). The number of reversals was recorded, as was the average number of perseverations per reversal. Perseverations were defined as serial touches on the newly-incorrect target following reversal, not including the first post-reversal incorrect touch (if one occurred). Thus, if an animal touched the incorrect target twice immediately following reversal, one perseveration would have been scored for that reversal. For both of the touch training tasks and for the DRL task, the number of sessions each animal took to achieve criterion and thus progress to the next task was recorded.

Detailed information on animal decision-making was also extracted (Bari et al., 2010; Phillips et al., 2018; Alsiö et al., 2019). During the PRL task, on any given trial, there were four possible outcomes: the animal touched the correct stimulus and was rewarded (a "win on correct" or "correct-win"), the animal touched the correct stimulus and was punished (a "loss on correct" or "correct-loss"), the animal touched the incorrect stimulus and was rewarded (a "win on incorrect" or "incorrect-win"), and the animal touched the incorrect stimulus and was punished (a "loss on incorrect" or "incorrect-loss"). Thus, there were four possible contexts for the animal's decision on the next trial, all involving varying histories of reward and punishment, including immediate-past (win/loss) and historical (correct/incorrect). Each pair of trials was examined to determine how the animal on average responded to each of these different contexts. The animal's choices in the 'win' (rewarded) contexts were described as the proportion of trials on which the animal 'stayed' on the same target, i.e. selected the same stimulus as in the previous trial. The animal's choices in the 'lose' (punished) contexts were usually described as the proportion of trials on which the animal 'shifted' to the alternative target, i.e. selected the stimulus not selected in the previous trial.

3.2.4 Data analysis

Parametric methods were used for analyses of arena-based behavioural testing, intra-stress sucrose preference, predicting session-level task performance from correct-win stay proportion and incorrect-loss shift proportion, and trial-by-trial analyses predicting whether the animal chose the same target as a previous trial. Nonparametric methods were used in all other cases.

Model structures are reported in the statistics tables in Appendix 7.1.1. For intra-stress analyses, models included baseline as a predictor (Senn, 2006; Landau et al., 2018; Wan, 2021). For PRL analyses, a baseline for each metric was created for each animal by taking the median over its final pre-stress PRL sessions. For SPT analysis, each animal's total pre-stress sucrose preference was used as its baseline, defined as the total sucrose solution consumed over the three pre-stress tests as a percentage of the total amount of sucrose and water consumed in that time. For body weight analysis, each animal's median weight over the thirteen days before the first stress day was used as the baseline. Before inclusion in models, baseline variables were first mean-centred.

Body weight was measured at varying intervals from PND 20 through sacrifice. For analysis of pre-stress body weight, age was divided into bins of seven days each, starting

from PND 20, and each animal's weight measurements were averaged for each bin. Because animals commenced adult stress at different ages, weights taken during adult stress were excluded from calculation of per-animal averages, and analysis extended only to the bin starting at PND 258, because large numbers of animals progressively commenced the adulthood stress shortly after that age. Because weight was not linear over time, age was treated as a categorical variable. For post-stress analysis, no binning was performed, and stress day was treated as a continuous variable because animals' weights were linear over time.

For PR and PRL analyses, all models treated session number as a continuous variable, both to minimise type II error by conserving degrees of freedom and because no frankly non-linear relationships between session number and any response variables were apparent on exploratory data visualisation. Where a two-term interaction involving group and session was significant with no significant higher-order interactions, this was followed up with a pairwise comparison between groups at both the first and last session. Where a three-term interaction involving group, gender, and session was significant, a new model was fit for each gender. Where a four-term interaction involving group, gender, session, and past trial outcome was significant, up to two sets of pairwise comparisons were performed. Pairwise comparisons were always between the two groups (MS and control), within the combinations of the levels of gender (male and female) and past trial outcome (reward or punishment). First, pairwise comparisons using *emtrends* were performed to compare the slopes across session number. Where slope comparisons were significant, pairwise comparisons using *emmeans* were performed to compare the EMMs at the first and last sessions. Where slope comparisons were non-significant, main effects of group were assessed: pairwise comparisons were performed using *emmeans* to compare the EMMs at the average session number between groups.

To probe the influence of historical trial outcomes on future decision making, three logistic mixed-effects models were constructed. These modelled the probability of an animal selecting the same target it selected on the previous 1, 2, and 3 trials, based on the interaction between group, gender, session number, and trial outcome (reward or punishment). Because multiple observations from each session were included in the dataset, two random intercepts were modelled: subject and session within subject. Post-hoc comparisons were performed using two-sample z-tests, first of the slope across session and then between EMMs in the form of the log odds of choosing the same target as on the previous trial.

3.3 Results

3.3.1 RMS did not have long-lasting effects on body weight

Animal body weight before adulthood stress was analysed from PND 20 through PND 258. No significant or trend effects involving group were identified (Appendix 7.1.1.1). Regardless, because visualization (Figure 1) of bootstrapped estimated marginal means suggested possible group differences over the first several age bins, and because of the possibility that the much more numerous and higher-variance bins with no apparent group differences could potentially compromise the sensitivity of the ANOVA to capture a small number of early group differences, exploratory post-hoc testing was performed. This revealed significant group differences at PND 20 ($t_{108.4}$ = 0.62, p < 1e-4), PND 27 ($t_{137.8}$ = 1.17, p = 0.023), PND 34 ($t_{137.8}$ = 1.33, p = 0.01), and PND 41 ($t_{108.4}$ = 0.73, p = 2e-4), with RMS animals having a lower body weight at these early post-RMS timepoints. Body weight of RMS animals recovered well before young adulthood, and ultimately RMS animals had non-significantly higher body weights throughout almost all of adulthood.

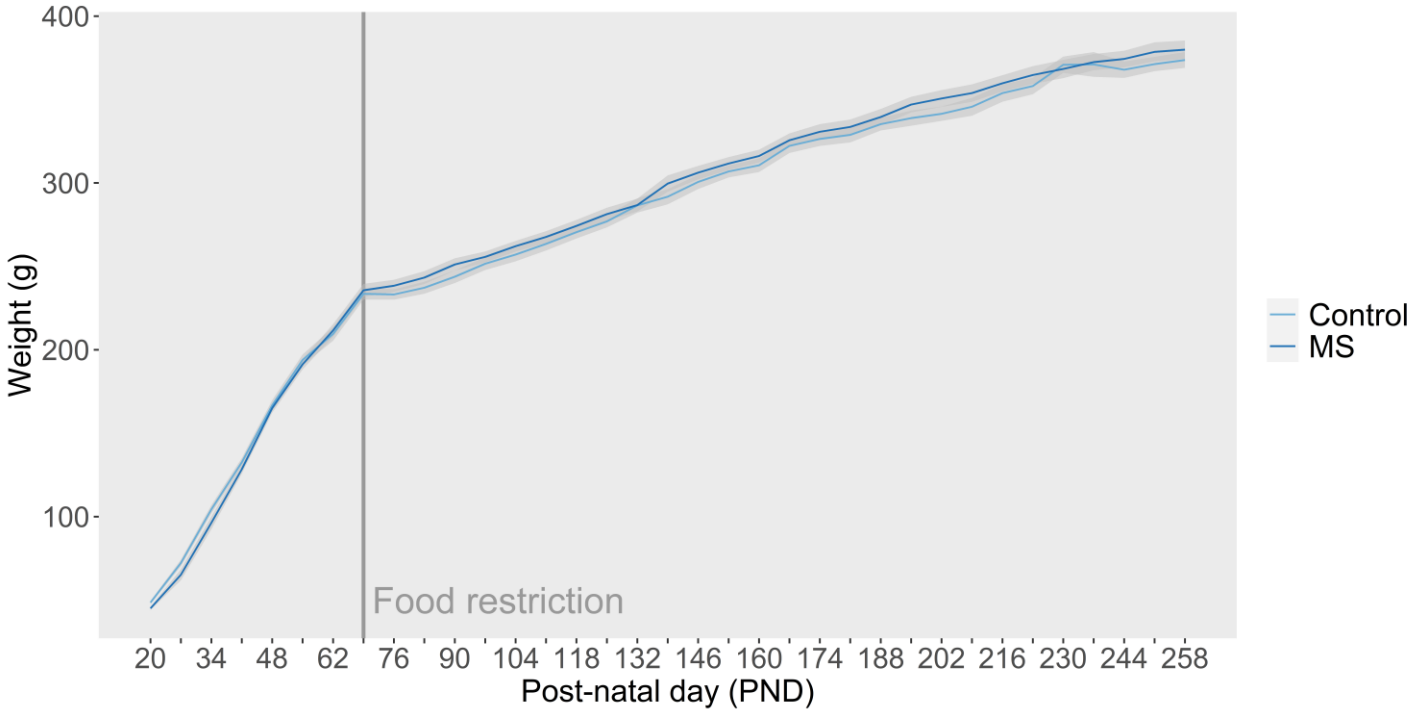

Figure 1. Repeated maternal separation (MS) did not have long lasting effects on body weight prior to adult stress. Animals were weighed periodically from PND 20 through to sacrifice. For the analysis of weights before adult stress, animals' pre-stress weights were averaged within seven-day bins. Mixed-effects ANOVA revealed no main or interaction effects involving group (MS vs control), but exploratory post-hoc testing suggested that MS caused a lower weight over the first four weeks following its completion (n = 28-29 per group).

Body weight data were also examined for a differential effect of stress between groups. This analysis covaried for baseline weight, and revealed a trend ($F_{1,49.0}$ = 3.61, p = 0.064) main effect of group (Figure 2), with RMS animals having a lower body weight across the stress period (RMS 395.4 ± 2.4 g vs controls 400.5 ± 2.0 g).

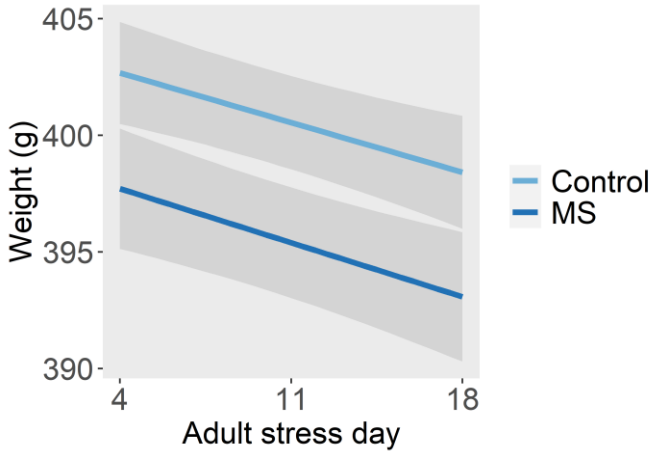

Figure 2. Repeated maternal separation (MS) did not cause significantly different body weight in the context of later-life stress. Animals were weighed at least four times (median: 6, maximum: 9) between stress days 4 and 18. Modelling adjusted for each animal's baseline, which was the median weight over the thirteen days preceding the stress day. A trend (p = 0.064) main effect of group was observed, with RMS animals having lower body weight in the context of later-life stress (n = 27 per group).

3.3.2 RMS did not affect anxiety-like behaviour or response to novelty

Roughly seven to eight weeks after the conclusion of maternal separation, a battery of tests sensitive to anxiety-like behaviour was conducted. The test battery included the elevated plus maze (EPM), the novelty preference (NP) test, and the novelty reactivity (NR) test. There was no main or interaction effect involving group on any of these measures (Figure 3).

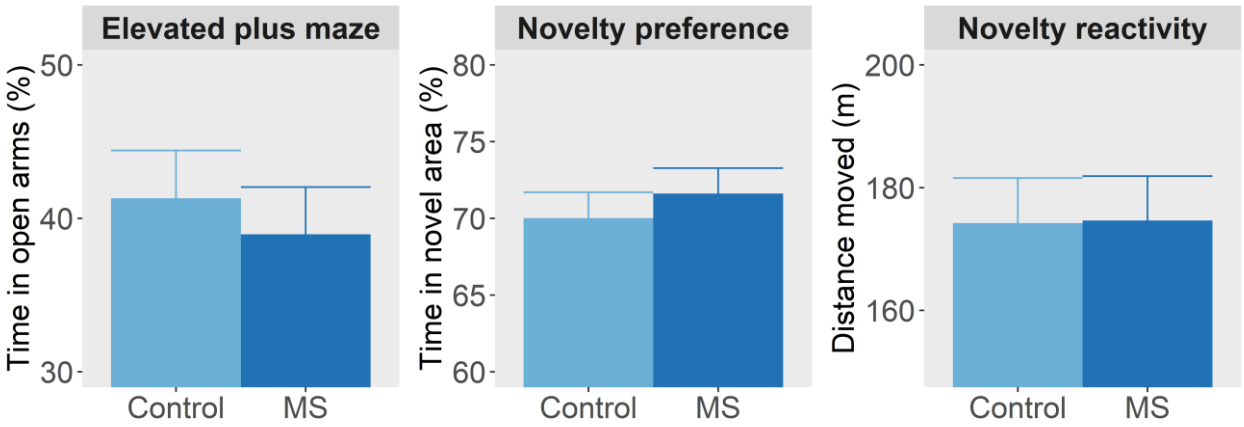

Figure 3. Repeated maternal separation (MS) did not have long-lasting effects on anxiety-like behaviour or response to novelty. Animals were tested for anxiety-like behaviour in early adulthood on the elevated plus maze (EPM), as well as for novel environment preference and locomotor reactivity to a novel environment. Results for the EPM are given as the time spent in the open arms as a percentage of the total time spent in either the open or closed arms across the five-minute test session. Novelty preference results represent the time spent in the novel chamber as a percentage of the time spent in either the novel or familiar chamber across the 15-minute test session. Novelty reactivity results are expressed as the total distance moved across the two-hour test session. There was no effect of maternal separation on any of these measures (n = 28-29 per group).

3.3.3 RMS did not affect sucrose preference

Preference for sucrose solution over water was measured both before and after adult stress in a series of two-bottle choice tests lasting four hours each. There were no significant or trend effects involving group at either timepoint (Figure 4), indicating that maternal separation had no persistent effect on sucrose preference or on susceptibility to change in sucrose preference in response to the adult stress.

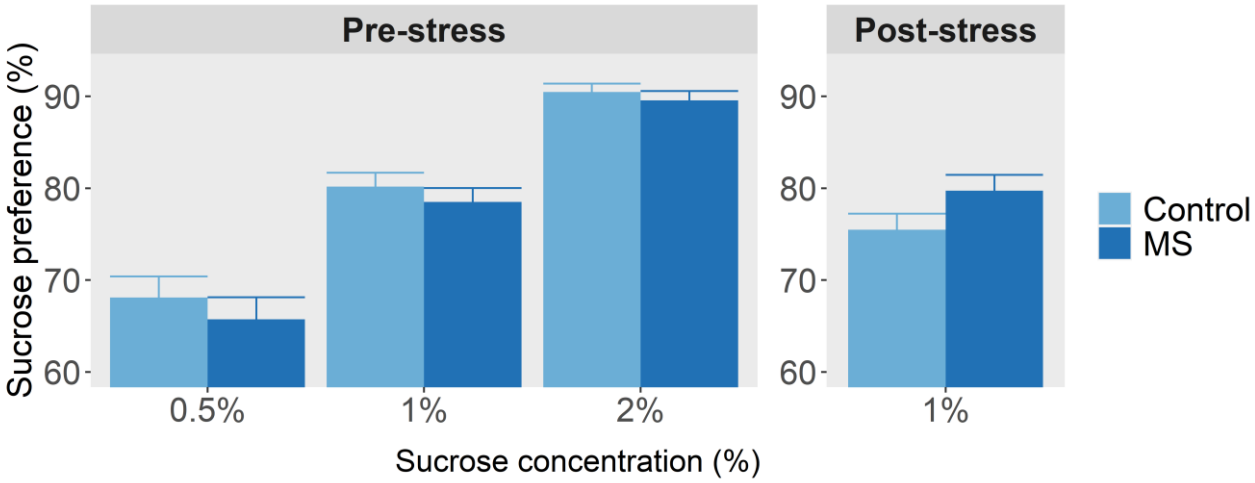

Figure 4. Repeated maternal separation (MS) did not affect sucrose preference. MS and control animals (n = 27-28 per group) were tested on the sucrose preference test in early adulthood in the absence of any recent stress and in late adulthood during a novel chronic stress. In early adulthood, three sucrose concentrations were used, whereas in late adulthood, only 1% sucrose was used. There were no differences between groups in any of the conditions examined.

3.3.4 RMS decreased latency to respond for reward but did not affect reward-motivated effort

Motivation for reward was assessed using progressive ratio schedules of reinforcement. Animals first progressed through one pre-training session and a variable number of fixed ratio 1 (FR1) and fixed ratio 5 (FR5) training sessions, before ultimately being tested on

progressive ratio (PR) 4, 8, and 16 schedules over nine sessions each. Only three of 58 animals were excluded from parts of this analysis: two completely and one from PR16 onwards, all due to illness (one did not recover from anaesthesia on PND 20, and two developed likely bowel obstructions of unknown origin in adulthood and so were euthanised). All other animals met criterion for both FR1 and FR5 and fully completed the PR stages. No significant differences were observed between groups in the number of sessions to criterion for FR1 or FR5 (Figure S3.1).

There was a main effect of group on latency to respond during both the PR4 task ($F_{1,52}$ = 5.17, p = 0.024) and PR16 task ($F_{1,51}$ = 5.36, p = 0.024) (Figure 5). On PR4, RMS animals were quicker to respond on average by 3.7 seconds, with response times of 15.8 ± 0.6 seconds compared to 19.5 ± 1.1 seconds for controls. On PR16, RMS animals were quicker to respond by 9.2 seconds, with response times of 33.3 ± 1.6 seconds compared to 42.5 ± 2.6 seconds for controls.

For PR8 there was a trend for a main effect of group ($F_{1,52}$ = 2.85, p = 0.099), with RMS animals being quicker to respond by 4.2 seconds, at 25.3 ± 1.5 seconds as opposed to 29.5 ± 1.3 seconds for controls.

There were no significant interactions involving group for latency to respond, and there were no significant main or interaction effects involving group for either the breakpoint or latency to collect reward.

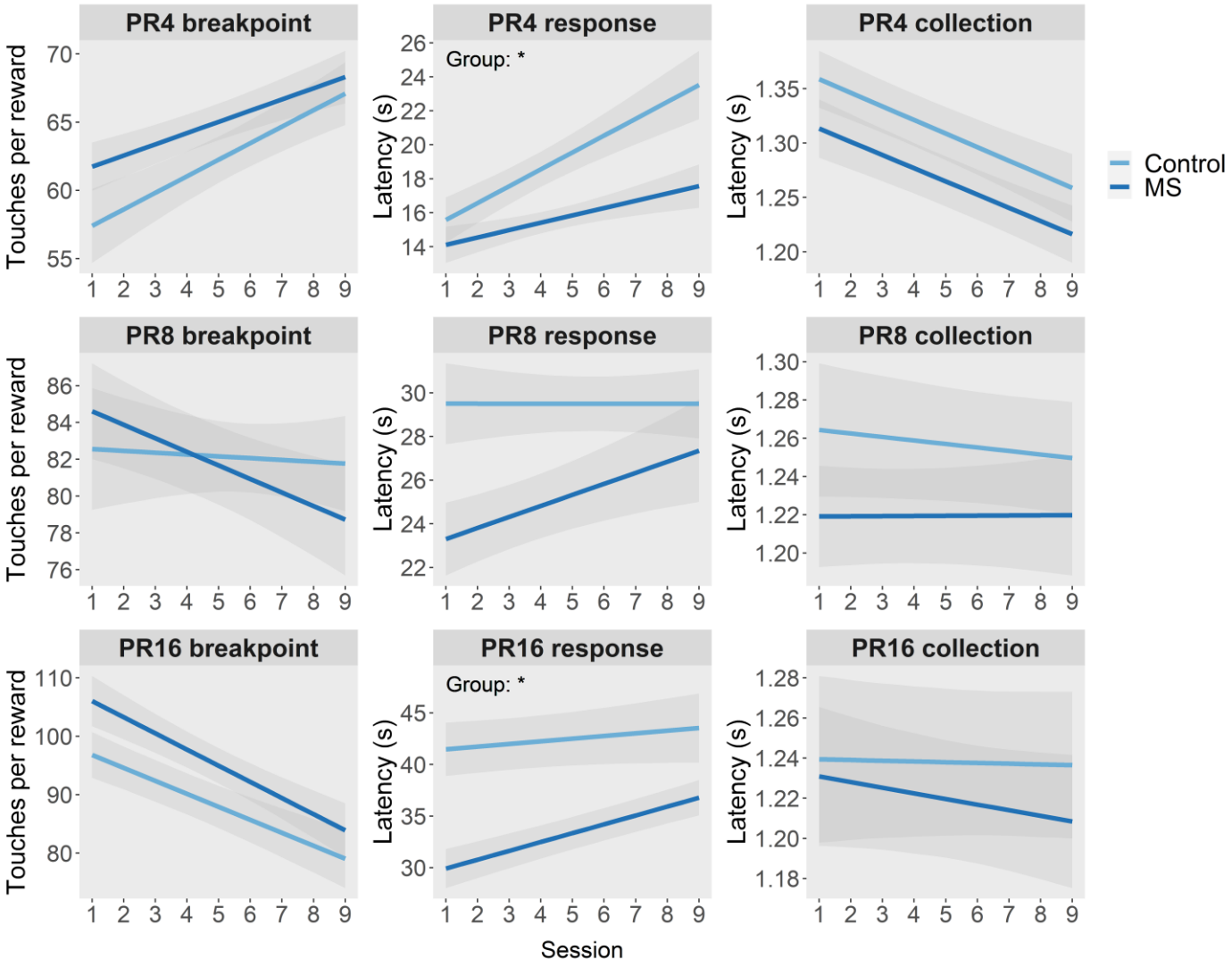

Figure 5. Repeated maternal separation (MS) decreased latency to respond for reward on a task with no decisional burden. Animals were tested in adulthood on the progressive ratio (PR) paradigm, a simple task in which they were presented with one touchscreen stimulus and could touch it an increasing number of times to earn a sucrose reward. They were advanced through three schedules of reinforcement with progressively increasing motivational demand: PR4, PR8, and PR16 (n = 27-28 per group for all tasks). While no differences were found between MS and control animals in the number of responses they were willing to complete to earn one reward, MS animals were consistently faster than controls in making their first touch on the stimulus following commencement of a new trial. There were no differences between groups in time to collect the sucrose pellet following the signalling of its delivery.

3.3.5 RMS did not affect acquisition of PRL training stages

Before commencing the PRL task, animals progressed through three training tasks by reaching a requisite level of performance on each: touch training A, touch training B, and deterministic reversal learning (DRL). Animals were then tested both before and during adult stress, allowing pre- and post-stress analysis. Pre-stress analysis was conducted over each

animal's first seven PRL sessions, while post-stress analysis used each animal's final five PRL sessions before the adult stress as a baseline, together with measurements on days 3, 6, and 11 post-stress.

Of the 58 animals in the study, 10 were excluded from the pre-stress analysis: three passed away before touch training commenced (causes described earlier), one became ill during the DRL stage (unwell-appearing without improvement so euthanised; cause not identified on pathological examination), one was incorrectly trained (DRL was skipped), and the remaining five (2/11 RMS females and 3/13 control females) acquired the tasks too slowly to have completed seven sessions of the PRL stage before the adult stress was scheduled to commence. For the post-stress analysis, one animal that had completed exactly five pre-stress PRL sessions was eligible for this analysis but not the pre-stress analysis, but an additional animal became ill during the stress period and so was excluded, for a total again of 48 included animals. Fourteen animals, balanced for group within each gender, underwent MRI scans on stress day eleven and so were not tested on that day; this missing data were handled statistically using mixed effects modelling.

Among animals included in the pre-stress analysis, there were no significant differences between RMS and control animals in the number of sessions to achieve criterion for the touch training B and DRL stages, while statistical analysis was not performed for Touch Training A sessions-to-criterion because all animals had the same value (i.e. 1 session) except two control females (Figure S3.2). Among animals included in the post-stress analysis, there was a trend ($F_{1,44} = 3.05$, $p = 0.088$) for a difference in the number of sessions completed by the time of adult stress onset, with RMS animals having slightly more experience on the PRL task, at 15.3 ± 0.9 sessions compared to 12.8 ± 0.9 sessions for controls.

3.3.6 RMS slowed decision making on the PRL task, and caused a long-lasting susceptibility to stress-induced exacerbation of this deficit

In both the pre- and post-stress PRL analyses, the effect of maternal separation on the following behavioural parameters was examined: overall task performance (correct target selection), trial count, latency to respond on a stimulus, latency to collect reward, incorrect-loss shift proportion, correct-loss shift proportion, incorrect-win stay proportion, correct-win stay proportion, number of reversals, and perseverations per reversal. Post-stress analyses

were intended to assess for differential responses to adult stress by RMS and control animals, so all post-stress test and descriptive statistics are adjusted for any influence of baseline (Senn, 2006; Landau et al., 2018; Wan, 2021).

Across the first seven PRL sessions, in the initial mixed-effects model involving the three main terms (group, gender, and session) plus full interactions, RMS had a significant main effect on latency to respond ($F_{1, 44} = 10.01$, $p = 0.002$) in the absence of any interaction with session or gender (Figure 6). Specifically, RMS increased the average time animals took to choose between the two stimuli by ~450 ms, from 1201.9 ± 46.5 ms to 1654.0 ± 89.2 ms, and this difference was consistent across sessions, even as both groups became quicker at responding with time.

RMS animals also had significantly greater latency to choose a stimulus during the adult stress period than controls ($F_{1,41.7} = 8.83$, $p = 0.004$), taking 1701.0 ± 98.8 ms to decide compared to 1279.8 ± 66.6 for controls, despite controlling for any baseline differences (Figure 4).

Despite the robust effects on latency to respond, there were no significant main or interaction effects involving group on latency to collect.

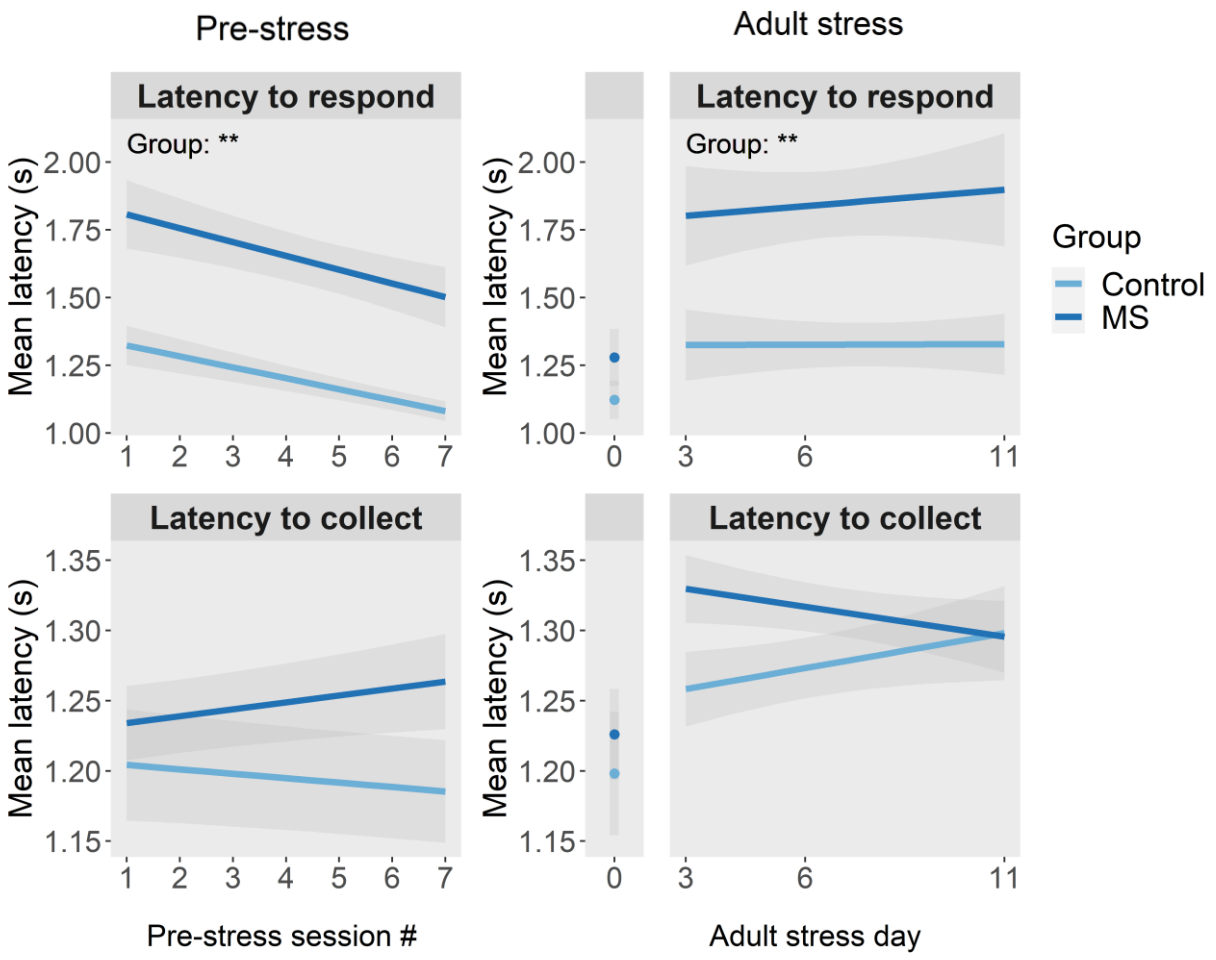

Figure 6. Repeated maternal separation (MS) caused a long-lasting slowing of decision making on the PRL task, and adult stress exacerbated this effect. Animals were tested on the spatial probabilistic reversal learning (PRL) paradigm, in which they were presented with two visually identical stimuli on a touchscreen and had to select one to respond on. The "correct" target was rewarded 80% of the time and punished (signalled non-reward) 20% of the time, while these probabilities were inverted for the "incorrect" target. After 8 consecutive responses on the correct target, the correct and incorrect targets were reversed. The number of reversals completed per session was typically between 2-5. Left panels: Across their first seven PRL sessions, MS animals (n = 24) were consistently slower than controls (n = 24) to respond on one of the two stimuli, while exhibiting a similar latency to collect reward as controls. Right panels: Animals underwent daily probabilistic reversal learning (PRL) testing until commencement of the adult stress, after which they were tested on two (MS n = 24-25, control n = 23) or three (MS n = 18, control n = 16) further sessions. Each animal's median performance across the final five sessions before commencement of adult stress was taken as its baseline (stress day 0). Regression lines shown across stress days 3-11 are adjusted for any influence of baseline, and thus differences represent differential effects of stress. Adult stress increased latency to respond for MS animals considerably more than it did for controls. Adult stress also increased latency to collect, but not significantly differently for MS and control animals.

3.3.7 RMS caused an initial desensitising of females and sensitising of males to the negative outcome on the PRL task, impairing and improving task performance respectively

Regarding the non-latency pre-stress analyses (Figures 7, 8, and S3.3), the group × gender × session interaction term was significant for two response variables: correct target selection ($F_{1,284} = 11.28$, $p = 0.001$) and shift proportion following incorrect losses ($F_{1,284} = 15.24$, $p < 0.0001$). Each three-way interaction was followed up by constructing a separate mixed-effects model for each gender.

The female-specific mixed-effects models (Figure 7) revealed a significant group × session interaction on shifting following a loss on the incorrect target ($F_{1,112} = 6.25$, $p = 0.015$), and a trend for a group × session interaction on overall task performance ($F_{1,112} = 4.17$, $p = 0.099$). Regarding the incorrect-loss shift proportion, maternally separated animals initially performed worse at session one, shifting only 54.1 ± 2.4 % of the time compared to 63.9 ± 1.6 % for controls ($t_{55.3} = 3.36$, $p = 0.006$), but by session seven there was no difference between groups, with RMS animals shifting 61.2 ± 2.2 % of the time and controls shifting 60.9 ± 1.3 % of the time ($t_{55.3} = -0.10$, $p = 0.91$). While post-hoc testing was not performed for overall task performance due to the trend significance level, similar trajectories were evident: RMS females initially touched the correct target only 61.6 ± 1.9 % of the time compared to controls at 65.8 ± 0.8 %, but by session seven this difference had closed, with RMS animals scoring 64.7 ± 0.9 % and controls scoring 65.4 ± 1.8 %.

For males, both response variables had significant group × session interactions (Figure 8). With respect to correct target selection ($F_{1,172} = 7.52$, $p = 0.004$) and shifting following losses on the incorrect target ($F_{1,172} = 9.86$, $p = 0.003$), the performance of maternally separated males worsened or remained constant while controls improved over time. Initially, RMS males achieved better task performance, selecting the correct stimulus 68.4 ± 0.8 % of the time compared to 65.4 ± 0.9 % of the time for controls ($t_{75.4} = -2.60$, $p = 0.030$), but by day seven this discrepancy had abated, with RMS animals scoring 68.2 ± 0.6 % and control animals scoring 69.3 ± 0.5 % ($t_{75.4} = 0.98$, $p = 0.20$). On incorrect-loss shift proportion, RMS animals initially performed non-significantly better, at 65.9 ± 1.6 % shifting vs 60.5 ± 2.1 % shifting for controls ($t_{91.7} = -2.25$, $p = 0.068$), but by day seven, controls performed

significantly better, at 66.5 ± 1.4 % shifting vs 61.3 ± 1.1 % shifting for RMS animals ($t_{91.7}$ = 2.16, p = 0.008).

There were no other main effects of group, and similarly the group × gender interaction term was not significant for any response variables in the pre-stress analysis.

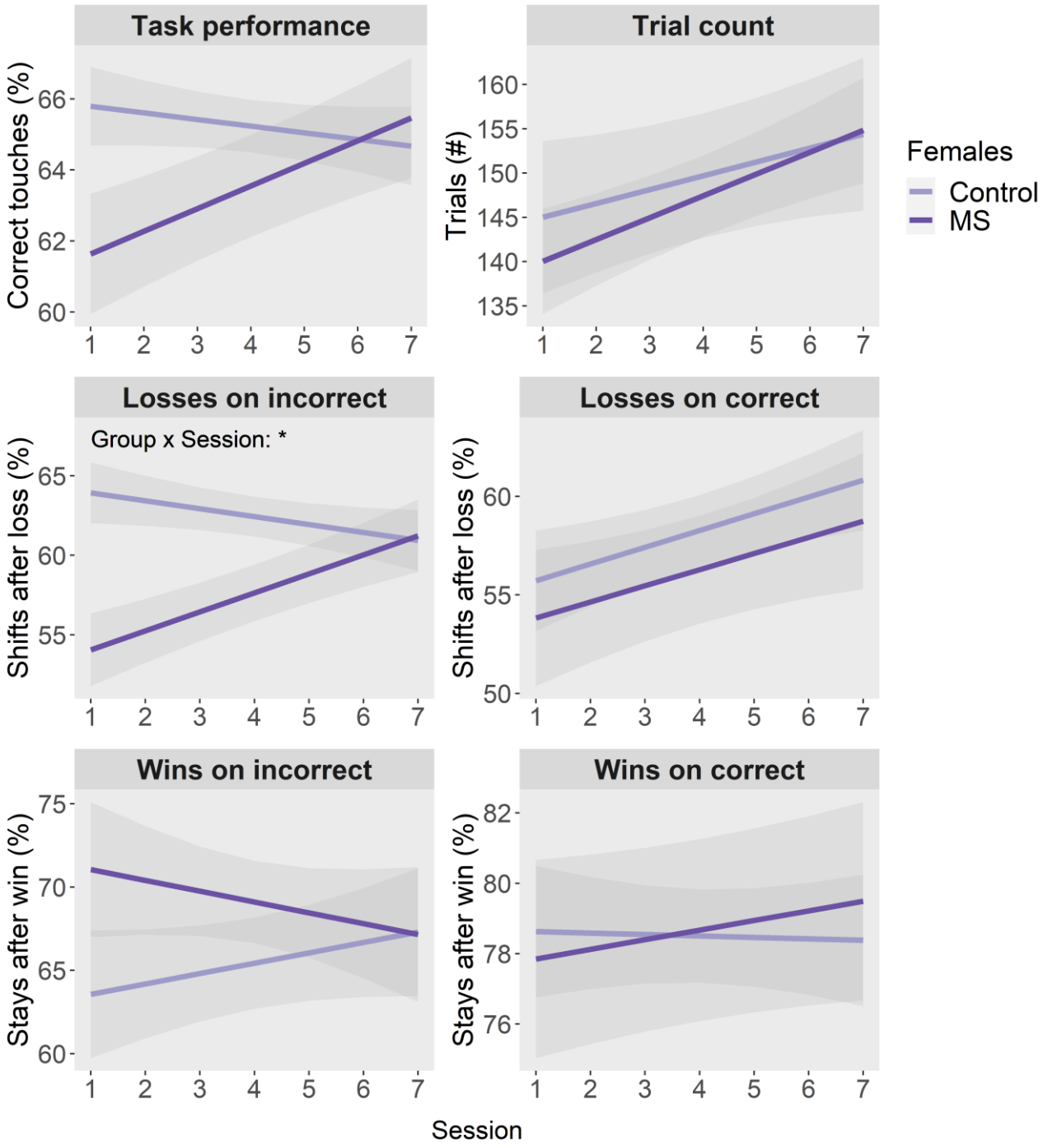

Figure 7. Repeated maternal separation (MS) females initially responded less appropriately to repeated non-reward. During the first of the seven initial probabilistic reversal learning (PRL) sessions, in situations where animals had selected the incorrect (i.e. historically 80% non-rewarded) target on the previous trial and again 'lost' (i.e. received no reward), MS females (n = 9) chose the incorrect target again more often than controls (n = 10). Correspondingly, on overall task performance, there was a trend for an interaction between group and session (p = 0.099). Groups did not differ on other measures of punishment or reward sensitivity, or in trials completed.

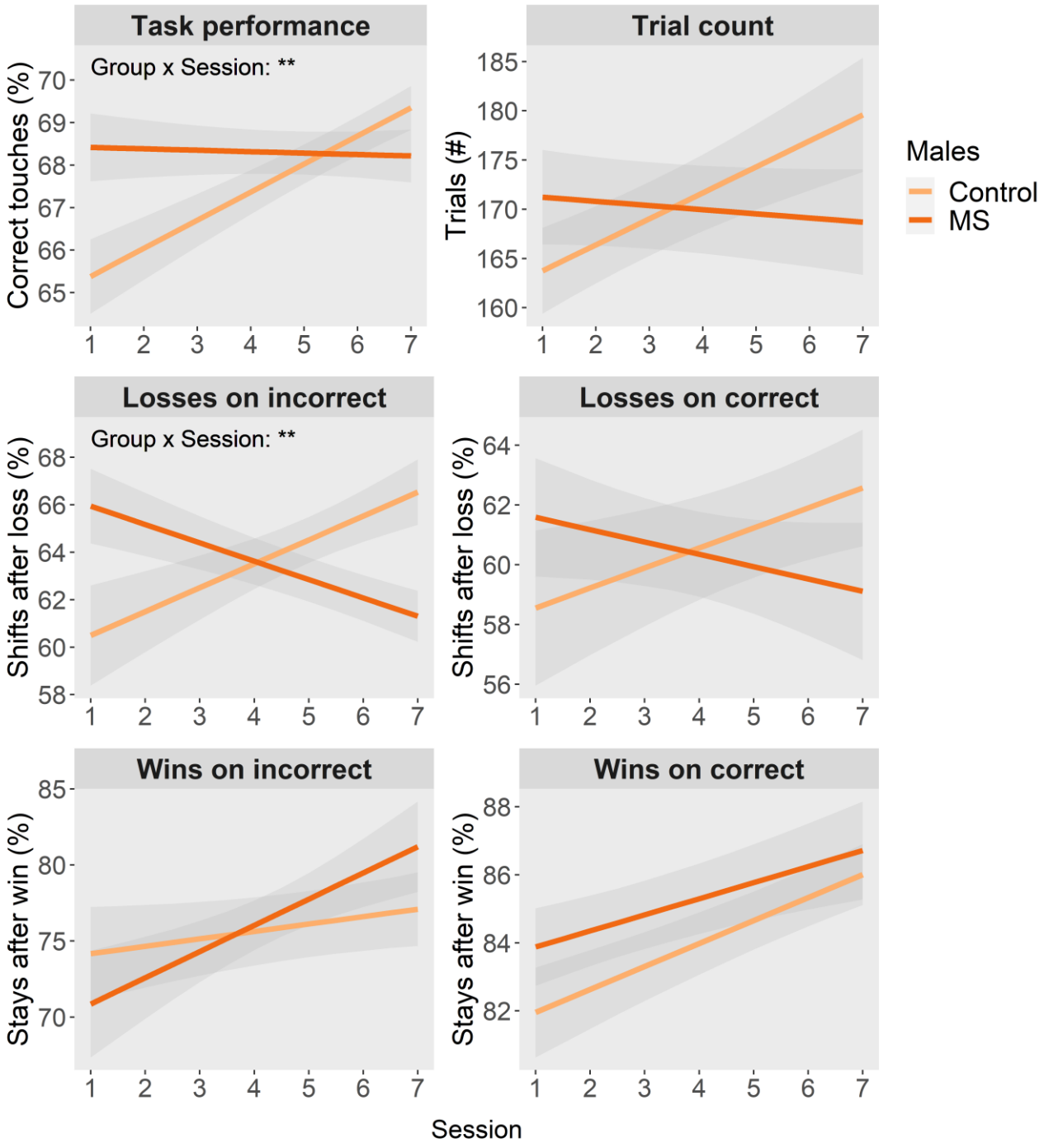

Figure 8. Repeated maternal separation (MS) males initially responded more appropriately to repeated non-reward. During the first of the seven initial probabilistic reversal learning (PRL) sessions, MS males (n = 15) selected the correct target more often than controls (n = 14). This seemed to be driven by the tendency of MS males to switch more frequently than controls to the correct target in the context of having selected the incorrect (i.e. historically 80% non-rewarded) target on the previous trial and then having 'lost' (i.e. received signalled non-reward, or 'punishment'). Groups did not differ on other measures of punishment or reward sensitivity, or in trials completed.

3.3.8 RMS caused a long-lasting susceptibility to stress-induced impairment of PRL task performance, likely driven predominantly by stress-induced desensitisation to the negative outcome

Regarding the non-latency intra-stress analyses (Figures 9 and S3.3), two significant effects involving group were identified, both after controlling for any influence of pre-stress baseline on intra-stress values of each metric.

For overall task performance, there was a significant interaction between group and stress day ($F_{1,88.4} = 6.82$, $p = 0.003$). Initially, at stress day three, RMS animals performed worse, with 63.1 ± 0.8 % correct touches vs controls with 66.2 ± 0.7 % correct ($t_{107.5} = 2.60$, $p = 0.009$), but performance of RMS animals recovered such that by day eleven there was a trend for worse performance by controls (RMS 67.3 ± 1.1 % vs control 65.1 ± 0.7 % correct, $t_{118.6} = -1.54$, $p = 0.083$).

For incorrect-loss shift proportion, there was a trend for a group by stress day interaction that followed the same trajectory as for correct target selection ($F_{1,87.9} = 3.24$, $p = 0.055$). RMS animals initially performed worse, with 60.2 ± 1.2 % shifting compared to 64.4 ± 1.4 % shifting, but ultimately had similar shift proportions, with RMS animals at 65.4 ± 2.0 % compared to 62.8 ± 1.6 % for controls.

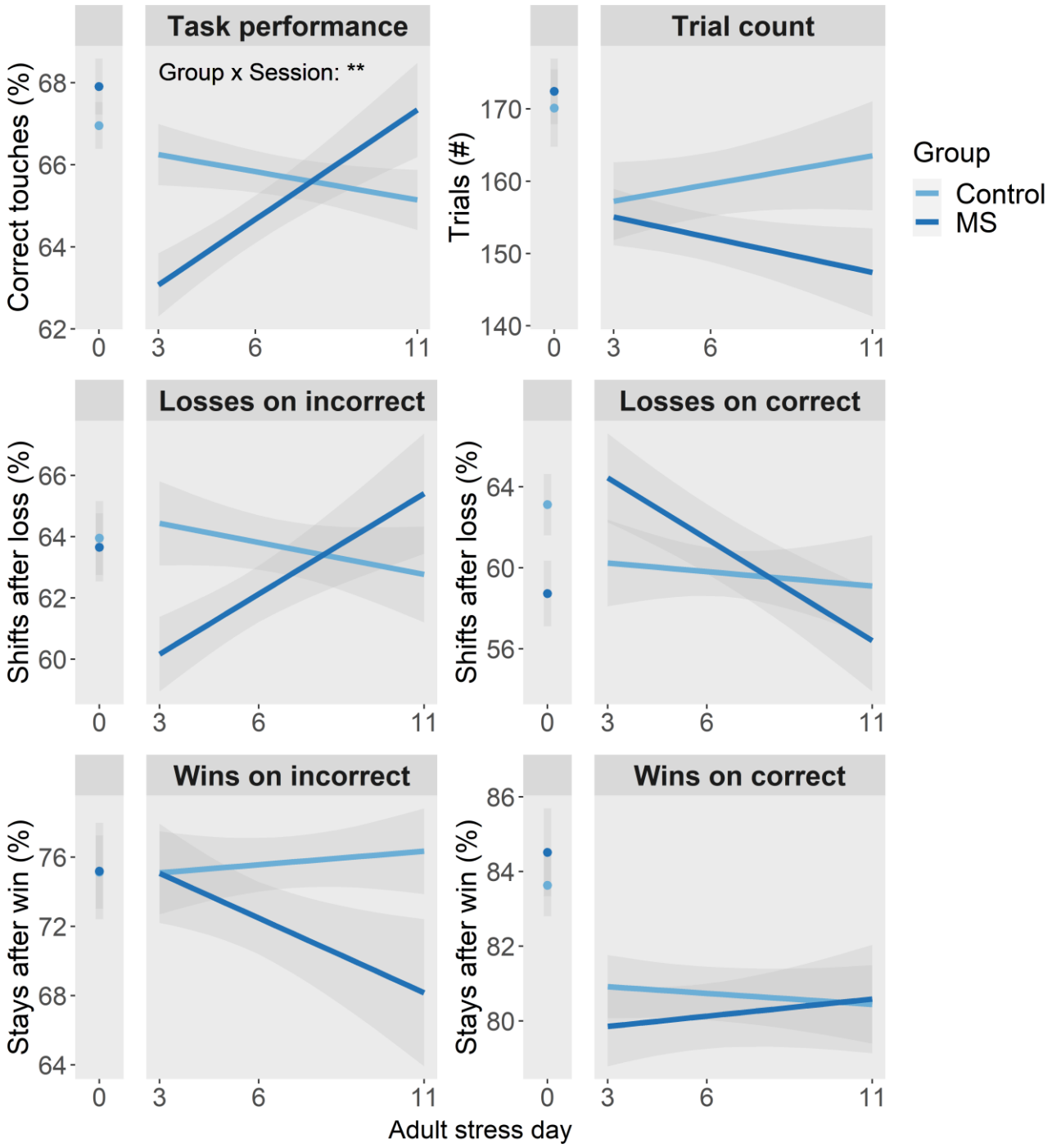

Figure 9. Repeated maternal separation (MS) conferred a long-lasting susceptibility to greater stress-induced impairment of PRL performance. Following application of the adult stress, there was a significant group × session effect on task performance and a trend for a group × session effect on incorrect-loss shift proportion. The former interaction revealed a significant effect of group at day 3 of stress. No significant effects involving group were observed on the trial count or target choice in any of the other three decisional contexts. At stress days 3-6, MS n = 24-25 and control n = 23. At stress day 11, MS n = 18 and control n = 16.

3.3.9 Incorrect-loss shift proportion and correct-win stay proportion are specific and sensitive indices of punishment and reward sensitivity

As noted above, in my study, there were effects within both genders of RMS on incorrect-loss shift proportion, but not on correct-loss shift proportion. Both metrics, along with last-trial-loss shift proportion, have been interpreted as representing punishment sensitivity, so a brief discussion and further analyses are provided to probe the discrepancy.

In the PRL literature relating to stress and depression, incorrect-loss shift proportion has only occasionally been calculated (Dickstein et al., 2010). Simultaneously, correct-loss shift proportion or count has been frequently calculated and often interpreted as reflecting sensitivity to negative feedback (Murphy et al., 2003; Taylor Tavares et al., 2008; Dickstein et al., 2010; Dombrovski et al., 2010; Dombrovski et al., 2015). The rationale is that subjects that shift more frequently than others following 'misleading' punishment on the correct target might potentially be interpreted as being inappropriately hypersensitive to the loss, because a less sensitive subject would ignore the negative feedback and re-select the historically rewarded correct target. However, while increases in this metric could indeed be driven by increased sensitivity to punishment, they could equally be driven by decreased sensitivity to reward. If reward has any influence on future decision-making beyond the immediate-next trial, then an animal who is less sensitive to reward than another animal would be less likely to choose the correct target again after a correct-loss, even if both animals are equally sensitive to punishment.

Both correct-loss shift proportion and incorrect-loss shift proportion are calculated using trial pairs where there was a loss on the first trial, so the discrepancy must be explained by the outcomes of previous trials. One possible explanation is that RMS and control animals are specifically differentially influenced by the historical punishment typically associated with the incorrect target, while somehow not being differentially sensitive to immediate-past punishment given the lack of any RMS effect on correct-loss shift proportion. In other words, the hypothesis is that past punishment, but not current punishment, affects the groups' future decision making differently. Another explanation is that the historical reward associated with the correct target is influencing future decision making to such a degree that it obscures between-group differences in sensitivity to punishment that would otherwise be apparent in correct-loss shift proportion.

To probe these possible explanations, I constructed three mixed effects logistic regression models, each of which predicted the probability of an animal selecting the same target as it selected on the past 1, 2, and 3 trials, based on group, gender, session number, and the outcome of the past trial (reward or punishment). This modelling yielded two key insights.

Firstly, where animals were punished on the immediate-past trial, there were significant effects of group (for females) or group × session (for males) that were extremely similar to the effects of group or group × session on incorrect-loss shift behaviour, whereas there was no differential effect of 2^{nd}- or 3^{rd}-last trial punishment on the future decisions of RMS and control animals (Figure 10 or see Appendices 7.1.1.8 and 7.1.1.9 for detailed statistics). This provides strong evidence against the hypothesis that the discrepancy between the incorrect-loss shift and correct-loss shift results is due to a differential impact of historical punishment between groups.

Secondly, for both groups and in both genders, the effect of historical reward on future decision making was greater than the effect of immediate-past punishment (indicated by the larger distance of the reward trendlines than the punishment trendlines from the dashed chance-decision line), suggesting that the correct-loss shift metric may actually be influenced more by reward sensitivity than punishment sensitivity. Indeed, this hypothesis was supported by the higher degree of correlation of correct-loss shift proportion with correct-win stay proportion ($\rho = 0.28$) than with incorrect-loss shift proportion ($\rho = 0.18$) (Figure 11). To follow this up, I conducted a multiple regression in which I predicted correct-loss shift proportion from the correct-win stay and incorrect-loss shift proportions, across all pre-stress sessions. There was a highly significant effect of reward sensitivity ($F_{1,333} = 31.02$, $p = 5e\text{-}08$), while punishment sensitivity was not even significantly predictive ($F_{1,333} = 3.01$, $p = 0.084$). Even last-trial-loss shift was corrupted to some extent by reward sensitivity, because although incorrect-lost shift ($F_{1,333} = 763.61$, $p = 3e\text{-}88$) predicted a much greater portion of the signal, correct-win stay ($F_{1,333} = 14.41$, $p = 2e\text{-}04$) was also significantly predictive. This was not surprising, given that roughly one third (34%) of all losses were correct-losses. Meanwhile, last-trial-win stay proportion was a similarly pure measure to correct-win-stay proportion of reward sensitivity ($F_{1,333} = 5004.22$, $p = <2e\text{-}16$), given that incorrect-loss shift had no ability to predict it ($F_{1,333} = 0.87$, $p = 0.35$). Again, this made sense, given that 89% of all wins were correct-wins, so there was little historical punishment that could have obscured the reward sensitivity signal.

Altogether, these results support the idea that incorrect-loss shift proportion is a more sensitive and specific index of sensitivity to negative outcomes than both correct-loss shift proportion and conventional (any target) last-trial-loss shift proportion. Because both of these latter metrics are calculated using trial pairs where the first trial is or may be the correct target, they will both be impacted by between-subject differences in reward sensitivity. The influence of historical reward on future decision making makes these metrics comparatively noisy and also vulnerable to confounding by between-group differences in reward sensitivity. Similarly, correct-win stay proportion should represent a relatively sensitive and specific measure of reward sensitivity compared to incorrect-win stay proportion and conventional last-trial-win stay proportion.

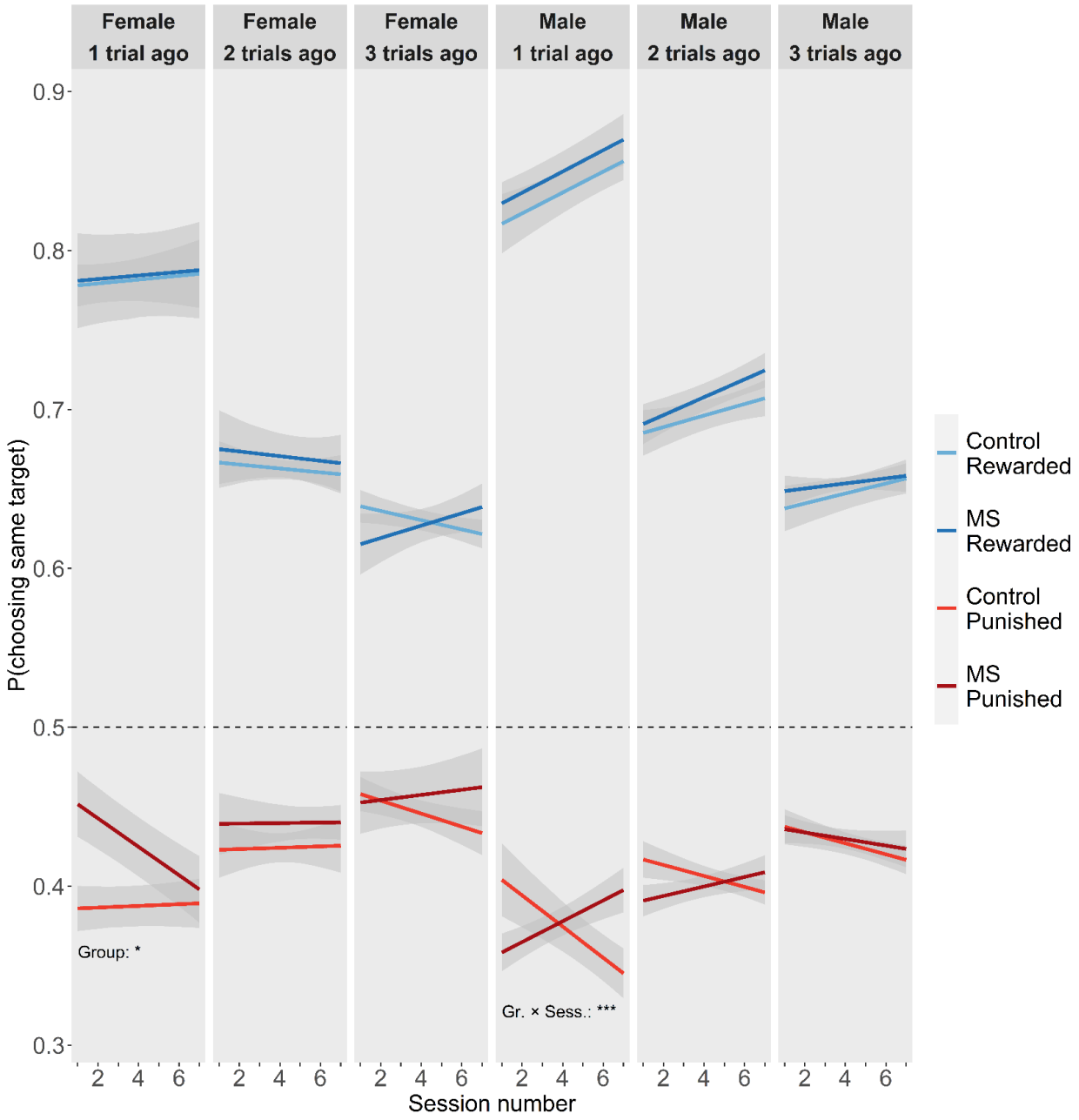

Figure 10. Repeated maternal separation (MS) animals were not differentially influenced by historical (2nd-last-trial or 3rd-last-trial) punishment or reward, and historical reward had a larger influence on future decision making than immediate-past punishment for both groups. Three logistic regression models were constructed which sought to predict animals' decision making based on whether they were punished or rewarded one, two, and three trials ago, as well as based on group, gender, and session number. The significant effects involving group that emerged in this modelling were limited to the model examining the influence of the immediate-past trial outcome, and these effects closely mirrored the described effects on incorrect-loss shift proportion. This figure demonstrates that even historical reward has a larger impact on future decision making than immediate-past non-reward, which likely limits the ability of correct-loss shift proportion to capture a clear punishment sensitivity signal.

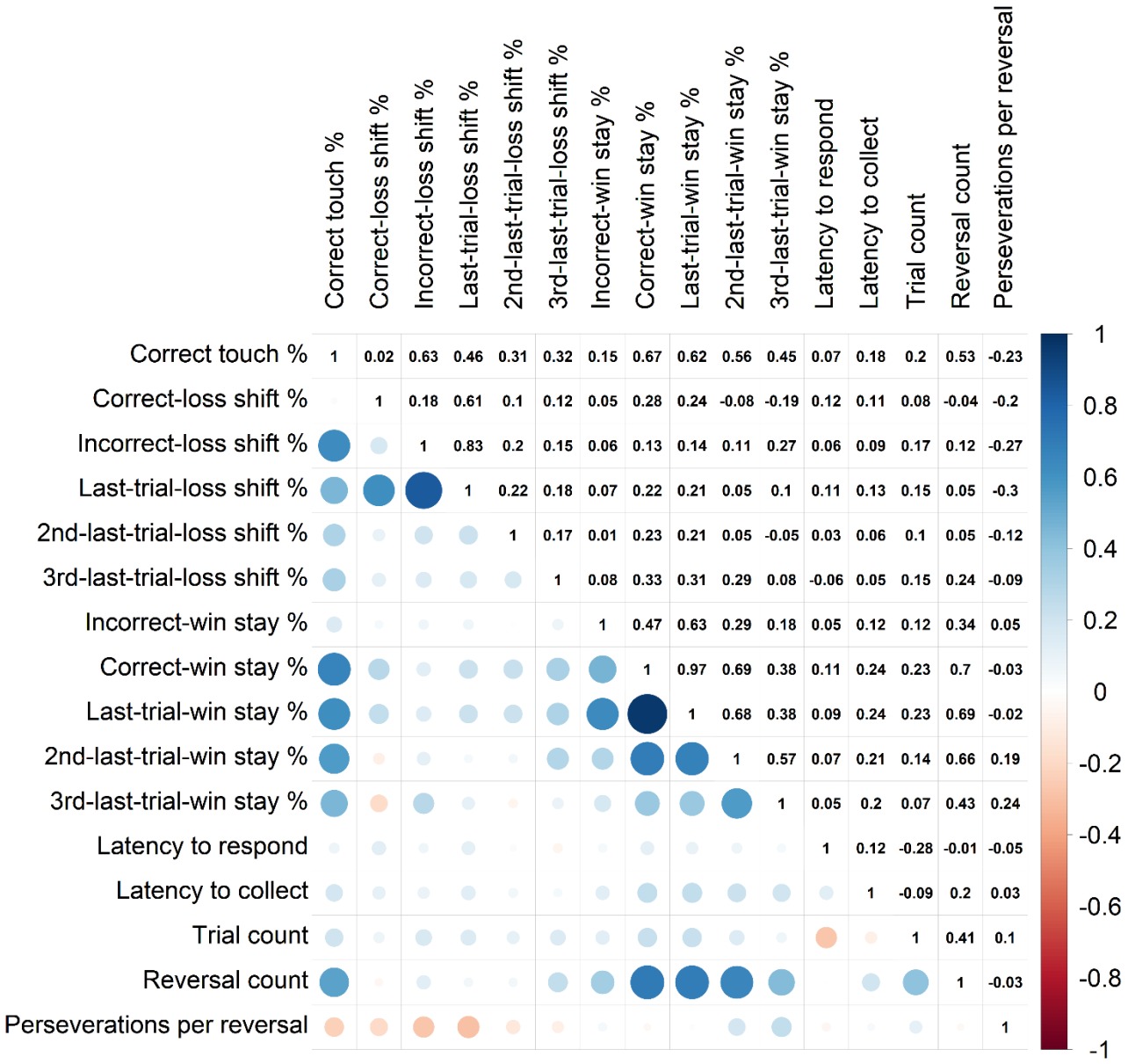

Figure 11. Correlations between session-level probabilistic reversal learning (PRL) metrics calculated using pre-stress sessions. This plot of pairwise spearman's rho yields several important insights. Firstly, task performance (correct touch %) is most strongly predicted by correct-win stay proportion and incorrect-loss shift proportion. Secondly, incorrect-loss shift proportion and correct-win stay proportion are negligibly correlated with one another, indicating that reward and punishment sensitivity are separable aspects of animal behaviour that can be measured using the PRL task. Thirdly, correct-loss shift proportion, which is widely interpreted as a measure of punishment sensitivity, has a greater association with correct-win stay proportion than with incorrect-loss shift proportion, indicating a greater influence on that metric of reward sensitivity than of punishment sensitivity. Finally, latency to respond, which is robustly increased in RMS animals and is increased more in those animals than in controls by adult stress, is orthogonal to all other behavioural metrics.

3.3.10 Punishment sensitivity and reward sensitivity are minimally correlated and together explain most of the variability in PRL task performance

Across all pre-stress sessions, correct-win stay proportion had only a weak correlation (ρ = 0.13) with incorrect-loss shift proportion, indicating that reward sensitivity and punishment sensitivity are minimally related and represent distinct aspects of animal behaviour, even though punishment is signalled non-reward. Exploratory data visualisation revealed that both variables had a fully linear relationship with correct touch proportion. In a linear model in which only these two terms were used to predict correct touch proportion, the adjusted R-squared indicated that these metrics together accounted for 81.04% of the variability in task performance. The F statistic for correct-win stay proportion ($F_{1,333}$ = 699.56, p = 8e-84) was 25% larger than the statistic for incorrect-loss shift proportion ($F_{1,333}$ = 559.59, p = 3e-73), indicating that differences in reward sensitivity between animals and between sessions accounted for a slightly larger proportion of the variability in task performance than punishment sensitivity did.

3.4 Discussion

3.4.1 RMS, weight, and energy balance

Here, I reported that RMS animals had a lower body weight over the first four weeks following conclusion of RMS, although these findings were only significant on post-hoc testing conducted despite the absence of an interaction effect between group and age.

These findings are broadly consistent with those of previous investigators. Studies generally report that RMS animals have a lower body weight at or shortly after the conclusion of RMS (Desbonnet et al., 2010; Meagher et al., 2010; Nakamura et al., 2011; Grassi-Oliveira et al., 2016; Arabameri et al., 2017; Moya-Pérez et al., 2017), although no difference is sometimes found (Rüedi-Bettschen et al., 2006; Chocyk et al., 2013). Where body weight in later life has been reported, these studies have often reported normalisation of body weight by young adulthood (Desbonnet et al., 2010; Grassi-Oliveira et al., 2016; Moya-Pérez et al., 2017; Huang et al., 2021), although other studies have reported that RMS animals were still underweight by young adulthood (Nakamura et al., 2011; Paternain et al.,

2012), while other studies reported increased weight of RMS animals in adulthood (Avitsur and Sheridan, 2009; Kiank et al., 2009).

In the present study, it is possible that both lower energy intake and higher energy expenditure contributed to the initially lower weight. In a recent systematic review, it was reported that where studies have assessed the effect of RMS in rats on arched-back (active) nursing and/or passive nursing, the majority of studies found that dams demonstrated increased active nursing and no change to passive nursing (Orso et al., 2019). This includes studies that have exhaustively measured nursing behaviour at numerous times of day, which have consistently reported more than double the frequency of arched-back nursing compared to control dams over the immediate-post-RMS observation periods (Rüedi-Bettschen et al., 2006; Couto et al., 2012; Chocyk et al., 2013; Biggio et al., 2014), but which have also reported elevated arched-back nursing up to seven hours later (Rüedi-Bettschen et al., 2006; Couto et al., 2012; Chocyk et al., 2013), and even in the hour before the next RMS episode was due to commence (Rüedi-Bettschen et al., 2006; Chocyk et al., 2013). Further, because these increases in arched-back nursing have been reported to co-occur with normal levels of passive nursing, even where many observation periods per day have been used, RMS animals appear to receive increased total nursing across the whole non-separation period compared to controls (Chocyk et al., 2013). In the two of these studies that reported body weight at the conclusion of RMS, this increased nursing was evidently sufficient to overcome the missed nursing time, as body weight in RMS animals was equal to controls (Rüedi-Bettschen et al., 2006; Chocyk et al., 2013). However, these statistics regarding maternal behaviour all refer to the periods where both RMS and control animals were in their home cages with their respective dams and did not attempt to subtract the nursing missed while RMS animals were undergoing separation. Further, separation duration in most of these studies was three hours, which is considerably shorter than the present six hours. Even though nursing is considerably increased by RMS, it is possible that where nursing is restricted to only 75% of a 24-hour period, total energy consumption is still less than that of a control animal.

Further evidence that under-consumption relative to controls may be playing a role in the relatively lower weight comes from adulthood chronic stressors. Chronic stress applied to adult rats has consistently been reported to cause weight loss or reduced weight gain, such as in the chronic unpredictable stress paradigm (Hodes et al., 2015; Park et al., 2017; Ramaker and Dulawa, 2017), the chronic footshock paradigm (Gerrits et al., 2005; Dagytė et al., 2010; Bobrovskaya et al., 2013), or chronic restraint (Harris et al., 2002). However, in many cases,

such as for chronic unpredictable stress (Varga et al., 2011; Pastor-Ciurana et al., 2014), chronic footshock (Dagytė et al., 2010), and chronic restraint (Martí et al., 1994; Martí et al., 1999; Harris et al., 2002; Jeong et al., 2013; Pastor-Ciurana et al., 2014), lower food intake has been reported alongside the lower body weight, indicating that stress can suppress efforts to consume food.

Aside from reduced energy intake, the other possible explanation for the weight loss often found in the short term in RMS animals is increased energy expenditure during the RMS period. No studies have directly measured energy expenditure or mitochondrial oxygen consumption in RMS animals, although a few studies have measured these parameters in other chronic stress models. Such studies have either used indirect calorimetry to assess energy expenditure, which depends on the use of an enclosed cage together with careful monitoring of oxygen and carbon dioxide levels, or have examined mitochondrial oxygen consumption. In one study using the chronic unpredictable stress (CUS) paradigm, it was reported that CUS animals exhibited less locomotor activity per 24 hours, together with less total energy expenditure per 24 hours, but that energy expenditure during periods of immobility (e.g. sleep), was no different to controls (Li et al., 2019). In another study, oxygen consumption by forebrain mitochondria was examined following 14 days of daily restraint stress, and reduced oxygen consumption was reported in restrained animals (Kambe and Miyata, 2015). A third study found that CMS animals had increased energy expenditure during a 2-hour indirect calorimetry measurement, but unlike the animals in the earlier study, these animals were found to have increased rather than decreased locomotor activity (García-Díaz et al., 2007). Altogether, these findings suggest that whether or not RMS results in increased, decreased, or no change to daily locomotor activity will likely be the primary driver of whether or not RMS animals have increased, decreased, or no change respectively to their daily energy expenditure.

Unfortunately, there are no data describing the relative daily locomotor activity of RMS pups compared to control pups. However, ultrasonic vocalizations (USVs) also require energy to produce and have been studied in RMS animals. USVs are a form of pup behaviour that serve to command the attention of the dam and are expressed under conditions of pup distress (Kaidbey et al., 2019; Burenkova et al., 2020). While several studies have measured differences in USV expression between RMS and control pups while pups from both groups are separated for testing (Ognibene et al., 2007; Kaidbey et al., 2019; Granata et al., 2021; Park et al., 2021), to my knowledge only one study has examined USVs before, during, and

after RMS in comparison to control animals kept in their home cage during USV recording (Burenkova et al., 2020). In this study, USVs were higher in RMS animals than controls during the 30 minutes before an RMS episode was due to start, over a 30-minute separation period, during 5 minutes in a novel environment, and during the first 30 minutes following reunion with the dam. USVs were highest during the brief novel environment exposure, followed by the beginning of the separation and post-reunion periods. Altogether, it seems plausible that, particularly during this time of near-total dependence on their mothers to meet basic physiological needs, RMS animals may have greater energy expenditure during the RMS period, but uncertainty remains, especially given findings in adult chronic stress models of eventually-reduced daily locomotor activity (Li et al., 2019).

3.4.2 RMS, anxiety-like behaviour, and response to novelty

Here, I used the elevated plus maze (EPM) to measure anxiety-like behaviour so that if it was affected by RMS, the findings from the PRL testing could be interpreted in this context. I found no effect of RMS on anxiety-like behaviour on this task. Findings regarding the effects of RMS on anxiety-like behaviour in classic tests, such as the EPM, OFT, and light-dark box are deeply inconsistent, as discussed previously by others (Rees et al., 2006). In adulthood, where no further stressor was documented, some studies have reported that RMS animals had increased anxiety-like behaviour on the OFT, as indicated by lower time, entries, or distance moved in the centre region (Huot et al., 2001; Roque et al., 2014; Shu et al., 2015), but many studies have reported no effect on EPM open arm time (Kaneko et al., 1994; Maniam and Morris, 2010b; Borges-Aguiar et al., 2018) or OFT centre activity (Shalev and Kafkafi, 2002; Harrison et al., 2014; Roque et al., 2014; Dimatelis et al., 2016; Borges-Aguiar et al., 2018), and decreased anxiety-like behaviour on the EPM and light-dark box has even been reported (Zhang et al., 2014). Even results regarding the short-term effects of RMS on anxiety-like behaviour are conflicting. In the absence of further stress, there are some reports of increased anxiety-like behaviour on the EPM (Salzberg et al., 2007; Veenema et al., 2008; Amini-Khoei et al., 2019; Dallé et al., 2020) and light-dark box (Majcher-Maślanka et al., 2019), but decreased anxiety has also been reported (Pierce et al., 2014). A recent systematic review of findings in mice that did not stratify findings by time since MS or the presence or absence of other stressors nevertheless summarised this situation well. While 60% of studies in C57BL/6 or BALB/c mice reported increased anxiety-like behaviour in RMS or SEMS animals on one or more tests, 35% reported the opposite: that maternally separated animals exhibited reduced anxiety-like behaviour (Tractenberg et al., 2016).

In addition to the EPM, I also used two other arena-based tasks, the NRT and the NPT, both intended as exploratory measurements to probe whether RMS has effects on response to novelty, which has relevance to the propensity to develop addiction. I observed no effect of RMS on either of these tasks. While novelty preference has not been examined in RMS animals before, several investigators have measured locomotor response to novelty and returned inconsistent results. Into adulthood without the application of any further stress, while RMS has occasionally been reported to either increase (Kaneko et al., 1994) or decrease (Shu et al., 2015) distance moved in an open field, most studies have reported no effect (Shalev and Kafkafi, 2002; O'Mahony et al., 2009; Harrison et al., 2014; Roque et al., 2014; Dimatelis et al., 2016; Gracia-Rubio et al., 2016). In the short-term without further stress, results are inconsistent, with increased (Gracia-Rubio et al., 2016) and decreased (Amini-Khoei et al., 2019) novelty-induced locomotor activity having been reported.

No clear cause for these discrepancies has been identified. Methodological differences between studies are presumably central to the variability in results, but at present it remains unclear which of these many differences are primarily responsible. One likely contributor to the variance is the nature of the control group – specifically, the degree to which control animals were handled during early and later life, if at all. For example, although the EPM is sensitive to inter-individual differences in unconditioned fear of open spaces and heights, handling history and stress exposure history have both been repeatedly shown to also affect putative anxiety-like behaviour on the EPM (Walf and Frye, 2007). Thus, perhaps particularly once the eyelids unseal at PND 13-15 and pups begin to be able to see experimenter-associated visual cues preceding RMS episodes (Langston et al., 2010), those episodes could potentially result in an experimenter-associated conditioned fear response, particularly when compared to equally-handled but unseparated animals. Or, by contrast, despite the associated stress, the repeated handling involved in most RMS protocols could lead to RMS animals having a reduced fear response to future handling compared to animals that have a comparatively low or non-existent handling history. Indeed, compared to leaving animals completely undisturbed during rearing, “early handling”, which involves maternal separation for 5 minutes or less per day (often less than a minute), and even “animal facility rearing”, which typically involves weekly or twice weekly cage changes, have been reported to have beneficial effects on stress-related physiology and ethanol consumption (Nylander and Roman, 2013). Similarly, there is evidence that very brief periods of maternal separation, such as for 10 or 15 minutes, significantly reduce anxiety-like behaviour on the EPM and

OFT compared to non-handled animals (Maniam and Morris, 2010b; Oines et al., 2012). As Tractenberg et al. (2016) lay out in their meta-analysis, other possible culprits include whether pups were separated from one another duration separation episodes, the duration of separation, the genders studied, whether animals were provided with an external heat source during separation, whether separation and reunion occurred during the light or dark phase of the light cycle, which species and strain was used, how much environmental enrichment animals had in their home cage during early and later life, and whether animals were isolation-, pair-, or group-housed following weaning. Another possible contributor is methodological variation in the conduct of the behavioural tests themselves, such as in the lighting intensity and extent of habituation procedures utilised (Cosquer et al., 2005; Walf and Frye, 2007).

3.4.3 RMS and anhedonia

In the present experiment, RMS did not cause a long-lasting anhedonia. I found no differences between RMS and control animals in any of the following measures: sucrose preference at any sucrose concentration tested (including after the novel chronic adult stress), breakpoint on the progressive ratio task, and trial count on the PRL task. Adult RMS animals were just as motivated to take action to consume sucrose as controls were, both in a lower-effort context (the sucrose preference test) and higher-effort contexts (the progressive ratio and PRL tasks). These findings are largely consistent with the existing literature.

When the short-term (pre-adulthood) effects of RMS on the SPT have been examined, a depressive-like effect has commonly been reported (Kundakovic et al., 2013; Gracia-Rubio et al., 2016; Dallé et al., 2020), although in one case reduced sucrose preference was found only in males and not in females (Maniam and Morris, 2010b), and no effect has also been reported (Majcher-Maślanka et al., 2019). However, reported results concerning the long-term effects of RMS on SPT are quite different. The vast majority of studies have reported that RMS has no persistent effect on sucrose preference in the absence of further chronic stress (Shalev and Kafkafi, 2002; Zhang et al., 2013; Harrison et al., 2014; Hill et al., 2014; Chocyk et al., 2015; Feldcamp et al., 2016; Gracia-Rubio et al., 2016; Mrdalj et al., 2016; Borges-Aguiar et al., 2018; Wei et al., 2018; Stuart et al., 2019), although a depressive-like effect has been reported (Shu et al., 2015; Huang et al., 2021), and an anti-depressive-like effect has been reported in females (Chocyk et al., 2015).

Several studies have measured the effects of RMS on motivation for appetitive reward using PR schedules. All have done so in adulthood, and most have reported no long-lasting effect of RMS (Shalev and Kafkafi, 2002; Zhang et al., 2005; Cruz et al., 2008; Stuart et al., 2019; Thornton et al., 2021). There have been some reports of long-lasting alterations in effort expended to obtain appetitive reward (Rüedi-Bettschen et al., 2005; Kosten et al., 2006; Campbell et al., 2017), but there are some notable limitations. In one of these cases, where RMS was conducted during the dark phase of the light cycle, the control group had a mean body weight over 70 g larger than that of the RMS animals at the time of PR testing, indicating larger energy needs and potentially explaining the larger effort for caloric reward (Bosy-Westphal et al., 2004; Rüedi-Bettschen et al., 2005). In the same paper, no effect on PR was found in a separate cohort of animals where RMS was conducted in the light rather than dark phase, but the true cause for the discrepancy between the light and dark phase cohorts may have been that the light phase control group was not disproportionately heavy compared to the RMS group, unlike for the dark phase cohort. Indeed, apart from the reported effect on PR in the dark phase group, no other notable long-lasting effects of either type of RMS were reported on a variety of behavioural and physiological metrics, including weight. In Kosten et al. (2006), the daily duration and total length of RMS was shorter than almost all other RMS studies, at one hour per day over PND 2-9, and an effect was only found on the exponential PR schedule and not the linear PR5 schedule, even though the eventual breakpoints and reward counts were similar for these two schedules. And finally, one study found a trend effect for increased rather than decreased motivation for appetitive reward on PR (Carlyle et al., 2012).

The idea that RMS results in short-term but not long-term anhedonia is consistent with studies of the duration of the depressive-like effects of other chronic stressors, such as chronic unpredictable stress (CUS) and chronic social defeat stress (SDS). Systematic reviews have revealed that these stressors have been shown in many dozens of studies to have depressive-like effects on sucrose preference or sucrose consumption (Vasconcelos et al., 2015; Antoniuk et al., 2019), but studies of the time-course of this effect have shown that the depressive-like behaviour seems to worsen linearly over time with continued application of the stressor (Elizalde et al., 2008; Stedenfeld et al., 2011; Erburu et al., 2015; Papp et al., 2016; Gao et al., 2017), and then begin to recover linearly with time as soon as the stressor is removed, generally in a similar or shorter amount of time than the total duration of stressor application (D'Aquila et al., 1997; Orsetti et al., 2006; Elizalde et al., 2008; Kinn Rød et al.,

2012; Alves et al., 2017; Park et al., 2017). Similarly, chronic daily social defeat (Miczek et al., 2011; Bergamini et al., 2016) but not short-term intermittent social defeat (Miczek et al., 2011), chronic unpredictable stress (Iguchi et al., 2015; Spierling et al., 2017; Picard et al., 2021), chronic footshock (Scheggi et al., 2018), and chronic corticosterone (Olausson et al., 2013; Dieterich et al., 2019) have all been shown to acutely reduce motivation for reward on the PR task. However, again there is evidence that these effects are short-lived, because while CUS caused reduced lever presses for saccharin solution on a progressive ratio task at 2 and 11 days after CUS conclusion, these effects were no longer significant at 19 days, and that there was no trace of them by 4 weeks after CUS (Iguchi et al., 2015). Supporting mechanistic evidence has emerged from studies where the effect of both stressors on mesolimbic dopamine (DA) system functioning has been measured directly using ventral tegmental area self-stimulation paradigms. Dysfunction of the mesolimbic DA system, which has been robustly implicated in motivational anhedonia in both humans and animal models (Der-Avakian and Markou, 2010; Der-Avakian and Markou, 2012; Pizzagalli, 2014; Treadway et al., 2019), has been shown to progressively worsen with continued CUS or SDS application (Moreau et al., 1994; Gottschalk et al., 2018), and to then quickly recover linearly with time in under two weeks following stressor cessation (Moreau et al., 1994) or over four weeks of fluoxetine administration (Gottschalk et al., 2018). These converging lines of evidence suggest that given time, rodents usually recover from stress-induced anhedonia.

Altogether, it appears that the effects of chronic stress paradigms such as RMS, CUS, and SDS on anhedonia are unlikely to persist very long under most experimental protocols. Indeed, the research community seems largely aware of this, given the findings of an informal survey I conducted of 50 consecutive recent papers in 5 major psychiatry journals (Molecular Psychiatry, Biological Psychiatry, Neuropsychopharmacology, Translational Psychiatry, and Brain, Behaviour, and Immunity) where the authors described measuring ‘depressive’ or ‘depression-like’ behaviour in rodents. Among these 50 primary papers, only three classes of measure were used in more than three papers: sucrose preference or absolute sucrose consumption tests, the forced swim test, and social exploration or interaction tests. In almost 80% of cases, the authors chose to conduct their behavioural measurements during or within 1 week of the conclusion of the induction paradigm, whether it was a psychosocial stressor or another intervention like a pro-inflammatory stimulus. Further, among these comparisons, over 90% revealed a depression-like effect of the model relative to controls,

whereas for measurements taken over 1 week after the conclusion of the induction paradigm, the majority of comparisons were negative for a depressive-like effect of the model.

3.4.4 RMS and punishment and reward sensitivity

Here, I provided a detailed characterisation of the long-lasting PRL phenotype of RMS, no aspect of which has been previously described. RMS and control animals did not differ on the PRL task with respect to several traits measured: behavioural flexibility as measured by reversal count and perseverations per reversal, motivation for reward as measured by trial count, and locomotor functioning as measured by latency to collect reward. However, RMS had long-lasting effects on two distinct aspects of animals' behaviour on the PRL task. First, RMS animals, regardless of gender, were considerably slower to select between the two stimuli across all seven initial PRL sessions (see Section 3.4.5 for a discussion of this effect). Second, RMS desensitised females and sensitised males to the negative possible outcomes of their decisions on the task, as indicted by their initially lower and higher incorrect-loss shift proportion respectively. This decreased and increased sensitivity to punishment appeared to drive parallel differences in overall task performance, with RMS females initially performing non-significantly worse than controls and RMS males initially performing significantly better. The fact that these initial differences between groups later converged suggests that groups had differing cognitive responses to punishment at baseline that were eventually updated after hundreds of trials through learning, the rate of which may have been unchanged or differed only as a function of the baseline difference.

What is particularly fascinating about these alterations to RMS animals' response times and responding to negative outcomes is that they persisted late into adulthood despite the absence of classical "depression-like" or "anxiety-like" behaviour. It is worth examining the evidence regarding the relation of these phenotypes to depression and anxiety in humans and other animal models, in order to establish whether they may represent endophenotypes or vulnerability factors for one or both of these disorders. If they represent either, then RMS may be a useful model with which to interrogate disease-related mechanisms.

3.4.4.1 Punishment and reward sensitivity in human depression

Several studies have attempted to interrogate the relationship between depression symptoms or diagnosis and punishment or reward sensitivity on PRL. Unfortunately, such studies have often only reported metrics that provide insight into one of these properties, but not both, and they often have serious other limitations. Two separate studies using

computational reinforcement learning of PRL data collected from MDD patients (Mukherjee et al., 2020) and healthy subjects with no psychiatric disorders (Ogishima et al., 2020) have reported that depressive symptoms are associated with a lower estimate of the value sensitivity parameter. How this is generally interpreted is that depressed subjects care less than healthy subjects whether their actions result in the positive outcome or the negative outcome. This finding could be explained by depressed people exhibiting reduced "wanting" (with or without reduced "liking") of the positive outcome, reduced aversion to the negative outcome, or both. Mukherjee et al. (2020), representing the largest study of PRL in MDD to date, provides considerable evidence for reduced reward sensitivity in depressed subjects on the PRL task, including among other findings a significantly lower last-trial-win stay proportion on two different versions of the task. Unfortunately, punishment sensitivity was not robustly probed. Last-trial-loss shift proportion was reported, and was non-significantly lower in depressed subjects, suggesting the possibility of reduced punishment sensitivity. However, as discussed earlier, given that these subjects display lower reward sensitivity, they will be more likely to shift following correct-losses, so it is possible that the lower reward sensitivity is pushing the last-trial-loss shift proportion upward in depressed subjects, masking what would otherwise be a significant reduction in punishment sensitivity (e.g. a lower incorrect-loss-shift proportion). Indeed, in line with the hypothesis that depressed patients have a lower punishment sensitivity on PRL, a further study featuring a well-matched control group reported that MDD patients had a reduced last-trial-loss shift proportion (Bakic et al., 2017). In another study, in children and adolescents, while MDD patients had a lower correct-win stay proportion, they did not have a lower incorrect-loss-shift proportion, suggesting reduced reward sensitivity but unaffected punishment sensitivity (Dickstein et al., 2010). However, in this study, the MDD patients were highly heterogeneous, with most also having a diagnosis of one of four different anxiety disorders.

Prior to these relatively recent studies, the prevailing hypothesis was that MDD patients had elevated, rather than reduced or unchanged, sensitivity to punishment on the PRL task. Two early studies reported increased correct-loss-shift proportion in depressed patients, and in both cases this was interpreted as reflecting greater sensitivity to punishment (Murphy et al., 2003; Taylor Tavares et al., 2008). However, as discussed earlier, correct-loss-shift proportion is under the influence of both punishment and reward sensitivity, and neither study provided metrics capable of indicating sensitivity to reward, so it is unclear whether lower reward sensitivity may have been driving the higher correct-loss-shift proportion. Indeed, in

the one study of MDD where correct-loss behaviour was reported alongside correct-win and incorrect-loss behaviour, correct-loss-shift proportion was increased in MDD, but correct-win-stay proportion was decreased while incorrect-loss-shift proportion was unaffected, suggesting that the larger correct-loss-shift proportion was a result of lower reward sensitivity rather than higher punishment sensitivity (Dickstein et al., 2010). In another study, among several small groups of elderly depressed patients, the group with a history of suicide attempts had increased last-trial-loss shift count (Dombrovski et al., 2010). However, because shift tendency was not reported as a proportion of losses, it is unclear whether this result was simply driven by a greater number of losses, which they likely exhibited given that those patients performed worse on the task than controls. In that study, the other two groups of depressed patients did not have a different mean last-trial-loss shift count to controls. Additionally, the depressed groups (and especially the suicide-attempter group) were disproportionately female and had a greater lifetime history of substance use disorders. These authors later found the same result, i.e. increased absolute last-trial-loss shifts, in a sample of roughly 30 depressed patients, but the same limitations apply, and additionally the depressed patients were significantly older than the controls, and it seems that many of the subjects were the same as those in the previous study (Dombrovski et al., 2015). Another two studies, also not using proportions and only reporting the confounded correct-loss behaviour, found no relationship between MDD presence and absolute correct-loss stays or shifts (Remijnse et al., 2009; Adoue et al., 2015).

Altogether, the weight of the evidence now suggests that MDD is associated with reduced sensitivity to reward on the PRL task, and either reduced or unaffected punishment sensitivity. Further studies and re-analysis of published studies will be useful in establishing greater certainty regarding the PRL phenotype(s) of MDD.

3.4.4.2 Punishment and reward sensitivity in human anxiety

To date, only one study of the relationship between anxiety and reward and punishment sensitivity on PRL has been conducted that has no major limitations (Xia et al., 2021). In this study, among university students with no neuropsychiatric diagnoses, 40 subjects (20 male, 20 female) with high trait anxiety were compared to 40 subjects with low trait anxiety (20 male, 20 female). High-anxiety individuals had a significantly lower last-trial-loss shift proportion (51% vs 64%), but with no difference in last-trial-win stay proportion. Given that most wins would likely have occurred on the correct target, the absence of a last-trial-win stay proportion difference suggests no group difference in reward sensitivity, which in turn

suggests that the lower last-trial-loss shift proportion truly reflects a lower punishment sensitivity in the high-anxiety group.

Other studies of the PRL task in anxiety are difficult to interpret. In a study of children and adolescents where the mean ages of the groups were between 12.5-13.5 years, no significant differences were reported in incorrect-loss shift proportion between subjects with anxiety disorders (n = 30) and controls (Dickstein et al., 2010). However, the anxiety disorder group was very heterogeneous, with less than half of the subjects having a diagnosis of generalized anxiety disorder, and most having social phobia, separation anxiety, or simple phobia. Further, the anxiety disorder group was 16% more female than the control group, although this difference was not statistically significant. In another study, there were no differences between a small group of generalized anxiety disorder patients in correct-loss shift count or correct-loss stay count (Szabó et al., 2013), but as previously discussed, these metrics are essentially uninterpretable because they are not proportions and are under considerable influence by both reward and punishment sensitivity.

Overall, the best evidence suggests that trait anxiety in humans is associated with lower negative outcome sensitivity on the PRL task, but more high-quality studies are required to definitively establish whether this is the case, and which anxiety disorders, if any, also exhibit this association.

3.4.4.3 Punishment and reward sensitivity in rodent depression models

The effects of animal models of depression and anxiety on PRL metrics have not been exhaustively investigated. In one study of chronic social defeat in mice, stressed animals had a lower correct-win stay proportion, together with a lower correct-loss shift proportion than controls (Bergamini et al., 2016). The lower reward sensitivity would, if anything, drive the correct-loss shift proportion upwards, so the fact that it was significantly reduced in stressed animals means that punishment sensitivity was considerably decreased as well. In the only other relevant study, mice subjected to chronic corticosterone administration had a trend for a lower correct-win stay proportion, with no suggestion of difference in correct-loss shift proportion (Dieterich et al., 2019), suggesting a possible small reduction in reward sensitivity but providing no insight into the presence or absence of an effect on punishment sensitivity.

3.4.4.4 In summary

Depression in humans and non-RMS rodent models is commonly associated with a reward sensitivity deficit on PRL and other tasks, which was not present in RMS animals of either

gender, indicating that there is a key element of the depressive PRL phenotype that RMS does not produce, at least not persistently. Reduced punishment sensitivity, however, is strongly associated with anxiety in humans, and there is some evidence that it may also be associated with depression. Given that RMS resulted in long-lasting sexually dimorphic effects on punishment sensitivity on the PRL task, it appears that the RMS may cause long-lasting alterations to cognitive processes relevant at a minimum to human anxiety, and possibly to human depression.

3.4.5 RMS and attentional control

On the PRL task, RMS animals were considerably slower than controls to respond on one of the two stimulus choices, across all seven initial PRL sessions. Simultaneously, there was no association (Spearman's $\rho = 0.07$) between latency to respond and task performance, or indeed between latency to respond and any other calculated PRL metric. Accordingly, there were many timepoints where RMS animals exhibited a prolonged latency to respond with no concurrent compromise to task performance. Further, there was no effect of RMS on latency to collect reward on any task, indicating that the latency to respond effect was not a function of differences in locomotor functioning. There is large body of evidence from the human and non-human primate literature in support of the hypothesis that the isolated response time effect is due to altered attentional control.

In one conceptualisation of attention as it relates to anxiety, there are two systems that underpin not attention in its entirety but specifically attentional control (Eysenck et al., 2007). There is a bottom-up system that is stimulus-driven, commands the initial orienting to information signalling possible threat, and is sometimes equated with vigilance (Shi et al., 2019). Then, there is a top-down system that controls when attention is disengaged from the stimulus. Subjects can either disengage from a stimulus: (1) relatively quickly, exhibiting "fast disengagement" or, in the case of threat, "avoidance", (2) at an equal pace to other subjects, or (3) relatively slowly, exhibiting "difficulty disengaging". In another conceptualisation of attentional control, there is an inhibition function of the central executive, responsible for limiting distraction from the task at hand by irrelevant information, and a shifting function, responsible for flexibly moving attention between relevant stimuli (Eysenck and Derakshan, 2011).

Attentional Control Theory (ACT) is an attempt to describe the relationship between anxiety and certain aspects of cognition. It arose in response to early data linking anxiety to

delayed responding without concomitant impairments in performance on certain tasks (Eysenck, 1979; Eysenck et al., 2007). ACT has two main tenets. Firstly, it posits that anxiety is associated with impaired attentional control, in both the inhibition and shifting functions, resulting in either premature or delayed disengagement from stimuli, particularly but not exclusively in the context of tasks where a subset of the stimuli are threat-associated or have a negative valence. Secondly, it argues that because the deficit in question can theoretically be overcome by simply spending more time before making a response, that anxiety will be associated with reduced task efficiency, defined as increased time (or other resources) expended relative to accuracy or performance level achieved. Thus, it claims that in many circumstances, task performance may be preserved while only response time is compromised.

Since it was first conceptualised decades ago (Eysenck, 1979), a large mass of evidence has accumulated that has largely supported both of these claims (Eysenck et al., 2007; Derakshan and Eysenck, 2009; Eysenck and Derakshan, 2011; Shi et al., 2019). For example, a recent meta-analysis of 58 studies involving over 8000 subjects concluded that anxiety (state and trait not analysed differentially) was associated with medium to large deficits in attentional control efficiency, and that this slower responding relative to achieved performance was exacerbated under conditions of higher cognitive load (Shi et al., 2019).

There is even some prior evidence that the PRL task specifically can capture anxiety-associated differences in attentional control. In one PRL study, subjects with generalised anxiety disorder (GAD) had slower responses on several trial types compared to subjects without GAD, but nevertheless achieved an equivalent number of correct responses (Szabó et al., 2013). In the only other PRL study in anxiety that reported analysis of response time, among a sample of undergraduate students, high trait anxiety was associated with faster responding by roughly 100 ms, but also with impaired performance, and thus likely unaffected efficiency (Xia et al., 2021).

In addition to its association with anxiety, impaired attentional control has also been associated directly with early life stress, both in humans and non-human primates. In one study, a large sample of children aged 6-12 performed the Flanker task, in which they had to identify the direction of the middle arrow in a row of arrows on a screen, and then indicate its direction using buttons (Fields et al., 2021). Attentional control was measured as the difference in response time between trials where the arrows all pointed in the same direction compared to where they pointed in different directions, i.e. where there were greater distracting stimuli. In this study, a greater number of caregiver changes was associated with

greater deficits in attentional control, i.e. a larger effect of distractor stimuli of slowing response time. Meanwhile, there was no relationship between caregiver changes and performance on the task. In another study, among a large sample of adults, greater exposure to an adverse early environment as measured by the Childhood History Questionnaire was associated with lower attentional control as measured by the Attentional Control Scale, which measures perceived ability to focus and limit distraction, and to flexibly shift attention between tasks (Crouch et al., 2012). Finally, in 4.5-5.5 year old macaques, early life maltreatment, consisting of rejection and physical abuse by the mother, was associated with globally slower reaction times on a dot-probe task involving a social threat visual stimulus, but not on a nearly-identical task involving no threat-associated stimulus (Morin et al., 2019). Interestingly, in this study, while there was evidence of an attentional bias (as measured by response time) towards the social threat-associated stimulus for both maltreated and control animals, there was no suggestion that this bias was increased or decreased by maltreatment. Thus, while the presence of the negatively valenced stimulus was responsible for the slowed responding, this effect did not seem to be mediated by an attentional bias towards or away from the threat-associated stimulus. It therefore did not seem to affect the inhibition function of attentional control (unless the presence of the threat stimulus resulted in equally greater distraction of attention to both the threat and non-threat stimulus), and perhaps instead affected the shifting function, which would have been necessarily recruited once the visual stimuli were removed and the cue was presented for the animal to respond on. Regardless of the precise mechanism, this study makes clear that early life stress interacts with the presence or absence of negatively valenced stimuli to modify attentional control and thus reaction time.

In addition to the effect of RMS on PRL response latency that I observed here, there was also an effect of RMS on response latency on the PR task. On that task, the inter-trial interval was 5 seconds, meaning that after each animal made a head entry for reward, the stimulus reappeared 5 seconds later, and they were free to begin responding on it anytime from that point onwards. On the PR4 and PR16 tasks, RMS animals were faster to resume responding on the stimulus than controls following the start of reward collection. There are two obvious possible explanations for this effect. One possibility is that RMS animals were more motivated for reward than controls. However, there was no evidence for this hypothesis from diverse other measures, including breakpoint on the PR4, PR8, or PR16 schedules, trial count on the PRL task, and sucrose preference on the SPT. Further, there was no evidence of

differential reward sensitivity on the PRL task, given the lack of effect on correct-win stay proportion. Thus, if RMS animals were more motivated for reward, the magnitude of this difference appeared to be so small as to be functionally insignificant. The other possible explanation is that RMS animals were less attentive to non-touchscreen stimuli during the period following pellet delivery and associated light and tone signalling. For example, it is possible that during this period, RMS animals spent less time searching the pellet receptacle for additional pellets, exploring the chamber, or simply processing stimuli. Given the volume of disconfirmatory evidence for the first hypothesis, this latter hypothesis appears comparatively likely, especially given that altered attention is also a plausible explanation for the PRL response latency effect. Unfortunately, none of the eight prior studies of RMS and PR for appetitive reward have reported latency to respond, much less probed animal behaviour following reward delivery, so additional experiments are certainly required to further investigate this latency effect.

Altogether, it seems likely that RMS had a long-lasting impact on attentional control, likely responsible at a minimum for slowing responding in the presence of a negatively valenced outcome and associated cues, but not itself meaningfully affecting performance. The precise cognitive mechanisms mediating the attentional effects of RMS are yet to be determined, just as there is still much uncertainty regarding the mechanisms mediating the neuro-cognitive relationship between anxiety and compromised task efficiency in humans (Eysenck and Derakshan, 2011; Berggren and Derakshan, 2013).

3.4.6 RMS and sensitisation to adult stress

In addition to measuring the long-lasting behavioural effects of RMS without any further stress, I also investigated whether RMS made animals susceptible to adult stress-induced behavioural changes. These measurements revealed that, compared to control animals, a novel subchronic footshock stressor caused a transient impairment in performance on the PRL task, with a near-significant concurrent effect of decreasing incorrect-loss shift proportion. Further, the adult stress caused a much larger slowing of the response time in RMS animals than it did in controls. Thus, the adult stress seemed to have the same effects on PRL in RMS males and females to those that RMS alone had in females. Meanwhile, the stressor had no differential impact on any other PRL metric, including trial count and latency to collect. Because PRL has never been conducted in RMS before, these second-hit (RMS then adult stress) results are entirely novel. However, because the parameters that were

differentially affected by the adult stress are the same as those that were affected by RMS alone, the interpretation is similar. It appears that RMS predisposed animals to a transient adult stress-induced desensitisation to negative feedback, with consequent transient impairment of task performance. Further, if attentional control deficits were responsible for the comparatively slow response time of RMS animals over their first seven sessions, then the second stress exacerbated these pre-existing attentional control deficits, given that it resulted in much greater slowing of responding in RMS animals than in controls, even at days 6 and 11 of stress when task performance was equal between the groups.

The other notable post-stress results were those with the potential to give insight into anhedonia. On PRL trial count, sucrose preference on stress day 17 or 18, and body weight (which should be capable of reflecting differential motivation to consume chow, if present, unless concurrent changes in energy expenditure cause confounding), there was no differential effect of the novel stress between RMS and control animals. Thus, there was no evidence from this experiment that RMS causes a greater susceptibility to adult stress-induced anhedonia. However, to gain insight into differential susceptibility to stress-induced anhedonia, it is probably more appropriate to examine studies in which the second hit is one that has been demonstrated to, if implemented well, be capable of inducing anhedonia if applied in isolation, i.e. without prior RMS. Several such studies have measured the differential effect of the second hit on sucrose preference, but none have measured it on tasks such as PR or PRL. Unfortunately, there is no consensus in the literature regarding the interaction between RMS and later-life stress. For example, following 3-4 weeks of chronic unpredictable stress, several studies have found no difference in sucrose preference between RMS and control females (Wei et al., 2018; Huang et al., 2021) or males (Mrdalj et al., 2016; Wei et al., 2018), but decreased (Zhang et al., 2013) and increased (Shu et al., 2015) preference has also been reported in males. Although not primarily acting via chronic stress, 3 weeks of around-the-clock corticosterone application via drinking water was reported to decrease sucrose preference to a greater degree in RMS animals than controls, but only in females and not males (Hill et al., 2014). These inconsistencies are likely a function of the many possible discrepancies in experimental protocols between these studies, such as in the RMS protocol (as previously discussed) and SPT protocol (for example, the degree of food and/or water deprivation applied prior to the test session). Altogether, it seems that under certain yet-to-be-determined conditions, RMS animals may be more susceptible to

developing anhedonia following an additional psychosocial or biological insult, but at present this is far from established.

3.5 Limitations

The results I have shown here imply that RMS animals had altered processing of negative information, potentially driving both altered learning from that negative information, and altered attentional control in the context of it. However, it was not possible using this task to determine precisely what the attentional disturbance consisted of, including whether attentional bias towards or away from stimuli associated with the negative outcome played a role. Additionally, it is not evident from these experiments alone whether or not the delayed responding in RMS animals is exclusive to decisional contexts involving negative outcomes and associated signals, or whether it would also have been evident on a task involving a similar number of potential distractor stimuli but without any having a negative valence. Indeed, in at least one study, humans with ELS have been shown to have delayed responding due to excessive distraction by even non-emotional distractor stimuli (Fields et al., 2021). Additional, carefully-designed studies are needed to resolve these questions regarding the precise effects of RMS on the directing of attention.

An additional limitation is the fact that there was not a no-shock (i.e. no adult stress) control condition. Regarding the measurements collected following the adult stress, the only significant effects of RMS were on PRL outcomes, for which baseline measurements were collected immediately before the adult stress, and these pre-adult-stress measurements are co-varied for in all behavioural post-adult-stress measurements. Throughout this thesis, I interpret these analyses as testing for a differential response to stress by RMS vs control animals. However, there are other possible explanations for these significant findings. Specifically, RMS and control animals may have had a differential response to the mere passage of time or to continued exposure to the behavioural tasks. In my view, these latter possibilities are unlikely because the differences between RMS and control animals are apparent at the very first post-stress measurement on the PRL task and then either remain stable in magnitude or are not present at any later time point. If there was a differential effect of time or task exposure, I would expect the difference between groups to increase over time rather than remain stable or decrease. However, these explanations cannot be definitively ruled out because all animals (RMS and control alike) received the adult stress.

3.6 Conclusion

In this chapter, I showed that RMS may cause long-lasting altered sensitivity to negative outcomes, with RMS males initially sensitised to the negative PRL outcome compared to controls, but with RMS females initially de-sensitised to the negative outcome compared to controls. Suggestive of altered attentional control, on the progressive ratio task, RMS animals were slightly faster than controls to respond for reward on the single available stimulus, while on the PRL task, RMS animals were much slower than controls at selecting between the two probabilistically rewarded stimuli, even where groups achieved equivalent performance. In response to a chronic adulthood stress, RMS animals exhibited greater de-sensitisation to the negative PRL outcome than controls, and their response time deficit was exacerbated.

In humans, reduced punishment sensitivity has been reported in both anxiety and depression, and attentional control deficits have been extensively positively associated with anxiety, yet here I show that rodent ELS caused the persistence of both of these effects in the absence of any long-term effects on conventional measures of anxiety- and depression-like behaviour. This suggests firstly that sophisticated cognitive measures should be used more frequently in translational studies of anxiety and depression. Secondly, these findings support the notion that impaired attentional control and altered sensitivity to punishment may be independent of other aspects of anxiety and depression and could potentially play a causal role in their development.

Finally, I provided novel insights regarding the accurate interpretation of the results of PRL testing. I first showed that correct-loss shift proportion is flawed as measure of punishment sensitivity, which is better measured with incorrect-loss shift proportion. Additionally, I showed that punishment and reward sensitivity are independently varying properties of animal behaviour that together account for almost all of the variability in animal success at the task.

4 Repeated maternal separation causes a long-lasting sensitisation of neuroimmune stress responsiveness

4.1 Introduction

Many studies have demonstrated that people with a history of ELS have higher inflammatory responses to acute stress, and higher peripheral pro-inflammatory signalling in general. It has repeatedly been shown that people who have experienced considerable early life stress have exaggerated immune responses to psychosocial stress in adulthood, with an acute stressor resulting in a larger change in circulating interleukin (IL)-6 (IL-6) than in control subjects (Carpenter et al., 2010; Gouin et al., 2012b; Janusek et al., 2017). Additionally, a recent large meta-analysis showed that individuals exposed to ELS had significantly elevated circulating IL-6, tumour necrosis factor alpha (TNF-a), and C-reactive protein (CRP) compared to controls, even in the absence of any specific laboratory stressor (Baumeister et al., 2016).

What is not clear, however, is whether this propensity to higher inflammation in later life, especially or perhaps exclusively in the face of subsequent stress, plays a causal role in the neuropsychiatric or physical health consequences of ELS. Such questions of causality are well-suited for investigation using animal models because elements of the immune system may be selectively targeted using pharmacological or genetic interventions during early life stress, adult stress, or both. In investigating the biological mechanisms linking ELS with its neuropsychiatric consequences specifically, research in animals holds the additional advantage compared to work in humans of allowing access to brain tissue at any point during and after ELS, enabling precise characterisation of its molecular and cellular consequences in the central nervous system (CNS).

Repeated maternal separation (RMS) represents a potentially useful model with which to study these effects. However, while many studies have examined the effects of RMS on the immune system, the results of these are seemingly inconsistent. Even within the same tissues, there are many reports of increased, decreased, or no change in the expression of many important cytokines (Kruschinski et al., 2008; Roque et al., 2016; Wang et al., 2017).

In this chapter, I sought to establish a clearer picture of the effects of RMS on the immune system. I did this in two ways: by measuring cytokines at various time-points following RMS, both without and with further stress, and by conducting a systematic review of others' findings.

In my own experiment, I measured cytokines only in plasma, because this is generally how inflammation associated with stress or psychopathology is measured in humans (Miller and Raison, 2016). However, there is considerable evidence that stress-induced inflammation is not limited to the blood (Pinheiro et al., 2015; Tang et al., 2017; Ganguly et al., 2019), so when conducting the systematic review of key immunological effects of RMS, I included findings from blood as well as non-blood tissue.

In my own experiment, I used a multiplex cytokine quantitation kit capable of measuring 23 cytokines and chemokines at once, specifically: interleukin (IL) 1 alpha (IL-1a), IL-1 beta (IL-1b), IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17A, IL-18, interferon gamma (IFN-g), tumour necrosis factor alpha (TNF-a), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth-regulated oncogene - keratinocyte chemoattractant (GRO-KC), macrophage colony-stimulating factor (M-CSF), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1a), macrophage inflammatory protein 3 alpha (MIP-3a), regulated upon activation, normal T cell expressed and secreted (RANTES), and vascular endothelial growth factor (VEGF). Most of these proteins, including IL-1a, IL-1b, IL-4, IL-5, IL-6, IL-7, IL-12, IL-13, IL-17A, IL-18, IFN-g, G-CSF, GM-CSF, MCP-1, MIP-1a, MIP-3a, RANTES, TNF-a, and VEGF are known to in some way, directly or indirectly, facilitate certain types of inflammatory responses (Dieu-Nosjean et al., 2000; Ferrara et al., 2003; Wynn, 2003; Maurer and Stebut, 2004; Roberts, 2005; Iwakura et al., 2008; Deshmane et al., 2009; Levy, 2009; Michel et al., 2012; Dinarello et al., 2013; Filippo et al., 2013; Francisco-Cruz et al., 2014; Miller and Raison, 2016; Bou-Dargham et al., 2017; Tait Wojno et al., 2019). However, some of these, particularly IL-4 and IL-13, shift immune responses away from a phagocyte-dominant response mediated by cytokines such as IL-1b, IL-6, and TNF-a, and instead towards an eosinophilic-type inflammation mediated by cytokines such as IL-5 and other molecules such as histamine, bradykinin, and leukotrienes (Yang et al., 2002; Wynn, 2003; Luzina et al., 2012; Chatterjee et al., 2014). IL-10, meanwhile, is known to have predominantly anti-inflammatory activity (Pestka et al., 2004; Sabat et al., 2010; Chatterjee et al., 2014), while IL-2 has nuanced roles as a regulator of the adaptive innate immune system,

important both for facilitating adaptive immune system responses and for facilitating adaptive immune tolerance (Malek, 2008). I chose to measure such a large array of cytokines because one goal of the joint behavioural and immunological characterisation of the RMS model is to identify possible pharmacological intervention targets, and there is little reason to expect that the most commonly measured cytokines will represent the best intervention targets (Miller and Raison, 2016).

In the systematic review, I limited my search to a manageable subset of the immunological parameters that have been measured in RMS. I sought to systematically identify and report on all findings of the effects of RMS on the four cytokines most commonly assayed in RMS studies (IL-1b, IL-6, IL-10, and TNF-a), as well as on interpretable measures of effects on microglia, the resident immune cells of the CNS (Graeber, 2010), including the density of microglia in brain tissue and their degree of activation. The systematic review was comparatively focused because there, the central goal was to understand when, where, and under what conditions RMS induced a pro-inflammatory response, rather than to identify particular mediators most implicated in such a response.

Regarding my experimental work, which was conducted before the systematic review, I hypothesized that RMS would increase pro-inflammatory signalling in the blood at all three measurement time points. This was based on meta-analytic findings indicating that humans with ELS had elevated circulating cytokines even in the absence of any specific laboratory stressor (Baumeister et al., 2016), together with reports that both RMS (Lennon et al., 2013; Do Prado et al., 2016; Banqueri et al., 2019) and foot shock (Deak et al., 2005; Maslanik et al., 2012; Maslanik et al., 2013) cause pro-inflammatory signalling in adulthood.

Subsequently, regarding the systematic review, I hypothesized despite our null results in blood that RMS would increase pro-inflammatory signalling in both blood and non-blood tissue in the presence of recent stress, but not in the absence of it. This was based on several positive results regarding the short-term effects of RMS, including in blood in one study (O'Malley et al., 2011; Roque et al., 2016; Li et al., 2017), and several null results regarding the long-term effects of RMS on cytokines in the absence of later-life stress (Kruschinski et al., 2008; Lennon et al., 2013; Grassi-Oliveira et al., 2016), together with evidence suggesting that the sympathetic nervous system (SNS) is sensitized by ELS (Loria et al., 2013) and that the SNS in turn can regulate neuroimmune physiology (Ulrich-Lai and Herman, 2009; Irwin and Cole, 2011; Weber et al., 2017). I sought to demonstrate this by comparing, through both my experimental work and in my systematic review, immunological measurements collected

under three conditions: (1) during or shortly following RMS, (2) long after RMS without any further stress, and (3) long after RMS but with further stress. Because of the possibility that measurements may differ between blood and non-blood tissue (Roque et al., 2016; Moya-Pérez et al., 2017; Wang et al., 2017), I treated these results separately in the systematic review.

4.2 Methods

4.2.1 Experimental methods

4.2.1.1 Sample collection

Blood was collected from each animal at three timepoints: PND 20 (the day after the conclusion of RMS), PND 54 (median; range: 53-55), and at sacrifice on PND 294 (median; range: 279-323). For the first two timepoints, animals were anaesthetised with isoflurane and venous access was gained via the sublingual vein. At sacrifice, animals were decapitated without anaesthesia (for rapid collection of non-ischemic brains), and truncal blood was immediately collected using a funnel. Blood was collected in ethylenediaminetetraacetic acid (EDTA)-coated tubes which were immediately placed on ice. Tubes were centrifuged within 30 minutes of sample collection at 15,000 g and 4 °C for 15 minutes. Plasma was collected and stored at -80 °C until the cytokine assay was performed.

4.2.1.2 Cytokine assay

For measurement of plasma cytokines, a magnetic bead-based multiplex assay capable of measuring 23 cytokines and chemokines at once was used (Cat. 12005641, Bio-Rad, Watford, UK). The assay was performed in accordance with the manufacturer's instructions, except that for incubation steps, an orbital shaker rather than microplate shaker was used, at 150 rpm rather than 850 rpm. In brief, the following reagents were sequentially added to the wells of a 96-well plate, with wash and incubation stages in between: capture antibody-coated magnetic beads, diluted samples or serially diluted standards, detection antibody, streptavidin-phycoerythrin, and assay buffer. The standards were diluted four-fold seven times, for eight total concentrations. All samples, standards, and blanks were plated in duplicate. The plate was read using a Luminex MAGPIX system (Austin, TX, USA).

4.2.1.3 Fluorescence analysis

The *drLumi* package (Sanz et al., 2017), designed specifically for analysis of Luminex multiplex bead immunoassays, was used for standard curve fitting, standard curve outlier detection, estimation of the lower and upper limits of quantitation (LLOQ and ULOQ), and estimation of sample concentration using the inverse standard curve function.

First, self-starter functions were used to fit a 5-parameter logistic regression model to the standard curve data for each analyte, predicting log median fluorescence index (MFI) from log concentration. Where convergence was not achieved, a 4-parameter logistic regression model was fit instead. Blanks were included in curve fitting by assuming a concentration half that of the most dilute standard. After initial curve fitting, outlying standard wells were detected by identifying wells with a standardised residual > 2, and where a well yielded an outlier result for at least one analyte, all standard curves were re-fit excluding that well. The lower and upper limits of detection (LLOD and ULOD) were defined as the upper and lower standard concentrations. The LLOQ and ULOQ bounded the range of concentrations in which the estimates of concentration using the inverse standard curve function had an expected coefficient of variation (CV) ≤ 0.2.

For each analyte, this curve fitting process was followed using five different subsets of standard data: all subsets included the blanks and the four lowest concentrations, but each subset other than the first one omitted an additional high-concentration level, such that the fifth subset excluded the fourth highest concentration levels. For each curve, the percentage of unusable samples was calculated, where an unusable sample was defined as one yielding an estimated concentration of analyte that was below the LLOQ or LLOD, above the ULOQ or ULOD, or incalculable because the average MFI was below the y-intercept and thus unable to be passed through the inverse function to yield the estimated concentration. The curve yielding the lowest proportion of unusable samples was selected for use in generating estimated concentrations. For a given analyte at a given timepoint, if none of the five standard curves yielded adequate performance, defined as ≤ 20% unusable samples and ≤ 30% samples that were either unusable or had a CV > 0.2, that analyte was excluded from analysis at that timepoint.

4.2.1.4 Data analysis

Estimated log concentrations of analyte per sample were exponentiated, multiplied by the sample dilution factor (either 4 or 4.5), and converted back to log (base 10) concentrations.

For each analyte, a simple linear regression was used to regress out any effect of plating order on the estimated log concentration.

Linear mixed-effects models were then constructed to analyse the effects of group, gender, and timepoint (PND 20, 54, or 294) on cytokine concentration. Model residuals were often non-normal, so non-parametric statistics were conducted in all cases, although parametric statistics are also provided in Appendix 7.1.2. Because cytokine concentration was often non-linear over time, timepoint was treated as a categorical predictor. For granulocyte colony-stimulating factor (G-CSF), because assay performance was only adequate for one timepoint, a fixed-effects linear model was used, and because model residuals were normal, only parametric statistics were calculated. Unusable samples as defined above were excluded from analyses.

4.2.2 Systematic review methods

A search was conducted of the PubMed and Embase databases via Scopus to identify publications for which the title, abstract, and keywords together contained any of the terms "maternal separation", "early deprivation", or "maternal deprivation", with at least one of the following additional terms: cytokine*, chemokine*, interleukin*, microglia*, monocyt*, "immune", "immunological", "immunity", "neuro-immunological", "mononuclear", "toll-like", and "toll like". Publications were limited to those classified as articles. The search was performed on 18 December 2019 and results were not restricted by date range. Articles were included if they performed either single-episode or repeated maternal separation (MS) in rats or mice and specifically reported on measures of microglial activation or density, or the expression of IL-1β, TNF-α, IL-6, or IL-10 in any tissue. Measurements were classified as reflecting short-term effects of MS if the measurement was taken within three weeks of the conclusion of MS, or as reflecting long-term effects if they were taken over three weeks after MS concluded. Three weeks was selected as the cut-off because many disruptions to behavioural and non-immune physiological parameters normalize by this time following chronic stress in rodents, but not reliably by earlier times (Zhao et al., 2012; Alves et al., 2017; Park et al., 2017; Jacobson et al., 2018). Short-term measurements were excluded from this review if they were taken from animals subjected to any potentially stressful procedure other than MS, except for intraperitoneal saline injection. Findings were excluded if the approximate age of the animals at the time of measurement was unclear (general descriptions such as "adulthood" were acceptable) or if potentially therapeutic interventions were

administered to animals, and articles were excluded if the full text was inaccessible. I did not restrict my search to particular tissues, because nervous-to-immune communication is thought to occur via altered autonomic nervous and neuro-endocrine signalling (Miller and Raison, 2016; Fleshner and Crane, 2017; Weber et al., 2017), and stress-induced effects on these pathways are known to occur concurrently in numerous organ systems and tissues throughout the body (Lupien et al., 2009; Ulrich-Lai and Herman, 2009). The search yielded 220 results, which were screened against the inclusion and exclusion criteria by the reading of abstracts and full text as appropriate. Ultimately, 46 articles met criteria for inclusion.

The findings of included articles were described in two ways. A written synthesis of the articles was created, and reference was made in writing to every finding that met the above criteria. The written descriptions and summaries were organized according to whether the findings measured short-term or long-term effects as defined above, and in the latter case, whether or not animals experienced stressful conditions or procedures following the conclusion of RMS. A semi-quantitative summary table was then created, containing the most commonly reported outcome (increase, decrease, or no change) for each cytokine under each time-stress condition: short-term without stress, long-term without stress, and long-term with stress. Microglial outcomes were not counted due to the low number of applicable studies. Because several studies reported a discrepancy between the short-term effect of RMS on cytokine expression in the blood compared to in a non-blood tissue (Roque et al., 2016; Moya-Pérez et al., 2017; Wang et al., 2017), measures in blood (plasma, serum, or supernatant of cultured whole blood) and non-blood tissue were treated separately. For each cytokine under each condition, each study's findings were reduced to a single summary finding for non-blood tissue and a single finding for blood. Where a study reported an applicable measurement and found an increase or decrease in expression in at least one non-blood tissue or blood product, that study was counted as supporting either an increase or decrease respectively in that particular tissue class, and otherwise the study was considered to support no change. Where a study reported the same measurement at two or more times within the same time-stress condition, only the first measurement was counted. No within-study conflicts were encountered using this process. The single most common outcome was reported for each combination of cytokine, time-stress condition, and tissue type, except where there was a tie or where two outcomes had the support of at least three studies, in which case both outcomes were reported. Where outcomes had the support of at least three studies, this was indicated as a measure of higher confidence in the summary outcome.

Reported measurements of cytokine expression in blood always refer to protein level. For non-blood tissues, because protein and mRNA results were generally concordant (Figure S4.1, and see Avitsur et al., 2006 and Ganguly et al., 2019), the assay substrate may not be specified in the main text but can be found in Figure S4.2, along with the species and gender of the animals in each included study.

4.3 Results

4.3.1 Experimental results

Blood was collected from RMS and control animals at PND 20, 54, and 294. A multiplex bead-based assay was then used to measure protein levels in plasma of 23 different cytokines. Some assayed cytokines, such as IL-1b, IL-6, and TNF-a, are known to be pro-inflammatory (Miller and Raison, 2016), while others, including IL-10 and IL-4, are known to have at least some anti-inflammatory activity (Yang et al., 2002; Pestka et al., 2004; Sabat et al., 2010; Luzina et al., 2012; Chatterjee et al., 2014). Assay performance varied across batches, permitting the analysis of some cytokines only at specific timepoints (Figure 1).

Linear mixed-effects models were conducted to assess the effect of group and gender on cytokine levels across timepoints (Figure 1). Ultimately, no significant main or interaction effects involving group were observed.

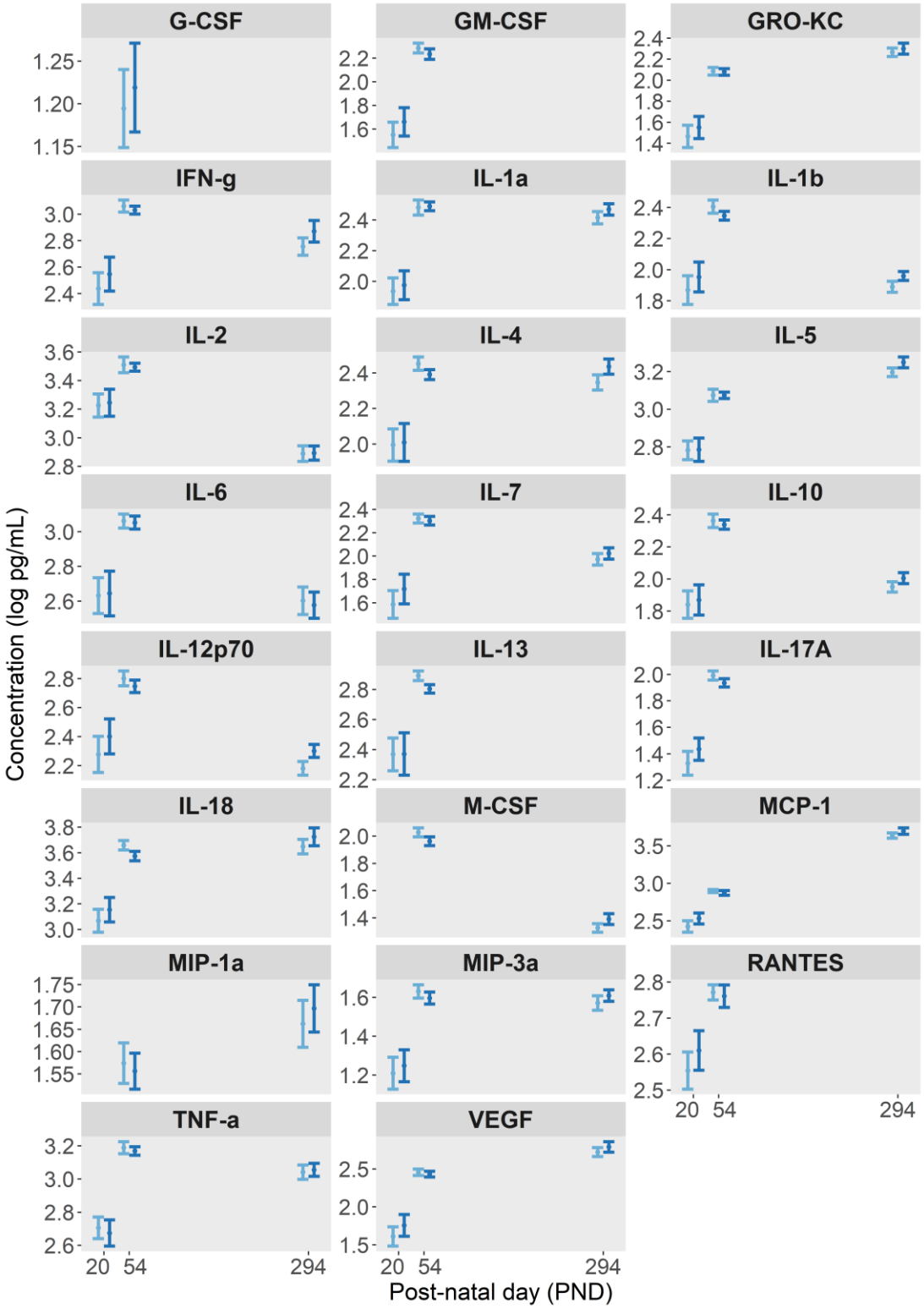

Figure 1. Maternal separation (MS) had no effect on pro- or anti-inflammatory cytokines in plasma at any timepoint. Plasma was collected at three timepoints for cytokine protein quantitation: PND 20 (MS n = 17-21, control n = 13-16), PND 54 (MS n = 16-17, control n = 19-22), PND 294 (MS n = 18-22, control n = 15-19). No significant differences between MS and control animals were identified.

4.3.2 Systematic review results

4.3.2.1 Overview

The most frequently reported effects of RMS on cytokine expression were determined and are displayed in Figure 2. In the short-term, RMS generally increases TNF-α, IL-6, and IL-10 in non-blood tissue while leaving these unaffected in blood (plasma, serum, or the supernatant of cultured whole blood). Without further stress, RMS has no long-term effect on cytokine expression in both non-blood tissues and blood. However, if further stress is applied, RMS animals exhibit increased IL-1β, TNF-α, IL-6, and IL-10 in non-blood tissue, although studies also regularly reported no change in TNF-α or IL-6, and a decrease in IL-10. In both contexts involving recent stress, increases in cytokine expression were much more commonly observed in non-blood tissue than in blood. The effects of RMS on microglia are described in the subsequent sections and summarised in Figure S4.3.

		Short-term	Long-term without stress	Long-term with stress
Solid tissue	IL-1β	↔ and ↓	↔	↑
	TNF-α	↑	↔	↑ and ↔
	IL-6	↑	↔	↑ and ↔
	IL-10	↑	↔	↑ and ↓
Blood	IL-1β	↔	↔	↑
	TNF-α	↔	↔	↔
	IL-6	↔	↔	↔
	IL-10	↔	↔	↔

Figure 2. Effects of repeated maternal separation (RMS) on cytokine expression. The most commonly reported outcomes (increase, decrease, or no change) are summarized for each cytokine under each condition. Dark blue shading indicates a high level of confidence (three or more studies supporting each outcome), whereas light blue shading indicates low confidence. Measurements were considered short-term if tissue samples were collected within three weeks after the conclusion of RMS, and long-term otherwise. In the absence of later-life stress, RMS most commonly had no long-term effect on the expression of any cytokine in either non-blood tissues or blood products. In the period shortly after RMS and when further stress was applied in later life, RMS animals most commonly had greater pro-inflammatory signalling than controls. Finally, pro-inflammatory signals

were much more likely to be observed in non-blood tissue than in blood products. IL-1b: interleukin-1b; IL-6: interleukin-6; IL-10: interleukin-10, TNF-a: tumour necrosis factor alpha.

4.3.2.2 Short-term effects

IL-1b

Several studies have examined the short-term effects of maternal separation on hippocampal IL-1b, in a range of different RMS and single-episode maternal separation (SEMS) cohorts. In animals subjected to RMS and sacrificed immediately after the final MS episode, it was found that hippocampal IL-1b mRNA levels were roughly 20 times higher than in animals that had never been stressed (Roque et al., 2016). In the same study, in animals sacrificed 24 hours after the final episode, IL-1b expression was still significantly elevated, but by this point the expression level was only roughly 2 to 2.5 times the level of the unstressed animals. In animals given IP saline injections immediately after their final RMS episode and sacrificed 90 minutes later, another report found a trend for increased hippocampal IL-1b protein in RMS animals, and suggested that the study may have been underpowered to detect this effect (Saavedra et al., 2017); here, because both RMS and control animals received IP injections, both groups experienced a brief stressor which may have reduced the statistical power to detect an RMS-induced effect on cytokine expression. A further study reported no effect of RMS on hippocampal IL-1b protein in animals sacrificed at an unclear duration following their final RMS episode, although it is worth noting that the study used a shorter RMS protocol than the other two studies and that a multiplex protein assay was used rather than a single-target protein or quantitative polymerase chain reaction (qPCR) assay (Giridharan et al., 2019). Animals sacrificed immediately after SEMS were not found to have increased hippocampal IL-1b expression relative to unstressed animals (Roque et al., 2016). However, among animals sacrificed immediately at the conclusion of an episode of maternal separation, those who had undergone RMS had roughly five times the hippocampal IL-1b level that SEMS animals had, suggesting that hippocampal IL-1b production in response to the acute stress of an MS episode increases progressively with repeated daily application of that acute stress (Roque et al., 2016). Altogether, in repeated maternal separation, these findings suggest that hippocampal IL-1b may undergo a daily cycling in which it peaks at the end of or shortly after each MS episode, and then rapidly returns towards normal until the stress is applied again. The daily peak seems to rise with each additional repetition such that eventually, after enough exposures, the daily elevation becomes so high that it does not normalize even by the start of the next day's episode.

The findings to date regarding IL-1b expression in other brain regions are quite different to the findings in the hippocampus. In the hypothalamus, in RMS animals sacrificed immediately after the final RMS episode, the same study that demonstrated a drastic increase in hippocampal IL-1b simultaneously found no effect of RMS on hypothalamic IL-1b (Roque et al., 2016). Another study found no effect of RMS on prefrontal cortex (PFC) IL-1b in animals sacrificed at an unclear time on the day of the final RMS episode (Giridharan et al., 2019). Two studies showed no effect of SEMS on hypothalamic IL-1b (Roque et al., 2016; Zajdel et al., 2019), although as mentioned above, no effect of SEMS was reported in the hippocampus either. In the prelimbic PFC (plPFC), at almost three weeks following the final RMS episode, one study found that IL-1b gene expression was decreased (Majcher-Maślanka et al., 2019). Another study performed an unusual whole-brain digestion at 48 hours after the final RMS episode, and though the statistical methodology used is unclear, including whether or not correction for multiple comparisons was performed on their many statistical tests, this study reported decreased IL-1b gene expression (Dimatelis et al., 2012). Overall, all these findings in the brain suggest that SEMS likely has no effect on brain IL-1b in any region, and that the effects of RMS are probably region-specific. Further work is required in animals exposed to at least two weeks of RMS and sacrificed immediately following the final RMS episode in order to determine whether regions other than the hippocampus may too undergo large increases in IL-1b expression followed by a rapid return to normal.

Three studies have measured the short-term effects of RMS on plasma IL-1b. In RMS animals sacrificed immediately after the final episode, IL-1b protein was significantly decreased to roughly 65-70% of the level of unstressed animals (Roque et al., 2016). By 24 hours after the final episode, RMS animals still had significantly lower IL-1b, although at approximately 80-85% the level of unstressed animals, possibly suggesting that this change to plasma IL-1b may quickly return to normal once the application of chronic stress is stopped (Roque et al., 2016). In another study, in animals given an IP saline injection shortly after the final episode and sacrificed 90 minutes later, no statistically significant effect on plasma IL-1b was reported (Saavedra et al., 2017). The effect of repeated maternal separation of decreasing the plasma IL-1b level was found to be something that developed with time, given that a single episode of maternal separation did not elicit this effect in animals sacrificed immediately after the episode, and in fact non-significantly increased the IL-1b plasma level compared to unstressed animals (Roque et al., 2016). These findings suggest that the effects of maternal separation on plasma IL-1b may to some extent inversely mirror

the effects on hippocampal IL-1b. There may be a daily cycling in which plasma IL-1b reaches its daily nadir during or shortly after each day's MS episode, but normalizes or near-normalizes by 24 hours later. The daily nadir may become progressively lower each day as more MS episodes are applied. Supporting the hypothesis that plasma IL-1b quickly normalizes following RMS conclusion, a further study found no effect of RMS on plasma IL-1b at 5 or 15 days following the final RMS episode (Grassi-Oliveira et al., 2016).

Only one study has looked at the short-term effects of MS on IL-1b in a tissue other than the brain or plasma. That study, in animals injected with saline and then either separated or kept with the dam for three hours until sacrifice, found no effect of SEMS on liver IL-1b mRNA (Zajdel et al., 2019).

TNF-a

Only two studies have so far examined the short-term effects of maternal separation in the hippocampus. The study by Roque et al. (2016) found in animals that underwent SEMS and were sacrificed immediately afterward that hippocampal TNF-a gene expression was approximately 2.5 times greater on average than in unstressed animals. This suggests that in stress-naïve animals, the first episode of maternal separation can rapidly increase hippocampal TNF-a expression. However, the study also showed, among animals that were sacrificed immediately after an episode of maternal separation, that those who had undergone repeated separation had roughly half the hippocampal TNF-a expression that animals who had just undergone their first-ever episode had. In fact, the mean hippocampal TNF-a level in RMS animals sacrificed immediately following the final episode was essentially the same as the mean level in animals that had never been stressed. This suggests that with repeated episodes of MS, the ability of each daily episode to raise TNF-a expression may decrease, which is the opposite of the finding regarding hippocampal IL-1b expression, which seems to increase with chronicity. Finally, this study did find a significant increase in hippocampal TNF-a in RMS animals at 24 hours following their final episode compared to animals that had never been stressed, although the magnitude of this difference was very small, at on average only 1.3 times the level of unstressed animals. Corroborating this finding, a further study reported increased hippocampal TNF-a in animals sacrificed at some point on the final day of RMS (Giridharan et al., 2019). Altogether, these results suggest that RMS probably increases hippocampal TNF-a, but that this increase may be modest and lagging compared to the increase in IL-1b, and that the peak daily hippocampal TNF-a level may decrease rather than increase with repeated exposure.

Two studies have measured the short-term effects of MS on TNF-a expression in brain regions other than the hippocampus. One study found increased TNF-a expression in the PFC in animals sacrificed at an unclear time after their final RMS episode (Giridharan et al., 2019). Another study found that RMS animals sacrificed immediately after their final episode had higher hypothalamic TNF-a than SEMS animals sacrificed at the same time, suggesting that hypothalamic TNF-a expression may increase with repeated MS exposures. However, importantly, this increase in RMS animals was not significant when compared either to animals that were never stressed or to animals that were sacrificed at 24 hours following their final RMS episode. Additionally, there was no suggestion of a difference in hypothalamic TNF-a between RMS animals sacrificed 24 hours after their final episode and never-stressed animals, suggesting that any increases in hypothalamic TNF-a normalize by 24 hours after the final episode. These findings suggest that RMS may cause modest increases in TNF-a expression in the hypothalamus and PFC, but, contrary to the hippocampal finding, that these increases may be greater at the conclusion of RMS rather than 24 hours later, and that peak expression may increase with chronicity.

The short-term effects of RMS have been examined in a variety of non-brain tissues. Studies to date have consistently found no significant effect of RMS or SEMS on plasma TNF-a (Barouei et al., 2015; Roque et al., 2016; Saavedra et al., 2017). In the liver, one study reported no effect of SEMS on TNF-a mRNA (Zajdel et al., 2019). In colon tissue, in animals that underwent RMS from only PND 5 through 9, an increase in TNF-a mRNA was reported at sacrifice immediately after the final MS episode (Li et al., 2017). However, these RMS animals also received daily intraperitoneal saline injections on each RMS day, while the most relevant control group did not. It is possible that this second methodological divergence might account for the differences in TNF-a expression, given that intraperitoneal injections involve trauma to the structures that the colon rests on, specifically the peritoneum and abdominal wall, and carry a risk of trauma even to the colon itself (Morton et al., 2001; Turner et al., 2011). Overall, to date, most studies find no short-term effect of MS on TNF-a expression in non-brain tissues, but more measurements are needed particularly in non-blood tissues at the conclusion of RMS.

IL-6

Most studies examining the short-term effects of MS on IL-6 have measured it in the blood. Roque et al. (2016) demonstrated that both RMS and SEMS cause similar increases in plasma IL-6 at the immediate conclusion of an MS episode, suggesting that IL-6 release into

plasma during acute stress may not habituate or potentiate with repeated application of that stress. This study also demonstrated that by 24 hours following the conclusion of an RMS episode, plasma IL-6 had decreased to below the level of unstressed animals. If these findings suggesting that plasma IL-6 rapidly changes from elevated to decreased within a 24-hour period following the conclusion of an MS episode are accurate, this implies that it possible to collect plasma at certain times within this 24-hour period that could lead experimenters to incorrectly conclude that MS is likely having no effect on plasma IL-6. Indeed, in one study in which animals were sacrificed at an unclear duration following their final RMS episode, no effect on serum IL-6 was observed (Moya-Pérez et al., 2017). Another study in which animals were given IP saline injections immediately after the final episode of RMS, and then sacrificed 90 minutes later, also reported no effect of RMS on plasma IL-6 (Saavedra et al., 2017). A final study measured plasma IL-6 ten days following RMS conclusion and found no effect of RMS (Barouei et al., 2015). Altogether, if RMS does increase plasma IL-6, this increase is likely very short-lived, perhaps detectable only at the conclusion of an MS episode.

The effects of RMS on IL-6 expression have been examined in a range of non-blood tissues. In the hypothalamus, among animals sacrificed immediately following an episode of MS, one study demonstrated that RMS animals had elevated hypothalamic IL-6 mRNA compared to unstressed animals, while SEMS animals did not (Roque et al., 2016). Interestingly, hypothalamic IL-6 was found to return to normal by 24 hours following the final RMS episode (Roque et al., 2016). Another study confirmed the lack of an effect of SEMS on hypothalamic IL-6, but also liver IL-6, even in animals sacrificed immediately following the episode (Zajdel et al., 2019). In the PFC, one study found increased IL-6 at sacrifice on the final day of RMS (Giridharan et al., 2019). Two studies of RMS have reported increased colon tissue IL-6 mRNA or protein, although in one of these studies the RMS animals received an IP saline injection before each episode, whereas the control animals did not (O'Malley et al., 2011; Li et al., 2017). One of these studies sacrificed animals on the day of their final episode (Li et al., 2017), whereas for the other study, the duration between the final MS episode and sacrifice is unclear, but given the methods of similar papers by the authors, it was likely a maximum of three days (O'Malley et al., 2011). Only in the hippocampus has RMS been found to not have an effect on IL-6 expression, even in animals sacrificed immediately following the final episode (Roque et al., 2016; Giridharan et al., 2019). Findings regarding the effect of MS on IL-6 in non-blood tissues generally seem

to suggest that RMS but not SEMS increases IL-6 expression in a variety of tissues, at least at the conclusion of the final RMS episode if not for much longer.

IL-10

Four studies have measured the short-term effects of RMS on IL-10 expression. In one study, in animals sacrificed on the day of their final RMS episode, no effect on IL-10 in serum was found, but an increase was observed in small intestine tissue (Moya-Pérez et al., 2017). In another study, plasma was collected from both males and females at 5, 15, and 35 days following RMS, and in almost all cases no effect on IL-10 was reported, with the exception being an isolated finding in males at 15 days following RMS of increased IL-10 (Grassi-Oliveira et al., 2016). In animals sacrificed on the final day of RMS, IL-10 was found to be increased in the PFC but unchanged in the hippocampus (Giridharan et al., 2019). One study found a decrease in IL-10 in RMS animals, specifically in the digested whole brain in animals sacrificed two days after RMS conclusion, although this study used qPCR arrays with 84 distinct gene expression targets and did not report key details of the statistical methods used, such as whether correction for multiple comparisons was performed (Dimatelis et al., 2012). Overall, studies investigating the short-term effects of MS on IL-10 expression have generally found either an increase or no change in RMS animals.

Microglial activation

Three studies examined microglial activation at some point within the three days after the conclusion of repeated maternal separation, and all three demonstrated increased microglial activation on morphological analysis, regardless of whether they used a binary classification system (Roque et al., 2016; Saavedra et al., 2017), or directly assessed soma area and arborization area (Baldy et al., 2018). This finding was consistent across both CNS regions examined: in the hippocampus, specifically the hilus (Roque et al., 2016; Saavedra et al., 2017) and CA3 (Saavedra et al., 2017), and in the medulla (Baldy et al., 2018). Together, these studies make it clear, at least in the short term in certain parts of the CNS, that maternal separation leads to microglial activation.

Microglia density

Four studies measured microglia density shortly after the conclusion of RMS. Two of these reports found that maternal separation decreased microglia density. One of these, looking at CA3 and the hippocampal hilus at PND 14 or 15 (Saavedra et al., 2017), found this decrease despite simultaneously finding greater microglial activation. The other study

measured only microglial cell density, and found it was decreased in MS animals in the plPFC at PND 35 (Majcher-Maślanka et al., 2019). An additional study examining the hippocampal hilus, again while simultaneously finding increased microglial activation, found no hint of an effect of RMS on microglial cell density at PND 15 (Roque et al., 2016). In the brainstem, specifically in the medulla, one study observed increased microglia density at PND 14-15 in RMS animals, although this finding was only marginally significant ($p = 0.03$), and was 60-300 fold less significant than this study's concurrent findings demonstrating increased average microglial activation (Baldy et al., 2018). Overall, there is no strong agreement in the literature regarding the effects of RMS on microglia density in the short-term, and further work is required to account for these different results.

4.3.2.3 Long-term effects in absence of later-life stress

IL-1b

All but one study of the long-term impact of RMS on IL-1b expression in animals not subjected to any further potentially stressful procedures have reported no effect. In the plasma, no effect was found of RMS on IL-1b protein at either PND 120 or 150 (Kruschinski et al., 2008). Similarly, studies have found no long-term effect on expression in the lung (Avitsur et al., 2006; Kruschinski et al., 2008), kidney (De Miguel et al., 2018), colon (Lennon et al., 2013), spinal cord (Genty et al., 2018), and hippocampus (Zhu et al., 2017). However, one older study in eight RMS and eight control animals reported increased IL-1b in RMS animals at roughly PND 84 in the colon, liver, and spleen (Barreau et al., 2004). This study quantified gene expression of IL-1b, IL-2, IL-4, IL-10, and interferon gamma using a conventional polymerase chain reaction procedure with gel electrophoresis and reported significant increases in RMS animals in all five targets in all three tissues examined with the exception of IL-2 in the liver. It is possible that these consistent increases in diverse targets and tissues may be an artefact of technical factors such as batch effects.

TNF-a

In the absence of later-life stress, almost all studies examining the long-term effects of RMS on TNF-a expression have found no significant effect, whether they have looked at the hippocampus (Zhu et al., 2017; Banqueri et al., 2019), the dorsal striatum (Banqueri et al., 2019), the PFC (Banqueri et al., 2019), the lung (Avitsur et al., 2006), the colon (Lennon et al., 2013; Pierce et al., 2014), the genitourinary tract (Pierce et al., 2014), splenocytes (Kiank et al., 2009), or plasma (Barouei et al., 2015; Grassi-Oliveira et al., 2016). Three studies from

two groups comprise the exceptions to this general lack of effect. Do Prado et al. (2016) sought to investigate the extent to which housing conditions could affect the long-term trajectory of certain inflammatory and behavioural parameters following RMS, so all animals were housed in either a non-enriched or enriched environment from PND 21 through to sacrifice at PND 56. This study reported that RMS males but not females had increased plasma TNF-a protein at sacrifice if they were subjected to the non-enriched condition, but that they had a normal TNF-a level if subjected to the enriched condition. The non-enriched condition was described as same-sex pair housing in "standard animal facility clear polypropylene cages". Pair housing differentiates these animals from those measured in more than 75% of the studies discussed in this review, which generally employed group housing. However, pair housing is less stressful than isolation housing (Westenbroek et al., 2005; Baker and Bielajew, 2007; Nakayasu and Ishii, 2008), which itself is much less stressful than the chronic stress paradigms commonly used to induce anhedonia in animals (Wallace et al., 2009; Sarkar and Kabbaj, 2016). Together with the description of the cages, it seems responsible to assume for the purposes of this review that the animals in the non-enriched environments were housed in reasonably typical conditions. However, it is possible that the environment that these animals experienced was substantially less enriched than the cage environment used in the other studies described here, perhaps even to the extent that it represented a stressor, or at least to the extent that the relative lack of enrichment and opportunity for social play prevented the normal recovery of the stress, reward, and/or neuro-immune circuitry from the effects of the significant chronic stress of RMS. Riba et al. (2017; 2018) reported in two publications that RMS animals seemingly not subjected to any further stressful procedures were found to have elevated small intestine TNF-a. The most unique things about these studies are the use of inbred C3H/HeN mice and sacrifice at PND 50, which is closer to the conclusion of RMS than almost all other studies of its long-term effects, and again these animals were pair-housed and the degree of enrichment provided is unclear. It is possible that the lesser opportunity for play resulted in slower recovery from the effects of the chronic stress of RMS than the recovery of the animals in other studies discussed here, and so may not have occurred by the relatively early time of sacrifice (Brenes Sáenz et al., 2006; Veena et al., 2009; Hui et al., 2011; Odeon and Acosta, 2019).

IL-6

In general, studies measuring IL-6 in adult animals that have experienced no stress in adolescence or adulthood have found no effect of RMS. A lack of a long-term effect has been

reported in the hippocampus (Zhu et al., 2017), dorsal striatum (Banqueri et al., 2019), PFC (Banqueri et al., 2019), plasma (Barouei et al., 2015), spinal cord (Genty et al., 2018), colon (Pierce et al., 2014; Fuentes et al., 2016), genitourinary tract (Pierce et al., 2014), and lung (Avitsur et al., 2006). Two studies, however, did report increased IL-6 mRNA in RMS animals in adulthood, specifically in the hippocampus (Banqueri et al., 2019) and colon tissue (Lennon et al., 2013), although in the latter study the difference was driven by the non-representative elevated measurements of two of nine RMS animals.

IL-10

Studies measuring IL-10 expression in animals not subjected to any stress since RMS have almost all found no effect, specifically in: plasma at PND 55 (Grassi-Oliveira et al., 2016), plasma at roughly PND 90 in animals housed in either a highly-enriched environment or a relatively unenriched one (Do Prado et al., 2016), the supernatant of splenocytes cultured with LPS (Kiank et al., 2009), and the colon and genitourinary tract (Pierce et al., 2014; Pierce et al., 2016). The one exception is the study by Barreau et al. (2004), which reported increased IL-10 in the colon, liver, and spleen at 12 weeks of age, and the limitations of which are described in the IL-1b section above.

Microglial activation

Only one study has so far examined microglial activation in adulthood in RMS animals not subjected to further stress, and that study found no effects at PND 55 of RMS on plPFC microglial soma area, summed microglial process length, or microglial process end-point count (Ganguly et al., 2018).

Microglia density

Two studies have examined the long-term impact of maternal separation on microglia density. One found no effect on microglia density in the plPFC at PND 55 (Ganguly et al., 2018), while another found increased microglia density in CA3, the dorsal striatum, and the nucleus accumbens (NAc) at PND 100 (Banqueri et al., 2019).

4.3.2.4 Long-term effects in presence of later-life stress

IL-1b

Studies measuring the effects of RMS on IL-1b expression in animals subjected to further stress near-universally report increased IL-1b expression in RMS animals, representing a stark contrast to the findings in unstressed animals.

Increased expression in RMS animals has been reported in: the hippocampus and colon in animals subjected to anxiogenic behavioural testing such as the forced swim test (Amini-Khoei et al., 2017; Amini-Khoei et al., 2019), the hippocampus, PFC, and serum in animals that received IP saline injections for three weeks followed by behavioural testing (Wang et al., 2017), the serum and cerebrospinal fluid in animals injected for two weeks followed by aversive behavioural testing (Réus et al., 2013), the hippocampus in animals subjected to a two-hour sevoflurane anaesthetic three to five days prior to sacrifice, but not among animals exposed to 100% oxygen for the same period (Zhu et al., 2017), the hypothalamic paraventricular nucleus in animals that underwent a procedure to assess sensitivity to pain shortly before sacrifice (Tang et al., 2017), the striatum in animals that underwent intracerebral injection of the neurotoxin 6-hydroxydopamine in adulthood (Dallé et al., 2017), the kidney among animals injected with LPS but not among those injected with saline (De Miguel et al., 2018), and the lung in animals inoculated with an influenza virus nine days prior to sacrifice, but not in those inoculated with saline (Avitsur et al., 2006).

Several studies have reported either no effect or decreased IL-1b expression in RMS animals, although usually in the context of psychosocial stressor that is more directly a physical inflammatory insult. In one study in which animals experienced just one day of isolation housing then a single IP injection of saline or low-dose LPS, together likely representing a very mild stress, no effect of RMS on serum IL-1b was found (Avitsur et al., 2013). However, in animals administered high-dose LPS, RMS males but not females had lower serum IL-1b than controls, suggesting that RMS animals may have been more responsive to the mild stress of new isolation housing, but that the suppressive effect of this greater pre-activation on the ability of immune cells to potently respond to LPS was so mild that it was only apparent statistically at one but not both LPS doses. A further study reported no long-term effect of RMS on IL-1b in LPS-injected animals, specifically in the serum in animals that experienced three weeks of ligature-induced periodontitis and then received an LPS injection two hours before sacrifice (Breivik et al., 2015). A final study reported that in control but not RMS animals, compression trauma to a large nerve increased IL-1b mRNA in the nearby spinal cord at four days following trauma, although by 21 days following trauma, there was no difference between groups (Genty et al., 2018).

TNF-a

In contrast to the findings in unstressed animals, most but not all studies where animals underwent some likely-stressful procedure in adolescence or adulthood have shown an

increase in TNF-a in RMS animals, although generally in non-blood tissues rather than the blood. Regarding just the brain, an increase in TNF-a expression in RMS animals was reported in: the hippocampus in animals that underwent intraperitoneal saline injections daily for two weeks in late adolescence or adulthood (Pinheiro et al., 2015), in animals that had undergone aversive behavioural testing including the forced swim test shortly before sacrifice (Amini-Khoei et al., 2017), in animals that had been recently anaesthetized for two hours with sevoflurane but not those simply exposed to 100% oxygen (Zhu et al., 2017), and in animals that had undergone two weeks of saline injections and then two weeks of two hours of daily restraint, although this difference was conveyed in a heat map and was not tested statistically (Han et al., 2019); the PFC in animals injected either repeatedly (Pinheiro et al., 2015) or, in males but not females, once four hours before sacrifice (Ganguly et al., 2019); the hypothalamic paraventricular nucleus (PVN) in animals that underwent an invasive assessment of sensory sensitivity to colorectal distension shortly before being sacrificed (Tang et al., 2017); the striatum in animals that underwent lesioning of the dopaminergic projections passing through the medial forebrain bundle (Dallé et al., 2017); and the NAc in males but not females injected with saline four hours before sacrifice (Ganguly et al., 2019). Several studies did not find any effect of RMS in the brain, however, in: the hippocampus or PFC in animals that had undergone three weeks of saline injections, although over two weeks had passed following the conclusion of the injections before these animals were sacrificed (Wang et al., 2017); the hippocampus in animals that had recently undergone the forced swim test and elevated plus maze (Amini-Khoei et al., 2019); and the medial PFC in animals that had undergone a poly-(I:C) injection five hours prior to sacrifice (Viola et al., 2019).

Reports measuring TNF-a expression in non-blood tissues other than the brain generally find either increased expression or no change in RMS animals. One study found increased TNF-a protein in RMS animals in large intestine tissue, among animals that had recently undergone the forced swim test and elevated plus maze testing (Amini-Khoei et al., 2019). Another study found in animals that underwent abdominal wall electrode implantation a week prior to sacrifice that RMS animals had increased TNF-a expression in the reproductive tract, although significant effects in the bladder and colon were not found (Pierce et al., 2014). Two studies examined the effect of RMS on lung TNF-a level. The first found no effect of RMS on TNF-a levels in lung tissue in animals that had undergone immune sensitization to an antigen by two injections finishing a week before sacrifice and then intratracheal instillation of that antigen shortly before sacrifice (Kruschinski et al., 2008). The second study found

lung TNF-a mRNA was increased in RMS males and decreased in RMS females, but found both effects only in animals that had been inoculated with an influenza virus rather than saline nine days prior to sacrifice (Avitsur et al., 2006). If the cumulative distress experienced during an active influenza infection is greater than that caused by a weekly injection and then intratracheal instillation of a substance, this might explain some of the discrepancy between the findings in the lung of these two studies. Another study found no effect on the LPS-induced production of TNF-a by splenocytes taken from animals that had recently undergone five days of restraint and acoustic noise stress for two to four hours daily (Kiank et al., 2009),

In most studies that have looked at TNF-a in blood in animals exposed to some sort of later-life stress, no effect of RMS has been shown. In two studies, the stress consisted simply of aversive behavioural testing, in one case the forced swim test (Desbonnet et al., 2010), and in another an open field test considered to be a "novel stress" (O'Mahony et al., 2009), and in both cases there was no effect of RMS on whole blood TNF-a level, with or without *ex vivo* stimulation with LPS (O'Mahony et al., 2009; Desbonnet et al., 2010) or concanavalin A (Desbonnet et al., 2010). Other studies reported no effect of RMS on: serum TNF-a in animals that had undergone three weeks of IP injections and then a variety of behavioural tests (Wang et al., 2017), plasma TNF-a in animals subjected to four days of 30 minutes of restraint or isolation stress immediately before sacrifice (Barouei et al., 2015), serum TNF-a in depression-prone rats that underwent the forced swim test and then were sacrificed roughly a week later (Carboni et al., 2010), and the serum in animals individually housed for 24 hours then given either saline or high-dose LPS two hours before sacrifice (Avitsur et al., 2013).

However, a few studies have found a long-term effect of RMS on plasma or serum TNF-a in the context of further stress. The study by Do Prado et al. (2016) described in the previous section reported elevated plasma TNF-a in RMS animals that had lived for the five weeks between weaning and sacrifice in a relatively unenriched environment, which may or may not have been stressful, but not in RMS animals that had lived in a highly enriched environment. Similarly, another study reported increased serum and CSF TNF-a in RMS animals sacrificed immediately after two weeks of daily IP injections culminating in the forced swim test on the final two days before sacrifice (Réus et al., 2013). In contrast, but still consistent with the hypothesis that RMS confers a vulnerability to psychosocial stress hyper-responsiveness, the study by Avitsur et al. (2013) reported decreased serum TNF-a animals housed in isolation for a day before receiving LPS two hours before sacrifice, although only in females but not males, and oddly only at the lower of the two LPS doses examined.

IL-6

Interestingly, in animals subjected to later-life stress, in contrast to the reasonably consistent findings of increased IL-1b and TNF-a in RMS animals, the findings regarding IL-6 are mixed. Many studies have found no effect on IL-6, even in non-blood tissues.

No effect of RMS was reported in: lung tissue, bronchoalveolar lavage fluid, or plasma in animals sensitized to and then inoculated with a respiratory antigen (Kruschinski et al., 2008; Vig et al., 2010), the genitourinary tract and colon in animals who had undergone abdominal wall surgery and visceral organ distension a week prior (Pierce et al., 2014), the bladder or colon in animals who had been subjected to water avoidance stress one or eight days prior respectively, which involved being placed on a body-sized platform surrounded by water for one hour (Fuentes et al., 2016; Pierce et al., 2016), the kidney or spleen in LPS-treated animals (De Miguel et al., 2018), the hypothalamic PVN in animals who had recently undergone colorectal distension for visual pain sensitivity scoring (Tang et al., 2017), the medial PFC in animals injected with poly-(I:C) five hours earlier (Viola et al., 2019), the spinal cord following trauma to a nearby nerve (Genty et al., 2018), plasma in animals subjected to once-daily exposure to 30 minutes of restraint or isolation stress for four days (Barouei et al., 2015), serum in animals isolated for 24 hours then injected with either saline, low-dose, or high-dose LPS (Avitsur et al., 2013), serum in depression-prone animals exposed to the forced swim test a week before sacrifice (Carboni et al., 2010), or whole blood cultured *ex vivo* with LPS in animals recently subjected to forced swim testing on two days or open field testing on four days (O'Mahony et al., 2009; Desbonnet et al., 2010).

However, there are a number of studies that have reported an increase in IL-6 in RMS animals, specifically in: the colon but not bladder or bladder but not colon respectively in animals that underwent one WAS exposure and sacrifice the day later or one WAS exposure a week before sacrifice and several tail vein bleeds over the same period (Pierce et al., 2016), the lung in animals inoculated with an influenza virus but not saline (Avitsur et al., 2006), the striatum in animals that received neurotoxic striatal lesions (Dallé et al., 2017), the hippocampus in animals that had undergone sevoflurane anaesthesia 3-5 days prior but not 100% oxygen exposure for the same duration (Zhu et al., 2017), the hippocampus in animals that received two weeks of IP injections then two weeks of restraint, albeit not tested statistically (Han et al., 2019), and whole blood cultured with concanavalin A but not saline from animals who had undergone recent repeated open field testing (Desbonnet et al., 2010).

Finally, one study reported decreased IL-6 expression in RMS animals, specifically in the medial PFC, five hours following a saline injection (Viola et al., 2019).

IL-10

Many studies of the effects of RMS expression on IL-10 expression in animals subjected to later-life stress have reported no effect. This has been found in: whole blood cultured with or without LPS or concanavalin A in animals that had undergone recent repeated forced swim or open field testing (O'Mahony et al., 2009; Desbonnet et al., 2010), serum in depression-prone animals the underwent forced-swim testing a week before sacrifice (Carboni et al., 2010), the serum in animals that experienced three weeks of dental pain and inflammation followed by LPS injection two hours before sacrifice (Breivik et al., 2015), to the colon in animals that had been subjected to a one hour water avoidance stress exposure either one or eight days prior (Pierce et al., 2016) or abdominal wall surgery a week prior (Pierce et al., 2014), the bladder in animals that had undergone a WAS exposure eight days prior (Pierce et al., 2016), the serum and PFC in animals that experienced three weeks of IP saline injections and then a range of behavioural tests (Wang et al., 2017), the PFC in animals exposed to two weeks of IP injections then brief behavioural testing (Pinheiro et al., 2015), or the lung or plasma in animals sensitized to ovalbumin by IP injection one and two weeks before sacrifice then challenged via tracheal instillation of ovalbumin a day before sacrifice (Kruschinski et al., 2008).

An increase in IL-10 in RMS animals has been reported in: the genitourinary tract in animals that underwent abdominal wall surgery then underwent testing for sensitivity of the tract to pain (Pierce et al., 2014), the hippocampus in animals subjected to two weeks of IP injections then brief behavioural testing (Pinheiro et al., 2015), and the bladder in animals exposed to water avoidance stress the day before sacrifice (Pierce et al., 2016). It is important to note that an increase in IL-10 does not imply an anti-inflammatory state. For example, one study reported in animals housed in unenriched environments since weaning that RMS animals had a mean plasma IL-10 level roughly 1.5 times that of controls (non-significant), but simultaneously had a mean plasma TNF-a level roughly 5 times that of controls (Do Prado et al., 2016). The study mentioned earlier in this paragraph that reported a significant increase in hippocampal IL-10 in MS animals subjected to repeated IP injections also reported a significant increase in hippocampal TNF-a of a similar magnitude (Pinheiro et al., 2015). That study unfortunately did not measure IL-1b, which in the hippocampus seems to be considerably more responsive to stress than TNF-a (Roque et al., 2016).

Finally, five studies have reported decreased IL-10 expression in RMS animals. Among animals that had undergone dopaminergic pathway lesioning, one study reported that RMS animals had decreased IL-10 mRNA in the striatum, concurrently with increased IL-1b, IL-6, and TNF-a (Dallé et al., 2017). In animals that had undergone three weeks of IP injections then various behavioural tests, it was reported that RMS animals had lower IL-10 in the ventral hippocampus, together with higher IL-1b (Wang et al., 2017). In animals subjected to five days of restraint and noise stress for two to four hours daily, when splenocytes were cultured with LPS, the splenocytes of RMS animals produced significantly less IL-10, along with non-significantly more TNF-a (Kiank et al., 2009). In animals injected daily with saline for two weeks and then exposed to the forced swim test on the final two days, serum IL-10 was found to be decreased in RMS animals (Réus et al., 2013). Finally, among animals implanted with abdominal wall electrodes five days prior to sacrifice and later tested for sensitivity to organ distension, RMS animals were reported to have decreased colon tissue IL-10, but increased interferon gamma (Shao et al., 2019).

Altogether, there are two main conclusions that can be drawn regarding IL-10 expression in RMS models. Firstly, RMS does not have a consistent effect on IL-10 expression in the face of later-life stress, and the drivers of this variability are currently unclear. Secondly, even where pro-inflammatory cytokines are elevated, IL-10 expression can be decreased, increased, or unchanged, suggesting that it should not be over-interpreted as a representative indicator of the inflammatory state of a tissue.

Microglial activation

Only one study has examined microglial activation in RMS animals in the context of later-life stress. This study, in which animals received daily intraperitoneal saline injections from PND 28-42, found at two weeks after the conclusion of the injections that RMS animals had slightly but significantly reduced hippocampal microglial process length and process number, consistent with a more activated phenotype on average (Han et al., 2019). Despite these findings, differences in microglial morphology between groups were not robust enough to also be apparent using a classification-based approach, as the study found no differences between MS and control animals in the proportion of microglia manually classified into any of three levels of activation (Han et al., 2019). However, this study also examined the impact of an additional stress following the two weeks of IP injections. Among animals subjected to two weeks of daily two-hour restraint stress from PND 42-56, the study reported the same effects of maternal separation on process length and number described above, but in this case,

the effects were robust enough to also be detected using a classification-based approach, in that MS animals had a larger proportion of highly activated microglia than controls.

Microglia density

One study examined the impact of maternal separation on microglia density in the context of an adulthood stressor. Specifically, it was found that maternal separation confers a vulnerability lasting at least into young adulthood to greater increases in spinal cord microglia in response to compression trauma of a nearby nerve (Mizoguchi et al., 2019).

4.4 Discussion

In this chapter, I sought to establish a clearer picture of the effects of ELS on the innate immune system, by conducting plasma cytokine measurements and systematically surveying others' measurements of cytokines and microglia in the most widely used animal model of ELS. My own measurements aligned entirely with the high-confidence findings of the systematic review, with both finding no effect in either the short term or long term with further stress of RMS on blood cytokine levels.

The systematic review, however, revealed additional, important findings. Overall, RMS did not appear to cause a persistent production of a pro-inflammatory state by immune cells, given that among instances where animals experienced no later-life stress, generally no long-term effect of RMS on cytokine expression was found. However, in the context of later-life stress, RMS animals very commonly exhibited a more pro-inflammatory cytokine expression profile than controls. The contrast between these two sets of findings suggests that RMS causes a long-lasting sensitization of the mechanism by which an active psychosocial stressor results in pro-inflammatory signalling in tissues (Figure 3). In this mechanism as presently understood, (1) the CNS makes an assessment of stressor intensity and generates a proportional systemic production of neurotransmitters and hormones, particularly noradrenaline and adrenaline, which then act directly and indirectly on (2) innate immune cells, which respond by increasing their production of pro-inflammatory cytokines (LeDoux, 2003; Roozendaal et al., 2009; Ulrich-Lai and Herman, 2009; Irwin and Cole, 2011; Hodes et al., 2015; Miller and Raison, 2016; Fleshner and Crane, 2017; Weber et al., 2017). It is possible that the sensitizing effects of RMS are mediated through modification of either or both parts of this mechanism. To my knowledge, only one study has been conducted to date that directly assessed these two possibilities. Kiank et al. (2009) harvested splenocytes, a rich collection of innate and adaptive immune cells, from RMS and control rats and performed an

ex vivo assessment of their cytokine production in response to LPS, avoiding the confounding of the stress caused by *in vivo* LPS administration. They found differences in cytokine production by immune cells between RMS and control animals only when both groups experienced later-life stress; in the absence of later-life stress, LPS-stimulated cytokine production by immune cells was completely unaffected by RMS. This suggests that rather than priming innate immune cells to effect a greater pro-inflammatory response to activating signals, RMS may instead result in greater nervous and/or neuroendocrine production of activating signals in response to later-life stress. Indeed, many studies in both humans and animals have reported long-lasting effects of ELS on the brain which could result in increased autonomic and endocrine responsiveness to stress. For example, RMS has been reported to increase neuron density in the amygdala (Gondré-Lewis et al., 2016; Bassey and Gondré-Lewis, 2019) and decrease parvalbumin-positive interneuron density in the mPFC (Wieck et al., 2013; Do Prado et al., 2016; Grassi-Oliveira et al., 2016), while human ELS has been shown to decrease dorsal mPFC volume in adulthood (van Harmelen et al., 2010). However, in rodent models of adulthood stress, stress-induced priming of immune cells has been demonstrated both to subsequent stressors (Audet et al., 2011) and inflammatory stimuli (Wohleb et al., 2012; Frank et al., 2014), indicating that further work is required to understand the role of immunological priming in the elevated neuroimmune responsiveness that follows early life stress.

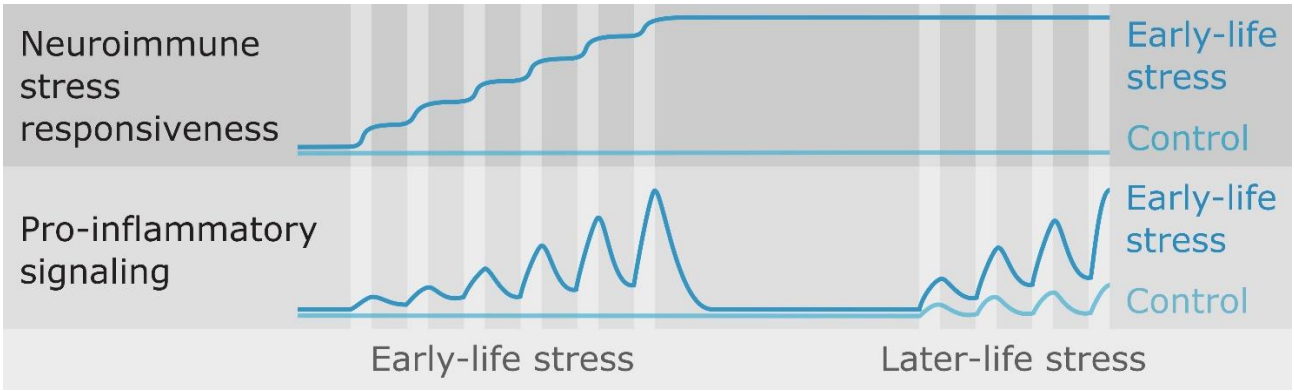

Figure 3. Hypothesized effects of early life stress (ELS). The findings of this review suggest that ELS exerts a long-lasting augmentation to individuals' physiological responsiveness to stressors. When exposed to stressors later in life, individuals with a history of ELS may exhibit elevated autonomic nervous or endocrine signalling, and/or elevated immune cell responses to that signalling, and in turn elevated pro-inflammatory cytokine expression.

Another implication of my findings is that the elevated inflammation that has been identified in humans with a history of ELS may be a direct result of (1) ongoing adulthood stress, on a background of (2) increased responsiveness to stress. Ongoing adulthood stress

may for example result from everyday occupational, financial, or relationship stressors, or from the consequences of one or more of the psychiatric disorders that individuals with a history of ELS are at increased risk of developing. It is also possible that individuals with a history of ELS exhibit a more intense stress–immune response to sample collection itself, in which case findings suggesting elevated inflammation should be interpreted not necessarily as reflecting a stable elevated baseline, but instead as suggesting increased variability and peak daily pro-inflammatory signalling, or increased average inflammation over time. These findings point towards opportunities for therapeutic intervention in patients with a history of ELS, aimed at preventing or treating disorders thought to be caused or exacerbated by inflammation, such as major depression and cardiovascular disease (Batten et al., 2004; Nanni et al., 2012; Steptoe and Kivimäki, 2012). Identification and reduction of ongoing stressors, as well as certain psychotherapeutic, meditation, and relaxation regimens, represent readily available non-pharmacological interventions that, given my findings, may be particularly beneficial for patients with a history of ELS (Pace et al., 2009; Antoni et al., 2012; Creswell et al., 2012; Morgan et al., 2014).

While I did not collect data directly examining the causal role of ELS-associated inflammation with respect to any particular disorders, my findings have some generalisable implications regarding causality. Where long-term non-immunological effects of RMS have been identified in animals not subjected to any further stress, such as neurobiological effects (Matthews et al., 2001), my results suggest that ongoing pro-inflammatory signalling may be unlikely to play a causal role in those effects. However, the immune system may still be causally involved in that the early life inflammation may have long-lasting effects on other systems or processes, for example neurodevelopment, that persist beyond the resolution of the early life inflammation (Knuesel et al., 2014; Estes and McAllister, 2016). Intervention studies targeting the immune system, particularly in early life, are necessary to elucidate the precise consequences of RMS-associated inflammation, and this review provides clear guidance for such studies. In RMS animals, it should be expected that pro-inflammatory signalling will peak during or immediately after early-life or later-life stress exposure, and rapidly decline in the absence of stress. Normalization of inflammatory signalling should be expected within several weeks if not within one day (Roque et al., 2016), although recovery may be slowed if animals are deprived of standard stress-relieving cage elements such as tunnels, nesting, and littermates (Do Prado et al., 2016). Therefore, both inflammation and its hypothesized consequences should be measured during or immediately after stress, and

interventions targeting the immune system will likely be most effective if administered during early-life or later-life stress, or both. Additionally, measurement of inflammatory signalling in non-blood tissues relevant to disorders of interest is encouraged as this appears to be more sensitive than measurement in blood. For three out of four cytokines, the most common short-term effect in non-blood tissues was an increase, whereas in the blood, no change was most common. Additionally, while most included studies measured cytokine expression in either non-blood tissue or blood, but not both, four studies did simultaneously measure a particular cytokine in both types of tissue, and three of these detected at least one change in non-blood tissue that was not detected in plasma (Roque et al., 2016; Moya-Pérez et al., 2017; Wang et al., 2017).

4.5 Limitations

Regarding my own experimental results, during the conduct of the assay, I performed the incubation steps using an orbital shaker at 150 rpm rather than a microplate shaker at 850 rpm as advised by the product manual. This approach was recommended to me by a highly experienced operator of the machine, but not of the specific assay kit I used, and I now have reason to believe that this discrepancy compromised assay performance. Specifically, data analysis often revealed considerable intra-duplicate variance exclusively at the highest standard curve concentrations, and fluorescence readings were often similar between the third highest concentrations, whereas they should have continued increasing with increasing analyte concentration. This suggests that there was under-incubation that particularly affected the very highest analyte concentrations, which in general were vastly above expected tissue concentrations. To overcome this issue, I generated multiple standard curves for each analyte and permitted only standard curves and individual measurements which passed stringent quality requirements, consistent with norms in the literature (Sanz et al., 2017). Most analytes at most timepoints met these standards. Additionally, the values I have observed here for many cytokines are similar to those others have observed (Carboni et al., 2010; Do Prado et al., 2016; Felger et al., 2016; Grassi-Oliveira et al., 2016). Finally, the negative results at all timepoints are wholly consistent with the results of my systematic review. For all these reasons, I have high confidence that the data I have presented are reliable.

Regarding the systematic review, the main limitation is that it was relatively focused. There are many more aspects of the immune system that have been studied in relation to RMS, and in obtaining a full understanding of the immunological effects of RMS, it will be

important to take account of those findings. However, the central aim of the systematic review was narrower: it was to robustly elucidate the circumstances and general tissue type in which RMS can be expected result in elevated inflammatory signalling.

4.6 Conclusion

In summary, my findings in this chapter suggest that ELS results in a long-lasting sensitization of the neuroimmune response to stress, and consequently a propensity to elevated inflammation in response to later-life stress. Further, the finding that for ELS to result in elevated inflammation in later life, subjects must generally be exposed to ongoing stress, suggests that non-pharmacological interventions aimed at reducing stressor exposure or stress responsiveness may be particularly beneficial in reducing inflammation in people with a history of ELS. Finally, my findings guide the future use of RMS to interrogate the causal roles of ELS and inflammation in disorders such as depression and cardiovascular disease.

5 Repeated maternal separation has long-lasting effects on amygdala volume

5.1 Introduction

The adverse psychiatric consequences of ELS are necessarily mediated by effects of ELS on the brain, and the adverse physical health consequences may be mediated by CNS effects too. Depression and anxiety disorders are now known to at once be disorders of the mind and of the brain (Hilbert et al., 2014; Otte et al., 2016). Corticolimbic appraisal of threat can, via sympathetic nervous activation of immune cells, potentiate inflammatory processes (Ulrich-Lai and Herman, 2009; Miller and Raison, 2016). Inflammation in turn is a key component of many of the physical illnesses associated with ELS (Kumar et al., 2015), and is currently thought to play a causal role in roughly a third of depression cases (Miller and Raison, 2016; Treadway et al., 2019). Thus, the central nervous consequences of ELS are important to understand, so that interventions may be best targeted in order to either prevent some of these outcomes, or to optimally treat them when they develop in individuals with a history of ELS exposure. One means of studying such consequences is the measurement of regional brain volumes (Whittle et al., 2013; Pagliaccio et al., 2014), which are capable of reflecting underlying alterations to neuronal or glial number or morphology (Fjell and Walhovd, 2010; Pirko et al., 2011; Heikkinen et al., 2017).

In this chapter, I sought to describe the short- and long-term effects of RMS on the volumes of key brain regions of possible relevance to the relationship between human ELS and subsequent depression and anxiety. Except for the amygdala, these regions were selected because of reported associations between their structure or function and these human conditions. Thus, these analyses were not driven by RMS-specific hypotheses, but instead constituted a descriptive, exploratory characterization, intended to probe for relationships which, if present, may be able to contribute to an understanding of the mechanistic relationships between human ELS and its consequences.

Regarding the amygdala, I hypothesized that RMS would increase its volume at all three time points, both because of findings of increased volume (Mehta et al., 2009; Tottenham et

al., 2010; Pechtel et al., 2014; van Rooij et al., 2020) and increased functional reactivity or connectivity (Steuwe et al., 2015; Peverill et al., 2019; van Rooij et al., 2020; Olsavsky et al., 2021) following human ELS, as well as reports of increased amygdala neuronal count following RMS (Gondré-Lewis et al., 2016; Hegde et al., 2020).

The other regions selected for measurement were: the hippocampus, because there is meta-analytic evidence of reduced volume in recurrent major depression (Schmaal et al., 2016), and only one prior well-powered study had been conducted in RMS (Hui et al., 2010), and another had shown reduced hippocampus volume in early life following RMS (Herpfer et al., 2012); the nucleus accumbens, because reduced reward reactivity has been reported in depression (Der-Avakian and Markou, 2012; Treadway et al., 2019), and because anxious avoidance critically depends on it (LeDoux and Daw, 2018); the dorsal striatum, because reduced caudate reactivity to reward has been reported in depression (Pizzagalli et al., 2009; Pizzagalli, 2014); and the insula and cingulate cortex because altered functional connectivity of both has been reported in both human depression (Sheline et al., 2010; Veer et al., 2010; Zeng et al., 2012) and anxiety, including in meta-analyses (Groenewold et al., 2013; Chavanne and Robinson, 2021).

Quantitation was limited to six regions for several reasons. Firstly, limiting the number of comparisons avoids the need to correct for multiple comparisons, which would reduce power. Secondly, I did not have strong reason to suspect other regional volumetric differences. Finally, although some rat brain atlases segment the brain into several dozen regions (Valdés-Hernández et al., 2011; Papp et al., 2014), these atlases have often been derived using several different structural and diffusion sequences simultaneously, sometimes *ex vivo* and thus in particularly high resolution. They thus have been derived using scans with tissue contrast that is impractical to obtain for a large number of live animals. Because many of the borders of the delineated structures are not visible on T1, magnetisation transfer (MT), or proton density (PD) images collected *in vivo*, algorithms which conduct registration, i.e. non-linearly transform subject images to a template image, cannot operate on these borders (Avants et al., 2011). Therefore, while an argument can be made that these regional delineations can still be useful for functional analyses, because they will generally refer to roughly the same region across subjects, many are not reliable for volumetric quantitation.

5.2 Methods

5.2.1 MRI scanning

Rats underwent MRI scanning at three timepoints: PND 20 (immediately following blood collection), PND 62 (median; range: 61-62), and PND 285 (median; range: 271-309; median days of stress before scan day: 11, range: 9-13).

For the PND 20 scan, rats were kept under isoflurane anaesthesia from before blood collection to the end of the MRI scan. One to two percent isoflurane was delivered in 100% oxygen at 1 L/min. During the scan, rats were monitored using a pulse oximeter, respiratory tracer pad, and rectal temperature probe (SA Instruments, Stony Brook, NY, USA). Vital signs (heart rate, respiratory rate, oxygen saturation, body temperature) were maintained at age- and gender-specific norms by adjustment of anaesthesia depth and the temperature of an adjustable heat pad secured atop the animal.

5.2.2 Image acquisition

MRI images were acquired using a 9.4 Tesla (T) horizontal bore MRI scanner (BioSpec 94/20, Bruker, Coventry, UK). A three-dimensional gradient echo sequence was used to acquire structural images. The field of view of 25.60 × 20.48 × 30.72 mm^3 was constrained within a matrix of 160 × 128 × 192 voxels, each with an isotropic resolution of 160 μm^3. Three different image types were obtained, with each providing unique tissue contrast: magnetisation transfer (MT) images, proton density (PD) images, and T1-weighted images. PD images were acquired using a relaxation time (TR) of 25 ms and an echo time (TE) of 2.41 ms, with a flip angle (FA) of 6°. MT images were acquired using the same parameters, but with the additional application of 4 ms radiofrequency (RF) pulses with a Gaussian shape (2 kHz frequency offset, bandwidth 685 Hz, 10 uT magnitude). T1 images were acquired using a TR of 18 ms, a TE of 2.41 ms, and an FA of 40°. Acquisition was accelerated by a factor of 1.55. Images were acquired every 2.1ms until 6 MT, 6 T1, and 8 PD images were acquired, and then for each image type, a single average image was produced.

5.2.3 Image pre-processing

All MRI processing operations were performed using tools included in: the Advanced Normalization Tools (ANTs) toolbox v2.3.4 (Avants et al., 2011; Tustison et al., 2014),

FMRIB Software Library (FSL) v6.0 (Smith et al., 2004; Jenkinson et al., 2012), MRTrix3 v3.0.4 (Tournier et al., 2019), and convert3d v1.1.0 (Yushkevich et al., 2006).

First, bias field correction was performed using *N4BiasFieldCorrection* (ANTs) (Tustison et al., 2010) on MT, T1, and PD images. Between 6-18 bias-corrected images were generated for each input image, each using a different b-spline mesh, and then corrected images were averaged without normalization to produce the final bias-corrected image.

Because MT, T1, and PD images from the same animal at a given timepoint were not perfectly aligned with each other, the rigid transformation from bias-corrected T1 and PD to MT space was found for each set of those three images using *antsRegistration*. This transformation was then converted to MRTrix3 format using *c3d_affine_tool* (convert3d) and *transformconvert* (MRTrix3), and then applied to the header of the bias-corrected T1 or PD image using *mrtransform* (MRTrix3) to avoid resampling.

5.2.4 Generation of study templates

Tri-modal template generation was performed using *antsMultivariateTemplateConstruction2* (ANTs). At each timepoint (PND 20, 62, and 285), one animal's scan was selected to act as the initial reference space, and then sequentially for all other scanned animals, the three bias-corrected images (MT, T1, PD) were concurrently rigidly registered into the reference space. The transformed images were averaged with normalization to produce three modality- and study-specific template images (ANTs study templates), and then each animal's original bias-corrected images were non-linearly registered to the new templates. This average-and-register step was performed a total of four times in sequence: twice with a three-stage symmetric normalization (SyN) registration, and then twice with a four-stage SyN registration involving 30-50 iterations at the final stage (Avants et al., 2008). In all multi-modal registrations, the MT, T1, and PD images were given relative weights of 2:1:1 in calculation of the image metric, due to the superior grey-white matter contrast in the MT images.

Extensive optimisation of registration parameters, including whether and where to use brain masks, was performed. Ultimately, regarding masking and brain extraction, the following methods were used: For the PND 20 timepoint only, the template brain was manually masked, and the mask then inverse warped into subject space. Each subject brain mask was manually edited for accuracy, and then the entire template generation process was repeated using the brain-extracted images. For the PND 62 and 285 timepoints, template

generation was performed using whole-head images and without supplying *antsRegistration* with any masks. A final, additional registration to the template was then calculated for use in analyses. This registration used whole-head images, but the affine part was performed by supplying *antsRegistration* a mask of the fixed image only, while the non-linear part used no masks.

5.2.5 ROI volume quantitation

Region of interest (ROI) masks were derived from the Tohoku University Rat Brain Atlas, which was kindly provided by Dr Akira Sumiyoshi of the National Institutes for Quantum and Radiological Science and Technology, Japan (personal correspondence, January 2021). The creation of this atlas is described in Liang et al. (2018), although it has since been modified. The current atlas is a composite of 46 × 2 unilateral cortical ROIs from the original Tohoku University cortex-only atlas (Valdés-Hernández et al., 2011), together with 26 × 2 unilateral subcortical ROIs derived from the Calabrese et al. (2013a) atlas, and 3 × 2 unilateral cortical ROIs derived from the Schwarz et al. (2006) atlas. The Valdés-Hernández et al. (2011) and Calabrese et al. (2013a) segmentations were based on Paxinos and Watson (2007) while the Schwarz (2006) segmentations were based on Paxinos and Watson (1998).

First, all ROI masks were extracted from the atlas. Then, the Cg1 and Cg2 masks dividing the cingulate cortex were merged into one, and the six masks dividing the insular cortex were merged. Non-linear transforms were found from the Tohoku template image to the PND 285 MT study template image, and from the PND 285 template to the PND 20 and PND 62 templates. These transforms were used to warp select ROI masks into each of the three study template spaces. A mask of grey and white matter was created for each study template, based initially on the range of voxel intensity values across these two tissue classes, then with further manual editing for accuracy. These intensity masks were applied to the ROI masks to remove any aberrant voxels in CSF, meningeal tissue, or air spaces. ROI visualizations were produced using MRIcroGL (Rorden and Brett, 2000).

Six ROIs were selected for volume quantitation, including four subcortical ROIs (amygdala, nucleus accumbens, dorsal striatum, and hippocampal formation), and two cortical ROIs (cingulate cortex and insula). The amygdala mask encompasses all amygdaloid nuclei delineated in Paxinos and Watson (2007), including the basolateral, central, medial, intercalated, lateral, posterolateral cortical, and posteromedial cortical amygdaloid nuclei (Calabrese et al., 2013b). The nucleus accumbens (NAc) mask encompasses both the core

and the shell. Sub-segmentation of ROIs was not attempted because their internal boundaries are not visible using any of the three contrasts acquired here. Non-linear registration depends on intensity boundaries or gradients and cannot attend to invisible boundaries, and thus volume quantitation would be unreliable due to the considerable or predominant influence of the affine part of the registration on the size of the final, subject-space ROI mask.

ROI masks were inverse-warped from each study template space back to subject space. The grey-white matter mask for each template space was also inverse-warped to subject space, to provide an index of total brain volume (TBV). All warping of ROI masks from the PND 285 template space onward was performed using linear interpolation, and ultimately ROI volumes were calculated by multiplying the mean intensity value (range $1 \geq x > 0$) of non-zero voxels by the total volume (in mm^3) occupied by non-zero voxels.

5.2.6 Data analysis

A separate linear mixed-effects model was constructed for each ROI, which included the full interaction of group, gender, and timepoint, as well as TBV and its interaction with timepoint. Timepoint was treated as a categorical variable because there were only three timepoints and ROI volume was never linear across them. TBV was mean-centred around the mean TBV at each timepoint. For some ROIs, model residuals were modestly non-normal; for consistency, non-parametric statistics were calculated and presented in the main text for all models, but parametric statistics are similar and are also presented in Appendix 7.1.3.

5.3 Results

5.3.1 RMS increased amygdala size in late adulthood during adulthood stress

RMS and control animals underwent MRI scans at three timepoints: the day following conclusion of MS (PND 20), in early adulthood (PND 62) in the absence of recent stress, and late adulthood (PND 285) during the application of a chronic adulthood stressor. Masks of six regions of interest (Figures 1 and 2) were warped into study template space for each of these timepoints, and from there into subject space for volume quantitation.

Results are presented in Figure 3. In the mixed-effects model for amygdala volume, there was a significant group × timepoint interaction ($F_{2,63.1} = 4.78$, $p = 0.016$). On post-hoc testing within timepoints, there was a significant difference at PND 285 ($t_{94.7} = -2.26$, $p = 0.043$),

with RMS animals having an amygdala volume of 33.8 ± 0.173 mm^3 compared to 33.4 ± 0.164 mm^3 for controls. There were no differences at PND 62 ($t_{95.2}$ = -0.83, p = 0.319), where RMS animals had an amygdala volume of 29.5 ± 0.118 mm^3 and controls had a volume of 29.3 ± 0.126 mm^3, or at PND 20 ($t_{103.4}$ = 1.61, p = 0.143), where RMS animals had a volume of 27.7 ± 0.162 mm^3 and controls had a volume of 28.1 ± 0.241 mm^3. There were no other significant interactions involving group (Appendix 7.1.3).

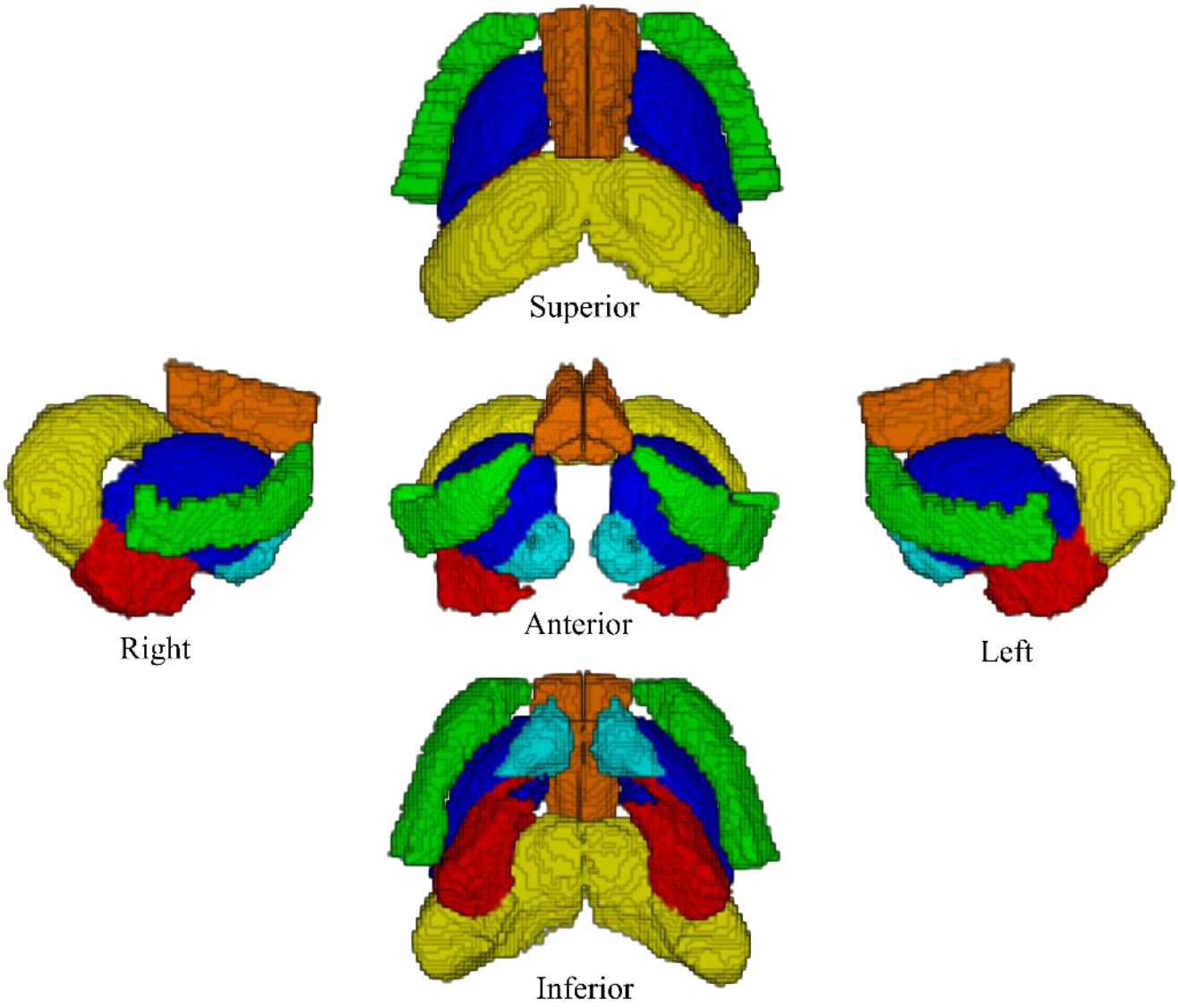

Figure 1. Three-dimensional renderings of region of interest (ROI) masks in PND 285 study template space. ROI masks were obtained by registering the Tohoku University Rat Brain Atlas template image to the PND 285 study template image, and then warping the Tohoku masks into study template space. The six ROIs depicted are: insula (green), cingulate cortex (orange), dorsal striatum (dark blue), ventral striatum (light blue), amygdala (red), and hippocampus (yellow).

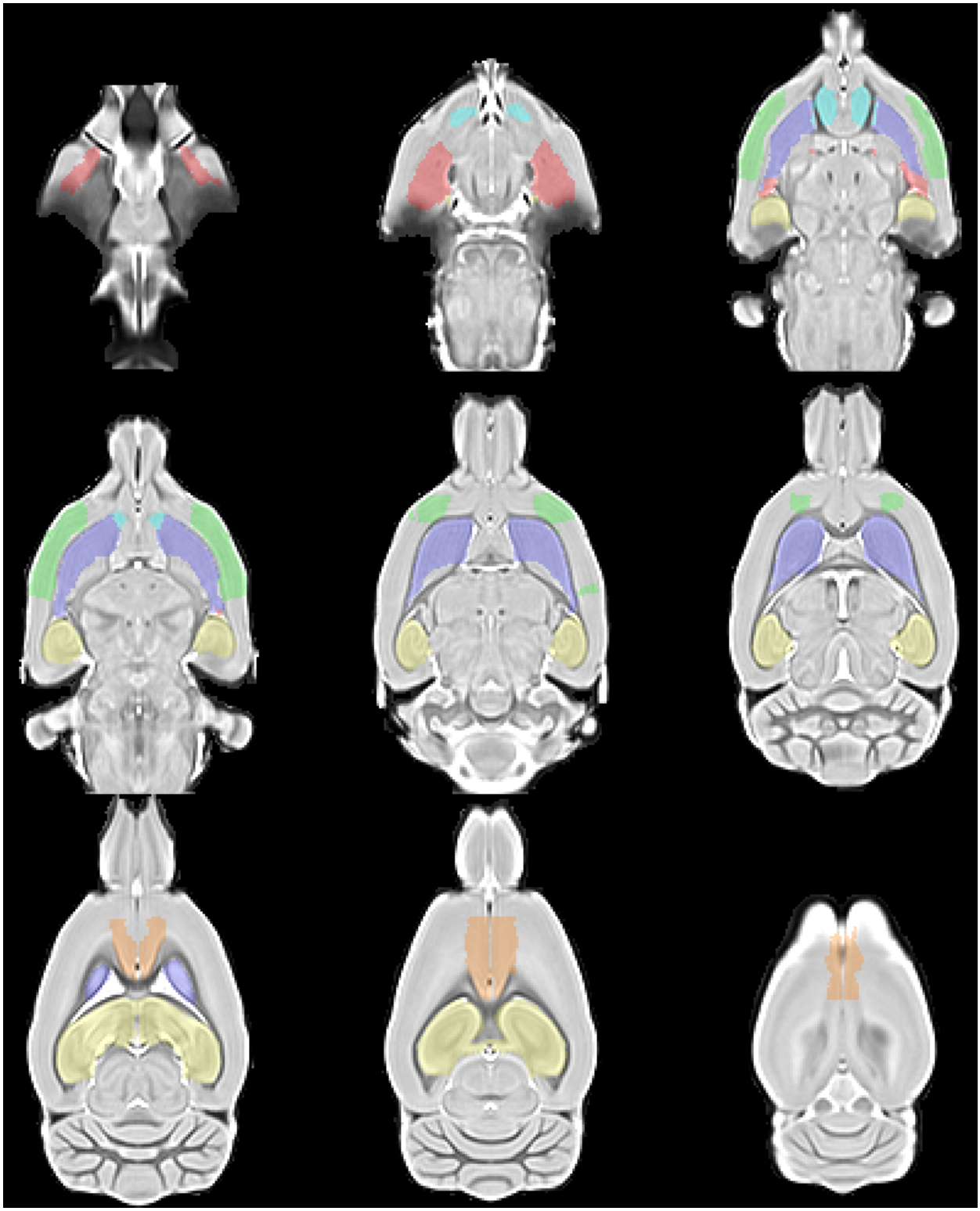

Figure 2. Axial sections of the post-natal day (PND) 285 magnetisation transfer (MT) study template, overlaid with the region of interest (ROI) masks. The six ROIs depicted are: insula (green), cingulate cortex (orange), dorsal striatum (dark blue), ventral striatum (light blue), amygdala (red), and hippocampus (yellow).

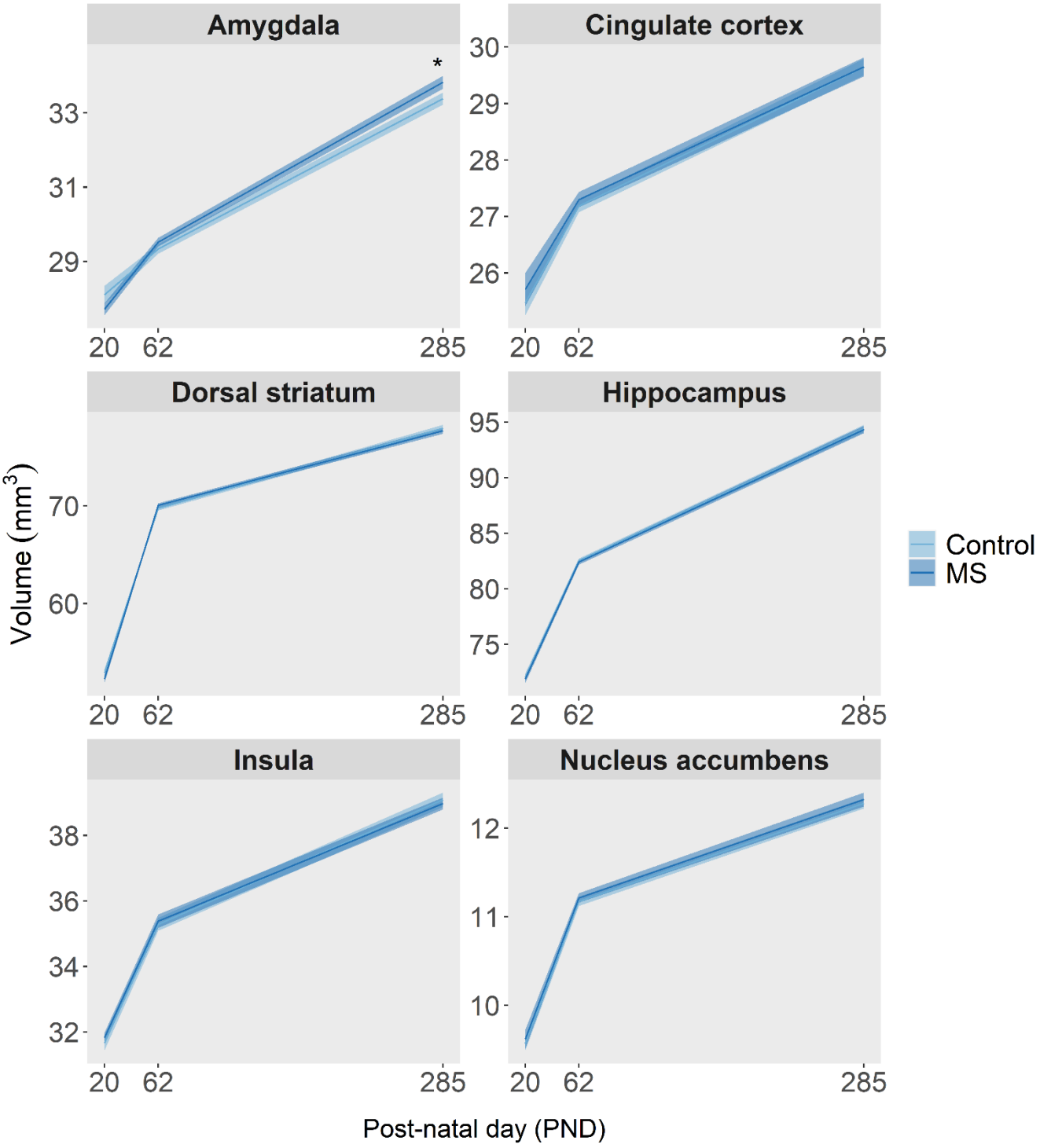

Figure 3. Effects of maternal separation (MS) on regional brain volumes. Animals underwent MRI scanning on PND 20 (MS n = 17, control n = 12), PND 62 (MS n = 22, control n = 20), and PND 285 (MS n = 21, control n = 23), and this data was analysed to quantify the volumes of six regions of interest. Maternal separation resulted in increased grey matter volume in the amygdala at the timepoint in late adulthood during adulthood stress, but not in early adulthood or immediately following MS.

5.4 Discussion

Here, I reported that RMS animals had a larger amygdala in late adulthood in the context of a later-life stress. Below, I will interrogate a breadth of literature in order to set this finding in context and probe the psychiatric, neuroendocrine, and autonomic nervous implications of larger or smaller amygdala volumes.

5.4.1 RMS and regional brain volumes

Six studies of regional brain structure following RMS have been conducted. To date, there has been a focus on the hippocampus, which is well-delineated on any structural MRI sequence and thus it is among the easiest structures to quantify the volume of. Thus, all six studies have, at a minimum, measured the effect of RMS on either whole hippocampus volume or dorsal and ventral hippocampus volume. Of the five that performed measurements in adulthood, all reported no effect of RMS on hippocampal volumes (Hui et al., 2010; Hui et al., 2011; Herpfer et al., 2012; González-Pardo et al., 2019; Guan et al., 2020), even in the context of large sample sizes (Hui et al., 2010). In one of these studies, in which early deprivation (i.e. separation of pups from one another in addition to from the dam) was conducted, reduced hippocampus volume was reported earlier in life, at both PND 15 and PND 30, but by PND 70 there was no difference between groups (Herpfer et al., 2012). However, another study reported no effect of RMS on dorsal or ventral hippocampus volume at PND 40, in either of two mouse strains examined and in either gender (Reshetnikov et al., 2021). Regarding volumetric analyses in regions other than the hippocampus, one study found no effect of RMS on whole cortex volume or motor cortex thickness at PND 40 (Reshetnikov et al., 2021), while another reported no effect of RMS on the volume of the dorsal striatum or medial prefrontal cortex (combined infralimbic and prelimbic cortex) at PND 90 (González-Pardo et al., 2019).

To summarise: To date, only a small number of ROIs have been examined for volumetric effects of RMS, specifically: whole hippocampus, dorsal hippocampus, ventral hippocampus, whole cortex, motor cortex, dorsal striatum, and combined infralimbic and prelimbic cortex. All measured ROIs have been reported to be unaffected, with the exception of one report of a short-lived effect of decreased hippocampal volume. Most volumetric measurements have occurred in adulthood.

5.4.2 RMS and amygdala neuronal morphology

While the volume of the amygdala has never before been measured following RMS, one possible cause of brain region enlargement is altered neuronal morphology, which has been previously examined in several studies. In one study, RMS animals were reported to have 25% more neurons in the amygdala as a whole at age PND 70 (Gondre-Lewis 2016). In that paper, each experimental group was evenly split between males and females, but analyses did not model the effect of gender. In study using only male animals, RMS was reported to increase spine density on basolateral amygdala principal neurons in adulthood (PND 60-70) by 20% among animals reared in enriched home cage conditions, and by 30% in animals reared in standard conditions (Hegde et al., 2020). When projection neurons from frontal cortex to the basolateral amygdala (BLA) were identified and their bouton density in the amygdala was measured, RMS animals were reported to have greater frontal-BLA innervation at PND 35, although this effect was not statistically significant at PND 67-75 (Thomas et al., 2020). Again in males, RMS animals were found to have greater total dendritic length and greater dendritic branch count at PND 84 (Koe et al., 2016), although in the only report of dendritic or axonal morphology in the amygdala specifically in females, neither effect was found (Lee et al., 2020). Altogether, while there is a paucity of evidence concerning the effects in females, RMS at least in males appears to result in long-lasting increases in the microstructural connectivity of the amygdala, which could potentially contribute to the finding of a larger amygdala in RMS animals in late adulthood.

5.4.3 Non-RMS rodent manipulations and the amygdala

To my knowledge, only two studies have conducted volumetry of the amygdala in non-RMS models of chronic stress in rodents. In one of these studies, mice subjected to 5 weeks of chronic unpredictable stress (CUS) were found to have an enlarged amygdala even after adjustment for total brain volume, which did not differ between groups (Nikolova et al., 2018). In the same article, investigators reported measuring regional brain volumes and conducting structural covariance analyses in a large sample of young adults, comparing those in the top 25% of the distribution of childhood maltreatment scores to those in the bottom 25%. While not finding a relationship between human ELS and amygdala volume, they found patterns of volumetric covariance between brain regions in humans with high ELS that strongly paralleled those they identified in CUS mice, with the volumes of other brain regions collectively varying much more strongly with amygdala volume in both humans with high

ELS compared to those with low ELS and in mice exposed to CUS compared to those unexposed (Nikolova et al., 2018). In another study, Sprague Dawley (SD) rats and Fisher 344 (F344) rats, which are known to be much more resistant to habituation of the corticosterone response to repeated exposure to the same stressor than SD rats, were both exposed to a 30 min stressor for two weeks, and then an *ex vivo* brain MRI scan was performed (Bourgin et al., 2015). There, in line with many of the findings from microscopy discussed below, it was reported that stress decreased hippocampal and medical prefrontal cortex (mPFC) volumes across both strains, while simultaneously greatly increasing amygdala volume in the F344 strain (Bourgin et al., 2015).

Other relevant findings have come from microscopy. Firstly, glucocorticoid administration has been repeatedly reported to facilitate synaptic plasticity in the amygdala while impairing it elsewhere. For example, acute and chronic administration of corticosterone to mice have both been reported to increase dendritic length and number of dendritic branch points in the BLA (Mitra and Sapolsky, 2008). In another study, chronic corticosterone was reported to decrease dendritic spine density in the hippocampal CA1 subfield, the infralimbic cortex, and the orbitofrontal cortex, while increasing it in the amygdala (Gourley et al., 2013). Interestingly, in this latter study, most of these effects, including the effect in the amygdala, were also shown to normalize after a 1-week washout period. In another case, chronic corticosterone administration to rats via their drinking water for three weeks was reported to decrease hippocampal expression of the synaptic proteins GluR1 and synaptophysin, while increasing them in the lateral amygdala (Monsey et al., 2014). Here, in contrast to the earlier study, while the hippocampal changes normalized following a 2-week washout period, the amygdala changes only became larger.

Corticosterone-based paradigms are only so informative, however, given that resultant levels of corticosterone may not correspond well to the glucocorticoid levels that typically result from chronic stress in humans or animals, and that the elevation does not result from HPA axis activation but instead will act to suppress it. Nevertheless, in this case, i.e. with respect to effects on dendritic morphology, chronic stress paradigms appear to have the same result as corticosterone administration. Using electron microscopy, chronic unpredictable stress (CUS) has been reported to simultaneously increase the thickness of the postsynaptic density and the length of the presynaptic active zone in the BLA, while decreasing both of these metrics in the prelimbic cortex (Li et al., 2015). In another study, CUS was reported to increase dendritic length of amygdala neurons, while decreasing dendritic length in prefrontal

cortical neurons (Hill et al., 2011). In another case, chronic restraint stress was reported to reduce dendritic length and arborization in hippocampal CA3 subfield pyramidal neurons, while simultaneously increasing dendritic length in BLA pyramidal neurons and dendritic length and branching in BLA stellate neurons (Vyas et al., 2002). Indeed, a recent review of the effects of rodent chronic stress paradigms on dendritic morphology concluded that, in general, these paradigms result in dendritic atrophy and spine loss in the hippocampus and PFC, while simultaneously increasing dendritic spine density in the amygdala (Qiao et al., 2016).

5.4.4 Human ELS and amygdala volume

Numerous studies have reported a positive association between ELS and amygdala volume in childhood, adolescence, and adulthood. In a study of 18 adults exposed to ELS and 33 healthy controls, subjects retrospectively reported the presence or absence of ten types of maltreatment or neglect for each year of childhood and adolescence from age 6 to age 18 (Pechtel et al., 2014). The ELS subjects were found to have larger right but not left amygdalae in adulthood, and similarly, across all subjects, summed ELS across ages 6-18 was positively associated with larger right amygdala volume in adulthood, even after controlling for age, gender, current perceived stress, current anxiety symptoms, and current depression symptoms, none of which were significantly related to right amygdala volume (Pechtel et al., 2014). In another study, among 139 adolescents mostly aged between 12-13 years, lower socioeconomic status was significantly associated with higher right amygdala volume, and higher Childhood Trauma Questionnaire scores were non-significantly associated with larger right and left amygdala volumes (Whittle et al., 2013). Among 69 children and adolescents aged 8-14 years who lived in unsafe, low SES areas in Atlanta, Georgia, USA and who generally reported high levels of trauma exposure, PTSD symptoms, and depression symptoms, the number of self-reported potentially traumatic events (including exposure to violence, abuse, and serious injuries) was associated with significantly and non-significantly larger left and right amygdala volumes respectively (van Rooij et al., 2020). When adolescents aged roughly 16 years who were reared under extremely deprived conditions in institutions in Romania and then adopted into UK families were compared to age-matched non-institutionalised controls, they were found to have larger right amygdala volumes (Mehta et al., 2009). A similar study compared children aged roughly 10-12 years who were adopted from orphanages mostly in Asia but also in Eastern Europe into US families to age-matched never-institutionalised controls (Tottenham et al., 2010). That study

reported that children who had been adopted after 15 months of age ("late-adopted") had larger average bilateral amygdala volume than both early-adopted children and never-adopted controls, which in turn did not differ from one another in amygdala volume (Tottenham et al., 2010). In another case, maternal depression, which is associated with an increased rate of withdrawn, disengaged behaviours, was used as a putative proxy of emotional neglect (Lupien et al., 2011). Here, 10-year-old children who had been parented by depressed mothers had larger amygdalae than age matched unexposed controls. While the probability of antenatal depression represents a potential confound here (Buss et al., 2012; Graham et al., 2018), this study nonetheless adds to the large body of evidence suggesting a positive association between ELS and later-life amygdala volume.

However, several studies have also reported no relationship between amygdala volume and ELS exposure, or even a negative relationship. It has been observed, however, that many of these reports have come either from samples with high rates of psychiatric diagnoses, which introduces noise at a minimum, particularly if subjects across the sample have varied diagnoses, but also confounding if disorders that are more common in ELS-exposed subjects are themselves associated in some way with amygdala volume (Teicher and Samson, 2016). For example, in one study, the number of stressful life events experienced before age 3-5 years did not predict left or right amygdala at age 7-12 years after controlling for total brain volume (Pagliaccio et al., 2014). However, the 120 subjects in this study were all recruited based on their elevated risk at age 3-5 for depression, based on a parent-report checklist for the presence or absence of certain behaviours, and 75% of them had at least one of 16 different psychiatric diagnoses by the time of the MRI scan, representing considerable noise and potential for confounding. In another study, 110 post-institutionalised adolescents adopted from overseas into US families were compared with 62 never-institutionalised controls (Hodel et al., 2015). While control adolescents had a mean age of 12 years, adopted adolescents: had a mean age of 13, had experienced a mean of almost 12 years with their adoptive family, were from 15 different countries, and nearly 30% had an axis I psychiatric disorder. Mean left and right amygdala volume was technically lower than controls in late-adopted adolescents, but this difference was not significant after adjustment for total supratentorial brain volume (Hodel et al., 2015). In the same vein, while several studies have reported negative associations between ELS and amygdala volume, these have often come from small studies in adult subjects that were primarily intended to study disorders, such as borderline personality disorder (Driessen et al., 2000; Schmahl et al., 2003), dissociative

identity disorder (Vermetten et al., 2006), MDD (Malykhin et al., 2012), and substance use disorder (van Dam et al., 2014). All of these are conditions which have been robustly associated with ELS exposure meta-analytically, but which do not follow necessarily from it (Teicher and Samson, 2013).

Altogether, the balance of the purely clinical evidence likely supports the hypothesis that ELS, at least from some causes, can result in larger amygdalae in later life. However, most of the supportive studies have been small, and there have been conflicting findings, even though these studies have had clear limitations. Large studies, particularly those with the ability to look for a relationship between ELS and amygdala volume in subjects without psychopathology, or with sufficient inter-subject variability in psychopathology diagnosis that the influence of each diagnosis can be reliably modelled out, together with the use of rodent ELS paradigms, will be necessary to isolate the effects of ELS on the amygdala.

5.4.5 Depression and amygdala volume

Widely variable associations have been reported between amygdala volume and MDD. First, there were early reports of no relation (Bremner et al., 2000), increased amygdala volume (Lange and Irle, 2004), and decreased volume in females (Hastings et al., 2004). Then, an early meta-analysis suggested that unmedicated MDD patients have smaller amygdalae whereas medicated MDD patients have larger amygdale (Hamilton et al., 2008), but the reliability of this analysis is questionable given that it did not account for the influence of TBV in at least one included study, resulting in the true, null result of that study being misrepresented in the meta-analysis as a strong, significant result (Bremner et al., 2000). In contrast to the findings of the meta-analysis, a small study conducted shortly afterwards found that mediation-naïve first-episode MDD patients had larger amygdalae than controls (van Eijndhoven et al., 2009). Most recently, the Enhancing Neuro-Imaging Genetics Through Meta-Analysis (ENIGMA) consortium conducted a meta-analysis using the raw MRI data from over 1,700 MDD patients and 7,000 controls from 15 research samples, looking for associations between MDD or various MDD subgroups and the volumes of seven subcortical grey matter regions as well as of the lateral ventricles (Schmaal et al., 2016). Overall, there was no significant relationship between amygdala volume and MDD. However, sensitivity analysis revealed that early-onset MDD patients, i.e. patients with onset at age 21 or earlier, had a smaller amygdala than controls, while the amygdalae of late-onset MDD patients were no different in size to controls (Schmaal et al., 2016). However, the size

of the effect in early-onset patients was small, with a Cohen's d of -0.12, and after correction for multiple comparisons, it had only trend significance. No other sensitivity analyses revealed any significant effect on amygdala volume, including comparisons specifically of recurrent MDD to controls and first-episode MDD to controls, and sample-level analysis of the influence of antidepressant medication. Unfortunately, no analyses in this paper attempted to control for maltreatment history. However, the fact that there was only a significant relationship between MDD and amygdala size in subjects whose depression developed before or at age 21, which was only the case for 35% of patients, suggests that this subgroup may be enriched for chronic stress in adolescence, in which case this signal may really be an ELS signal rather than an MDD signal. Indeed, that observed volumetric changes in psychiatric disorders, and particularly in MDD, may actually be the result of failure to account for confounding introduced by maltreatment history continues to be strongly advocated by some investigators (Teicher and Samson, 2016). However, although demographic information was not provided in the ENIGMA meta-analysis for the early-onset subjects, because the mean age of MDD patients in most included studies ranged from 30-50 years and that early-onset patients were distributed across these studies, it is likely that early-onset also connotes a longer lifetime duration of MDD. Therefore, rather than reflecting ELS, it may be that the early-onset signal is one of high vs low total illness duration. On the other hand, the fact of no relationship with amygdala volume even specifically in individuals with recurrent MDD, who necessarily have longer illness histories than first-episode patients, provides some evidence against the illness duration hypothesis. Altogether, the extreme difficulty of finding a consistent, significant relationship between MDD and amygdala volume, particularly in the context of relative ease finding relationships with hippocampus volume, strongly suggests MDD is not itself associated with volumetric changes to the amygdala, and that any such relationships are likely a function of other factors that are themselves associated with MDD in some samples, such as ELS.

5.4.6 Anxiety and amygdala volume

Where studies of amygdala volume have reported significant associations with the presence of generalized anxiety disorder (GAD), these have all reported that GAD patients have larger amygdalae (Kolesar et al., 2019). Specifically, enlarged amygdalae have been reported in two samples of 16-17 adult GAD patients (Etkin et al., 2009; Schienle et al., 2011), and one sample of 12 child and adolescent GAD patients (de Bellis et al., 2000). However, there have also been several reports of no difference, and while some were in small

samples (Mohlman et al., 2009; Hettema et al., 2012; Makovac et al., 2016), at least one null result has come from a sample including 50 patients and 90 controls (Suor et al., 2020).

Similar to the case for GAD, results relating amygdala volume to trait and state anxiety are also variable. For example, the following associations have been reported: trait anxiety with larger left amygdala volume (Baur et al., 2012), trait and state anxiety with smaller left amygdala volume (Spampinato et al., 2009; Blackmon et al., 2011), and no association between trait anxiety and left or right amygdala volume (Kühn et al., 2011).

As with depression, the fact of statistically significant positive and negative associations between amygdala volume and anxiety likely points to confounding, in that the inconsistency may well be driven by sample-to-sample differences in ELS history and the prevalence of psychopathology. Indeed, none of the GAD studies that reported positive associations with amygdala volume reported on or controlled for ELS history.

5.4.7 Cortisol and amygdala volume

Most studies investigating the relationship between cortisol stress reactivity and amygdala volume in humans have reported a negative association. For example, higher cortisol response to stress was associated with a smaller right amygdala in a sample of over 40 children aged roughly 6 years (Fowler et al., 2021). In another study, a similarly negative relationship was identified between cortisol stress reactivity at ages 3-5 and left amygdala volume at ages 7-12 (Pagliaccio et al., 2014). Similarly, in healthy teenagers, elevated cortisol stress reactivity was associated with a smaller right amygdala (Klimes-Dougan et al., 2014). In another study, although the relationship was not identified among controls, some of whom had a psychiatric history, among adult patients with a history of exposure in as neonates to mothers with post-natal depression, a smaller amygdala was again associated with greater cortisol stress reactivity (Barry et al., 2017). In a study in 51 healthy adults aged between 23 and 83 years, investigators found negative a relationship between total daytime cortisol output over 10 days and amygdala volume, but only in older adults, i.e. above age roughly 50 years (Ennis et al., 2019). While there was no laboratory stressor in this study, total daytime cortisol output will necessarily capture cortisol reactivity to daily stressors. Putting stress reactivity aside, to my knowledge, no relationship has been identified between basal HPA output and amygdala volume. For example, in a recent study across a sample of 90 combat veterans, including some with and some without PTSD, no association was observed between morning salivary cortisol and amygdala volume (Babson et al., 2017).

Altogether, the central message from these studies is that greater stress reactivity is associated with a smaller amygdala, or, to frame it another way: that having a larger amygdala is associated with reduced stress reactivity as it relates to cortisol and the HPA axis. While this negative relationship might at first seem counter-intuitive, there are numerous plausible explanations, and indeed, the causality behind this relationship is presently entirely unclear (Pagliaccio et al., 2014; Fowler et al., 2021). There is a tendency in the literature to focus on two possibilities, which is perhaps aided by the fact that the "higher cortisol reactivity – smaller amygdala" framing of the relationship is prevalent: (1) that greater mean cortisol levels over time causes amygdala atrophy, or (2) that a smaller amygdala in some way facilitates greater cortisol release in response to stress. However, the "larger amygdala – lower cortisol reactivity" framing makes other possibilities more obvious: (3) that stressor exposure, perhaps particularly if it is in some way predictable, causes both decreased cortisol stress reactivity (i.e. blunted responding of the HPA system, whether due to habituation or an elevated baseline and thus a ceiling effect) and increased amygdala volume (due to greater recruitment leading to greater structural connectivity), or (4) that some other cause, such as genetic or epigenetic influences, is responsible for two parameters changing in opposing directions, again without the two themselves being directly causally related.

Two main lines of evidence support the first hypothesis. Firstly, it has been reported that glucocorticoids can be toxic to neurons, particularly hippocampal neurons, *in vivo* and *in vitro* (Sapolsky et al., 1986), and that glucocorticoids increase the vulnerability of hippocampal neurons to death following exposure to other neurotoxins including beta amyloid and glutamate (Behl et al., 1997). Secondly, exposure of humans to high levels of glucocorticoids not by virtue of hypothalamic or pituitary overactivity has been associated several times with smaller amygdalae. For example, among both male and female children, congenital adrenal hyperplasia, which results in a hypocortisolaemic state necessitating lifelong exogenous glucocorticoid therapy from an early age, was associated with larger amygdalae at a mean age of 10.5 years for boys and 8.6 years for girls (Merke et al., 2003). Among a small group of children with Cushing's syndrome, which involves elevated adrenocortical production of glucocorticoids, amygdala volumes were smaller than those of controls before surgical cure and still at one year after cure (Merke et al., 2005). And in a study among adults with rheumatic disease and/or asthma, those who had been treated with

glucocorticoids for between 7-360 months had smaller amygdalae than those who had never received glucocorticoid therapy (Brown et al., 2008).

Nevertheless, there are strong arguments against the glucocorticoid toxicity hypothesis being responsible for the negative relationship between amygdala volume and cortisol reactivity. Firstly, while the story is different for hippocampal neurons, as far as I am aware, there is no direct *in vitro* evidence that glucocorticoids are toxic to amygdala neurons. Second, while hyperglucocorticoidism not resulting from hypothalamic or pituitary activity has been associated with smaller amygdalae, the levels of circulating glucocorticoids are often necessarily or unintentionally supraphysiologic, and should not be assumed to be equal to glucocorticoid levels following stress (Merke et al., 2003; Brown et al., 2008). Additionally, a supraphysiologic level of glucocorticoids has numerous effects on the brain which can manifest in numerous emotion, mood, and cognitive disturbances (Sonino et al., 2010; Valassi et al., 2012), and which could potentially reduce alter brain functional connectivity in such a way as to reduce amygdala recruitment and thereby ultimately size (Blair et al., 2001; Rosenkranz et al., 2003; Shekhar et al., 2005). Further, the extensive pre-clinical evidence described earlier in this chapter concerning the effects of stress on amygdala spinogenesis, dendritic length, and volume conflict with this idea that it is amygdala atrophy, rather than growth, that results from stress. Finally, there is evidence that antenatal maternal stress increases rather than decreases amygdala volumes, which must necessarily occur via hormonal or possibly cytokine-mediated mechanisms. Higher early-pregnancy cortisol level predicted larger right amygdala volumes in offspring 7 years later, although only in girls and not boys (Buss et al., 2012). In another study, average maternal serum interleukin-6 (IL-6) across pregnancy was associated with larger right amygdalae and stronger bilateral amygdala connectivity to numerous other brain regions in infants at 4 weeks of age (Graham et al., 2018). However, this study did not measure antenatal maternal stress or serum cortisol, and so did not control for either in this analysis, which is important since psychosocial stress increases serum IL-6 (Pace et al., 2006; Janusek et al., 2017). This study may therefore offer additional support for the hypothesis that antenatal maternal stress increases offspring amygdala volume. Given the lack of direct neural connection to the mother and ability to process most of the stimuli she is processing, this relationship implicates seems to implicate blood-carried signalling molecules such as hormones or cytokines. Overall, there thus appears to be limited evidence for the idea that stress-evoked cortisol resulting in amygdala

atrophy is responsible for the negative relationship between amygdala volume and cortisol reactivity.

A more likely explanation is that repeated stressor exposure leads to increased corticolimbic structural connectivity and thus amygdala enlargement, while also, particularly in the context of recent repeated exposure to predictable stressors, causing habituation of the HPA axis response to further stress (Onaka, 2020). Under this hypothesis, the two effects are dissociable, with one system sensitising to stressors while the other is habituating, even though they may often occur together, and even though the sensitisation may involve increased amygdala connectivity to the PVN (Ulrich-Lai and Herman, 2009). Both may be adaptive – increased corticolimbic connectivity may function to process a wider range of stimuli more effectively for potential threat, while in the context of repeated exposure to stressors, an extremely overactive HPA axis could be detrimental to an organism (McEwen, 2012). Indeed, mechanisms of negative feedback on the HPA axis have been extensively described (Gjerstad et al., 2018; Onaka, 2020), and numerous studies have shown that either elevated baseline HPA activity or elevated stressor exposure can be associated with blunted cortisol responses to stress. For example, subjects with anorexia nervosa have been reported to have elevated cortisol at rest but blunted cortisol reactivity to the TSST (Schmalbach et al., 2020). In another case, among healthy subjects, higher exposure to a large range of stressors was associated with lower corticotropin-releasing hormone (CRH)-induced adrenocorticotropic hormone (ACTH) and cortisol (Zhang et al., 2020). Similarly, in a study of healthy subjects who had experienced varying numbers of stressful life events over the past 12 months, among subjects who scored highly in their tendency to suppress rather than express emotions, greater recent stressor exposure was associated with blunted TSST cortisol responding (Roos et al., 2018). In another study of healthy subjects, lifetime stressor count was associated with decreased cortisol response to the Trier Social Stress Test for Groups (TSST-G) (Lam et al., 2019). Similarly, among a sample of 30 healthy individuals, an index of allostatic load derived from 15 biochemical and clinical parameters was positively associated with chronic stress, and the 40% of subjects meeting a threshold for high allostatic load were found to have lower awakening cortisol and blunted cortisol reactivity to the TSTT (Juster et al., 2011).

The notion that a larger amygdala might imply blunted HPA axis responding at first appears to conflict with the findings in RMS animals, given that here I reported a larger amygdala in RMS animals, while RMS animals generally have elevated corticosterone

reactivity in adulthood. Specifically, 62% of studies of corticosterone reactivity in adulthood of RMS animals have reported increased reactivity, while 38% did not find a significant effect (Van Bodegom et al., 2017). One likely explanation for this discrepancy with the human literature is that HPA axis habituation may be relatively short-lived. Thus, in humans, where stressors may be experienced daily, individuals who have greater corticolimbic responses to stress will likely have greater total HPA axis stimulation, and thus habituation, and may thereby exhibit decreased cortisol responsiveness to stress unless the corticolimbic sensitisation exceeds the HPA axis habituation. In contrast, while animals subjected to ELS may have short-term habituation and a short-term reduction in corticosterone stress responsiveness, once the ELS paradigm has concluded, animals are often left unstressed to adulthood, reversing any habituation and enabling the enhanced corticolimbic responsiveness to give way to potent HPA axis responsiveness as well.

5.4.8 SNS activity, cytokines, and amygdala volume

The amygdala has direct and indirect projections to brainstem nuclei of the sympathetic nervous system (LeDoux, 2003; Roozendaal et al., 2009; Ulrich-Lai and Herman, 2009), which is the primary mediator of psychosocial stress-evoked pro-inflammatory signalling (Irwin and Cole, 2011; Hodes et al., 2015; Miller and Raison, 2016; Fleshner and Crane, 2017; Weber et al., 2017). Therefore, studies measuring the association between amygdala volume and pro-inflammatory signalling may provide additional insight into sympathetic nervous activity. Across a sample of 40 depressed subjects and 40 healthy controls, larger amygdala volumes were associated with higher plasma IL-6 protein, even after controlling for the influence of MDD on amygdala volume, which was not significant regardless (Frodl et al., 2012). Among a sample of 75 human immunodeficiency virus patients, amygdala volume had a significant positive association with pro-inflammatory MCP-1, and a trend negative association with anti-inflammatory IL-10, again suggesting that larger amygdalae are associated with a more pro-inflammatory cytokine profile (Gongvatana et al., 2014). In another study in psychiatrically healthy females, a negative relationship was found, with smaller amygdalae being associated with higher plasma IL-6, but this relationship was also identified for several other brain regions, including the putamen and caudate, likely because the investigators appear to not have adjusted for total brain volume or controlled for any covariates in these analyses (Ironside et al., 2020). Altogether, there is some evidence that larger amygdala volumes are associated with higher pro-inflammatory signalling, and a likely explanatory mechanism for this association is that larger amygdala volumes often reflect

CNS stress circuitry that is more strongly connected to sympathetic nuclei, facilitating greater sympathetic outflow to the immune system in response to daily stressors.

Several studies of yoga or mindfulness interventions provide a window into the relationship between amygdala volume and SNS reactivity, but also with stress. Among stressed but otherwise healthy subjects, reduction in perceived stress following an 8-week mindfulness-based stress reduction intervention was associated with reduction in right basolateral amygdala (BLA) volume (Hölzel et al., 2010). In line with this finding, in a large population-based observational study involving over 2,000 participants with MRI scans, including some at multiple timepoints, participation in meditation or yoga practice was associated cross-sectionally with a smaller right amygdala, and longitudinally with decreasing right amygdala size, even after controlling for the higher representation of women and depression among those who partook in yoga or meditation (Gotink et al., 2018). According to a recent systematic review, there is considerable evidence that these interventions, particularly yoga but also to a lesser degree for mindfulness-based stress reduction, reduce sympathetic relative to parasympathetic activity, as reflected in lower diastolic blood pressure following both interventions, and lower mean arterial pressure and resting heart rate following yoga interventions (Pascoe et al., 2017). The fact that benefit from these interventions on perceived stress has been associated with reductions in amygdala volume may thus imply an association between reduced amygdala volume and reduced sympathetic activity. Some explicit support for this hypothesis comes from another intervention study, here in subjects with Gulf War Illness (GWI), a condition characterised by a variable set of mood, cognitive, and physical symptoms which may be related to autonomic dysregulation (Mathersul et al., 2020). Across a small sample of GWI patients who received 10 weeks of either a yoga intervention of cognitive behavioural therapy (CBT), pre- to post-intervention reduction in self-reported symptoms of autonomic dysfunction was associated with reduction in right amygdala volume (Mathersul et al., 2020).

Adding further support for this hypothesis are studies that directly examined relationships between amygdala volume and sympathetic or parasympathetic activity. Among combat veterans with above-the-median exposure to threatening combat situations, higher amygdala volume was associated with higher heart rate (Kang et al., 2020). However, it is worth noting that this analysis did not adjust for PTSD diagnosis, which was itself associated with larger amygdalae among this group. In another study among healthy individuals, larger left amygdala volume predicted larger skin conductance responses to fear-conditioned stimuli,

reflecting greater sympathetic nervous reactivity (Cacciaglia et al., 2015). Further supportive evidence comes from a study comparing individuals with temporal lobe damage to healthy controls (Gläscher and Adolphs, 2003). Most such subjects had undergone a partial resection of their anterior temporal lobe, including of the amygdala, to manage intractable epilepsy, and therefore had unilateral amygdala lesions. Subjects with unilateral left lesions had equivalent skin conductance responses (SCR) to emotional visual stimuli, while those with right amygdala lesions or bilateral lesions had significantly reduced SCR. Altogether, larger amygdala volumes appear to be associated with greater SNS responsiveness.

5.5 Limitations

The main limitation in the analyses conducted here is that the structures that were measured all have subregions with distinct structural connectivity and functional roles, but the divisions between these subregions are not discernible on the MRI sequences we acquired. For example, the amygdala region of interest quantified consists of several distinct nuclei, and so though we can say that the amygdala as a whole was enlarged in RMS animals, there was no way to determine which particular nuclei mediated this enlargement. Further studies will help to resolve this question. Amygdala nuclei can be individually examined microscopically for evidence of enhanced microstructural connectivity. Additionally, MRI studies using more powerful scanners, multiple sequences optimised for contrast between amygdala nuclei, and *ex vivo* scanning may volumetric measurement of these regions.

5.6 Conclusion

Here, I reported that while RMS animals had equivalent amygdala volume to controls in young adulthood at age 2 months in the absence of later-life stress, they had increased amygdala volume at age 9.5 months in the presence of a later-life subchronic stressor. This implies that RMS interacted either with ongoing neurodevelopment between early and late adulthood or, perhaps more likely, with the presence of later-life stress, to ultimately result in detectable amygdala enlargement. While further experiments are necessary to identify which of these interactions was responsible for this finding, similar results have been reported in the clinical ELS literature, particularly in studies not enriched for psychopathology and therefore less burdened statistically by a low signal to noise ratio and confounding. Meanwhile, the preclinical literature is particularly precise in supplying likely mechanisms for this effect, with numerous reports of increased numbers of key elements of neuronal microstructural connectivity, including neurons, dendritic spines, dendritic branches, and presynaptic

boutons. If enhancements to corticolimbic connectivity, including amygdala connectivity, give way to potentiated responses to further stress, including at the microstructural level, this could explain the apparent interaction. While amygdala volume has no clear relationship to depression or anxiety, it seems to predict elevated sympathetic nervous stress reactivity and thus immunological stress reactivity, and, in the context of ongoing stress, blunted HPA stress reactivity.

6 General discussion

6.1 Summary of findings and interpretation

In this thesis, I described my own experiments that revealed novel important and related differences between animals that had been exposed to 6 hours of separation from their mother each day between ages 5-19 days and those who were unseparated. Additionally, in Chapter 4, I described a systematic review I conducted, representing the first systematic review to date on the neuroimmunology of RMS, that brought new clarity to questions that had previously been answered with a large array of inconsistent findings. The separation procedure referred to in all of these experiments is known to be stressful in that it reliably evokes a corticosterone response, and it is thought to do so through deprivation of the normal responding by the mother to pups' distress in response to their needs, including their nutritional and thermoregulatory needs.

In Chapter 5, I measured amygdala volume in RMS animals for the first time. I showed that at age 285 days, in the context of 14-16 recent exposures to a footshock chamber in which animals received 0-2 shocks, animals that had experienced ELS had larger amygdalae. I showed that amygdala volumes were not significantly different between RMS and control animals at PND 20 or PND 62, implying that the larger amygdala volume at PND 285 was the result of an interaction between RMS and either aging or with the later-life stress. I discussed a large number of findings indicating that both RMS and non-RMS chronic stressors cause increased microstructural connectivity of the amygdala, with it exhibiting more neurons, presynaptic boutons, dendritic branches, and dendritic spines, as well as greater dendritic length. I posited that increased connectivity to and from the amygdala at baseline could give way to greater subsequent increases in connectivity in response to further stress, and that these microscopic morphological changes likely explain the macroscopic volumetric change evident on MRI. I discussed that while amygdala volume has no clear relationship to anxiety or depression, there is nevertheless good evidence that it predicts elevated sympathetic nervous reactivity to threat, together with blunted cortisol reactivity to threat, with the latter finding likely a function of overstimulation of the hypothalamus by the amygdala and consequent habituation within the HPA axis.

In Chapter 4, I measured over 20 cytokines in plasma from RMS and control animals at PND 20, PND 54, and PND 294. Despite the final measurement occurring in the presence of

recent stress, I did not find a significant difference between RMS and control animals in the levels of any cytokine at any timepoint. The systematic review I conducted set these findings in context, revealing that they are consistent with others' results. In blood, the only findings with support from three or more studies were those of no effect of RMS. Meanwhile, in non-blood tissue, such as brain tissue, multiple studies reported increased pro-inflammatory cytokine protein or mRNA, both in the short-term following RMS and in the long-term with further stress. Regarding measurements taken more than three weeks after RMS without further stress, there was a robust consensus supporting no effect of RMS for all four cytokines examined.

These findings imply that RMS potentiates at least one component of the mechanism by which a stressor leads to inflammation. Neatly, this mechanism critically depends on the exact structure implicated in Chapter 5: the amygdala. Potential threat is appraised by the corticolimbic circuitry, and the amygdala is an essential mediator of this appraisal. Further, if a stimulus is evaluated as threatening, efferent projections from the amygdala indirectly and directly to brainstem sympathetic nuclei are then activated. Sympathetic nervous dissemination of noradrenaline and adrenaline results in the activation of immune cells, encouraging their release of pro-inflammatory mediators. In theory, the long-lasting effect of RMS of sensitising this neuroimmune axis could potentially have resulted from the sensitisation of any one of its components. However, my finding and others' findings suggestive of long-lasting effects of RMS, and ELS generally, on the amygdala strongly suggest that potentiated amygdala responding to stressors is likely a central mediator of this neuroimmune sensitisation.

In Chapter 3, I reported a series of novel findings on the PRL task suggestive of altered responding of RMS animals to negative outcomes and associated stimuli, despite simultaneously finding no effect on classical tests of anxiety- and depression-like behaviour. Specifically, I showed the RMS caused an initial sensitisation of males to the negative outcome on the PRL task, while desensitising females to it. I further showed that a second stressor caused a brief desensitisation to the negative outcome specifically in RMS animals, and that this effect did not depend on gender. I reported that desensitisation to the negative outcome led to impaired task performance, while sensitisation improved it. Further, I showed that RMS animals were considerably slower at responding on this task, and that this deficit was exacerbated by a second stressor. Finally, I showed that even when RMS animals were just as accurate in selecting the correct target, they were still taking longer to do so,

suggestive of a deficit in attentional control. The directing and shifting of attention in the context of negatively valenced stimuli is known to depend critically on the amygdala and the prefrontal cortex (Bishop, 2008, 2009; Cisler and Koster, 2010; Malter Cohen et al., 2013), both of which exerts bidirectional control over the other (Arnsten, 2009; Gold, 2015). If a disproportionately large influence of the amygdala on the allocation or shifting of attention is responsible for the delayed responding or the altered learning from negative information, this would again fit neatly with the other findings described in this thesis, together all pointing to RMS-induced alterations to corticolimbic processing of negative stimuli.

6.2 Future research directions

The findings in this thesis raise several important questions that warrant further follow-up. Broadly, while it is clear from the findings in this thesis that RMS has long-lasting behavioural, immunological, and neurobiological effects, important questions remain about the precise localisation and mechanisms behind these effects. Although it is apparent that RMS increases connectivity of the amygdala to other structures, it is unclear exactly which structures these are, and what the directionality of that connectivity is. Further, it is unclear precisely what the functional implications of these connectivity changes are, including whether and how they contribute to the behavioural and immunological effects observed here. While there are plausible hypotheses regarding these questions, sometimes with good evidentiary support, this support often comes not from studies of RMS itself but from human studies or from other rodent stress paradigms. Thus, careful studies of the effects of RMS on the microstructural and functional connectivity of the amygdala to other structures, and of the role of these effects in the changes observed in this thesis on the PRL task and on the immune response to stress, are warranted. Additionally, the possibility that neuroimmune sensitisation is not exclusively neural, for example because immune progenitor cells undergo epigenetic modification that results in long-lasting sensitisation of their progeny to activating signals, should be examined. Cells of the innate immune system should be extracted from RMS animals in adulthood and subjected to *in vitro* stimulation experiments using noradrenaline and so-called danger- or pathogen-associated molecular patterns. If the most effective way to minimize harmful stress-induced inflammation is by targeting the source of the problem, such studies will inform therapeutic approaches aimed at this goal. If immune cells are themselves sensitised to activating signals, pharmacological agents that limit activation of those cells may be particularly useful. For sensitization of the CNS, cognitive interventions aimed at altering responses to negatively valenced stimuli, such as cognitive behavioural therapy, may

be most appropriate. Further interventions likely to be useful in either case include non-pharmacological interventions aimed at limiting sympathetic nervous activation, such as meditation or deep breathing. Finally, several of the findings in this thesis are novel and should therefore be replicated.

6.3 Conclusions

The findings of this thesis collectively show that RMS has long-lasting effects on the physiological system that evaluates stimuli for potential threat and then effects behavioural, cognitive, immunological, and hormonal responses. I show that RMS interacts either with an additional stressor applied in later life, or with age, to result in enlargement of the amygdala, a critical component of the corticolimbic circuitry responsible for threat detection and response. I show that long after the application of RMS has concluded, RMS animals have potentiated immune responses to further stress, and suggest that hyper-responsiveness of the amygdala and broader corticolimbic circuitry to threat may play a key role in this long-lasting neuroimmune sensitisation. I show that RMS causes long-lasting gender dimorphic effects on learning from the negative outcome and associated stimuli on the PRL task, and that RMS animals are slower to respond on that task, even when their performance is unaffected. I hypothesise that altered corticolimbic processing of negatively valenced stimuli may be responsible for both behavioural effects, by affecting ability to learn from and shift attention towards or away from such stimuli. Finally, I show that RMS has face, etiological, construct, and predictive validity as a model of human ELS, and that therefore these findings in rodents are meaningful in understanding the human condition.

7 Appendices

7.1 Statistics tables

7.1.1 Behavioural analysis statistics

7.1.1.1 Body weight

Response (Weight [g])	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
Pre-stress	Group	0.50	1,50.0	0.48	0.48	C 274.2 ± 3.3; RMS 277.5 ± 3.3	C 274.2 ± 3.2; RMS 277.5 ± 3.4
Pre-stress	Gender	1023.52	1,50.0	6e-35	0	F 199.7 ± 3.5; M 352.0 ± 3.1	NA
Pre-stress	Age	2810.24	34,1535.1	0	NA	NA	NA
Pre-stress	G×G	0.10	1,50.0	0.76	0.75	NA	NA
Pre-stress	Group×Age	1.57	34,1535.1	0.020	0.21	NA	NA
Pre-stress	Gender×Age	524.71	34,1535.1	0	0	NA	NA
Pre-stress	G×G×Age	0.70	34,1535.1	0.91	0.44	NA	NA
Post-stress	Group	3.61	1,49.0	0.063	0.064	C 401.6 ± 2.3; RMS 396.3 ± 2.2	C 400.5 ± 2.0; RMS 395.4 ± 2.4
Post-stress	Gender	2.27	1,48.6	0.14	0.15	F 410.2 ± 8.5; M 387.7 ± 6.7	NA
Post-stress	SD	6.12	1,278.9	0.014	NA	NA	NA
Post-stress	Baseline	399.34	1,48.6	4e-25	NA	NA	NA
Post-stress	G×G	0.43	1,49.0	0.52	0.52	NA	NA
Post-stress	Group×SD	0.11	1,278.9	0.74	0.83	NA	NA
Post-stress	Gender×SD	9.70	1,278.9	0.002	0.045	NA	NA
Post-stress	Baseline×SD	16.39	1,278.9	7e-05	NA	NA	NA
Post-stress	G×G×SD	2.16	1,279.0	0.14	0.35	NA	NA

Abbreviations: G×G: group × gender; SD: stress day; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.1.1.2 Arena-based behavioural testing

Response	Predictor	F	Df	p (parametric)	EMMs (parametric)
EPM Time in open arms (%)	Group	0.32	1,53	0.58	C 41.3 ± 3.1; RMS 39.0 ± 3.1
	Gender	13.72	1,53	5e-04	F 48.2 ± 3.2; M 32.0 ± 3.0
	G×G	0.31	1,53	0.58	NA
NP Time in novel area (%)	Group	0.51	1,51	0.48	C 70.0 ± 1.7; RMS 71.6 ± 1.7
	Gender	8.71	1,51	0.005	F 67.3 ± 1.7; M 74.3 ± 1.6

	Familiar colour	5.67	1,51	0.021	NA
	Familiar texture	27.74	1,51	3e-06	NA
	G×G	1.30	1,51	0.26	NA
NR Distance moved (m)	Group	0.00	1,53	0.96	C 174.2 ± 7.3; RMS 174.7 ± 7.2
	Gender	1.52	1,53	0.22	F 180.8 ± 7.5; M 168.1 ± 7.1
	G×G	0.03	1,53	0.87	NA

Abbreviations: EPM: elevated plus maze; NP: novelty preference test; NR: novelty reactivity test; G×G: group × gender; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.1.1.3 Sucrose preference testing

Response	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
SPT PS Sucrose preference (%)	Group	0.94	1,51.9	0.34	0.34	C 79.6 ± 1.4; RMS 77.9 ± 1.4	C 79.6 ± 1.0; RMS 77.9 ± 1.1
	Conc.	98.51	2,103.3	1e-24	NA	NA	NA
	Gender	0.69	1,52.0	0.41	0.41	F 79.6 ± 1.4; M 77.9 ± 1.3	NA
	G×G	1.24	1,52.0	0.27	0.26	NA	NA
	Group×C	0.14	2,103.3	0.87	0.83	NA	NA
	Gender×C	0.19	2,103.3	0.83	0.80	NA	NA
	G×G×C	2.11	2,103.3	0.13	0.13	NA	NA
SPT IS Sucrose preference (%)	Group	2.68	1,48	0.11	NA	C 75.5 ± 1.7; RMS 79.7 ± 1.7	NA
	Gender	2.20	1,48	0.14	NA	F 79.5 ± 1.9; M 75.7 ± 1.6	NA
	Baseline	1.96	1,48	0.17	NA	NA	NA
	G×G	0.67	1,48	0.42	NA	NA	NA

Abbreviations: SPT: sucrose preference test; PS: post-stress (adulthood stress); IS: intra-stress (adulthood stress); G×G: group × gender; ×C: × sucrose concentration (categorical); EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.1.1.4 Operant behavioural testing session counts

Response	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)
FR1 STC	Group	0.27	1,52	0.61	0.55	C 2.82 +/- 0.32; RMS 3.05 +/- 0.33
	Gender	15.73	1,52	2e-04	4e-05	F 3.85 +/- 0.34; M 2.02 +/- 0.31
	G×G	0.05	1,52	0.83	0.90	NA
FR5 STC	Group	0.94	1,52	0.34	0.30	C 5.93 +/- 0.84; RMS 7.07 +/- 0.85
	Gender	5.30	1,52	0.025	0.018	F 7.88 +/- 0.88; M 5.12 +/- 0.84
	G×G	0.04	1,52	0.84	0.91	NA

TTB STC	Group	0.26	1,44	0.62	0.63	C 5.79 ± 0.81; RMS 5.08 ± 0.82
	Gender	14.10	1,44	5e-04	2e-04	F 7.59 ± 0.90; M 3.28 ± 0.72
	G×G	0.37	1,44	0.55	0.57	NA
DRL STC	Group	0.40	1,44	0.53	0.56	C 3.04 ± 0.25; RMS 2.87 ± 0.25
	Gender	4.08	1,44	0.050	0.052	F 3.32 ± 0.28; M 2.59 ± 0.22
	G×G	0.35	1,44	0.56	0.57	NA
PRL Pre-stress SC	Group	3.05	1,44	0.088	0.088	C 12.8 ± 0.9; RMS 15.3 ± 0.9
	Gender	6.37	1,44	0.015	0.016	F 12.4 ± 1.0; M 15.6 ± 0.8
	G×G	0.78	1,44	0.38	0.38	NA

Abbreviations: FR1: fixed ratio 1; FR5: fixed ratio 5; TTB: touch training B; DRL: deterministic reversal learning; PRL: deterministic reversal learning; STC: sessions to criterion; SC: session count; G×G: group × gender; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.1.1.5 Progressive ratio schedules of reinforcement

Response	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
PR4 Breakpoint	Group	1.07	1,52.0	0.31	0.31	C 62.3 ± 1.9; RMS 65.0 ± 1.9	C 62.2 ± 1.7; RMS 65.0 ± 1.3
	Gender	1.11	1,52.0	0.30	0.29	F 62.2 ± 2.0; M 65.1 ± 1.8	NA
	Session	28.75	1,444.0	1e-07	0.71	NA	NA
	G×G	0.04	1,52.0	0.84	0.83	NA	NA
	Group×S	0.84	1,444.0	0.36	0.52	NA	NA
	Gender×S	1.10	1,444.0	0.29	0.46	NA	NA
	G×G×S	0.93	1,444.0	0.34	0.49	NA	NA
PR4 Latency to collect (ms)	Group	1.11	1,52.0	0.30	0.30	C 1308.6 ± 28.8; RMS 1265.0 ± 29.1	C 1308.7 ± 24.3; RMS 1264.8 ± 22.9
	Gender	0.14	1,52.0	0.71	0.71	F 1279.1 ± 29.9; M 1294.5 ± 27.9	NA
	Session	50.78	1,444.0	4e-12	NA	NA	NA
	G×G	0.06	1,52.0	0.80	0.79	NA	NA
	Group×S	0.01	1,444.0	0.94	0.96	NA	NA
	Gender×S	2.09	1,444.0	0.15	0.32	NA	NA
	G×G×S	0.14	1,444.0	0.71	0.80	NA	NA
PR4 Latency to respond (s)	Group	5.17	1,52.0	0.027	0.024	C 19.5 ± 1.1; RMS 15.8 ± 1.1	C 19.5 ± 1.1; RMS 15.8 ± 0.6
	Gender	0.65	1,52.0	0.43	0.43	F 18.3 ± 1.2; M 17.0 ± 1.1	NA
	Session	33.59	1,444.0	1e-08	NA	NA	NA

	G×G	0.34	1,52.0	0.56	0.57	NA	NA
	Group×S	4.74	1,444.0	0.030	0.16	NA	NA
	Gender×S	0.43	1,444.0	0.51	0.69	NA	NA
	G×G×S	1.46	1,444.0	0.23	0.45	NA	NA
PR8 Breakpoint	Group	0.01	1,52.0	0.92	0.92	C 82.2 ± 2.5; RMS 81.7 ± 2.6	C 82.2 ± 1.9; RMS 81.7 ± 2.1
	Gender	0.19	1,52.0	0.67	0.67	F 81.1 ± 2.6; M 82.7 ± 2.5	NA
	Session	1.81	1,441.3	0.18	0.57	NA	NA
	G×G	0.05	1,52.0	0.82	0.83	NA	NA
	Group×S	0.97	1,441.2	0.32	0.43	NA	NA
	Gender×S	0.07	1,441.3	0.79	0.84	NA	NA
	G×G×S	1.31	1,441.3	0.25	0.36	NA	NA
PR8 Latency to collect (ms)	Group	0.58	1,52.0	0.45	0.46	C 1256.7 ± 33.8; RMS 1219.7 ± 34.2	C 1257.0 ± 29.7; RMS 1219.4 ± 25.1
	Gender	0.69	1,52.0	0.41	0.41	F 1258.2 ± 35.2; M 1218.3 ± 32.7	NA
	Session	0.18	1,441.1	0.67	NA	NA	NA
	G×G	0.02	1,52.0	0.88	0.87	NA	NA
	Group×S	0.25	1,441.1	0.62	0.67	NA	NA
	Gender×S	0.33	1,441.1	0.57	0.63	NA	NA
	G×G×S	0.29	1,441.1	0.59	0.65	NA	NA
PR8 Latency to respond (s)	Group	2.85	1,52.0	0.097	0.099	C 29.5 ± 1.7; RMS 25.3 ± 1.7	C 29.5 ± 1.3; RMS 25.3 ± 1.5
	Gender	0.09	1,52.0	0.77	0.77	F 27.0 ± 1.8; M 27.8 ± 1.7	NA
	Session	2.36	1,441.2	0.13	NA	NA	NA
	G×G	0.19	1,52.0	0.66	0.66	NA	NA
	Group×S	2.05	1,441.2	0.15	0.28	NA	NA
	Gender×S	1.33	1,441.2	0.25	0.39	NA	NA
	G×G×S	0.40	1,441.2	0.53	0.64	NA	NA
PR16 Breakpoint	Group	1.45	1,51.0	0.23	0.23	C 87.9 ± 4.3; RMS 95.0 ± 4.3	C 87.9 ± 3.6; RMS 95.0 ± 3.0
	Gender	0.37	1,51.0	0.55	0.55	F 93.3 ± 4.5; M 89.6 ± 4.1	NA
	Session	29.63	1,436.0	9e-08	0.92	NA	NA
	G×G	0.04	1,51.0	0.85	0.85	NA	NA
	Group×S	0.30	1,436.0	0.58	0.62	NA	NA
	Gender×S	0.01	1,436.0	0.91	0.92	NA	NA
	G×G×S	0.27	1,436.0	0.60	0.64	NA	NA

PR16 Latency to collect (ms)	Group	0.09	1,51.0	0.76	0.77	C 1237.6 ± 41.6; RMS 1220.2 ± 41.3	C 1238.0 ± 36.6; RMS 1219.6 ± 29.2
	Gender	0.07	1,51.0	0.79	0.78	F 1220.9 ± 43.3; M 1236.9 ± 39.5	NA
	Session	0.38	1,436.0	0.54	NA	NA	NA
	G×G	0.00	1,51.0	0.97	0.96	NA	NA
	Group×S	0.28	1,436.0	0.59	0.61	NA	NA
	Gender×S	0.10	1,436.0	0.76	0.76	NA	NA
	G×G×S	0.30	1,436.0	0.58	0.60	NA	NA
PR16 Latency to respond (s)	Group	5.36	1,51.0	0.025	0.024	C 42.5 ± 2.7; RMS 33.3 ± 2.7	C 42.5 ± 2.6; RMS 33.3 ± 1.6
	Gender	0.25	1,51.0	0.62	0.63	F 37.0 ± 2.8; M 38.8 ± 2.6	NA
	Session	4.79	1,436.0	0.029	NA	NA	NA
	G×G	1.13	1,51.0	0.29	0.28	NA	NA
	Group×S	1.39	1,436.0	0.24	0.14	NA	NA
	Gender×S	0.65	1,436.0	0.42	0.31	NA	NA
	G×G×S	0.94	1,436.0	0.33	0.22	NA	NA

Abbreviations: SPT: sucrose preference test; PS: post-stress (adulthood stress); IS: intra-stress (adulthood stress); G×G: group × gender; S: session; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.1.1.6 Probabilistic reversal learning (pre-stress)

Response	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
Correct-loss shift %	Group	0.21	1,44.0	0.65	0.65	C 59.4 ± 1.5; RMS 58.3 ± 1.5	NA
	Gender	2.27	1,44.0	0.14	0.14	F 57.3 ± 1.6; M 60.5 ± 1.3	NA
	Session	2.09	1,284.0	0.15	NA	NA	NA
	G×G	0.17	1,44.0	0.68	0.68	NA	NA
	Group×S	1.48	1,284.0	0.23	0.21	NA	NA
	Gender×S	1.54	1,284.0	0.22	0.21	NA	NA
	G×G×S	0.88	1,284.0	0.35	0.35	NA	NA
Correct-win stay %	Group	0.32	1,44.0	0.57	0.58	C 81.2 ± 1.1; RMS 82.0 ± 1.1	NA
	Gender	15.66	1,44.0	3e-04	1e-04	F 78.6 ± 1.2; M 84.6 ± 1.0	NA
	Session	5.87	1,284.0	0.016	NA	NA	NA

	G×G	0.14	1,44.0	0.71	0.71	NA	NA
	Group×S	0.00	1,284.0	1.00	1.00	NA	NA
	Gender×S	1.99	1,284.0	0.16	0.16	NA	NA
	G×G×S	0.63	1,284.0	0.43	0.43	NA	NA
Correct touch %	Group	0.01	1,44.0	0.91	0.91	C 66.3 ± 0.6; RMS 65.9 ± 0.6	NA
	Gender	16.96	1,44.0	2e-04	2e-04	F 64.4 ± 0.6; M 67.8 ± 0.5	NA
	Session	5.64	1,284.0	0.018	NA	NA	NA
	G×G	2.50	1,44.0	0.12	0.12	NA	NA
	Group×S	0.18	1,284.0	0.67	0.70	NA	NA
	Gender×S	0.20	1,284.0	0.65	0.69	NA	NA
	G×G×S	11.28	1,284.0	9e-04	0.001	NA	NA
Correct touch % [F]	Group	1.16	1,17.0	0.30	0.30	C 65.2 ± 1.1; RMS 63.5 ± 1.1	C 65.2 ± 0.7; RMS 63.5 ± 1.2
	Session	1.02	1,112.0	0.31	NA	NA	NA
	Group×S	4.17	1,112.0	0.044	0.099	NA	NA
Correct touch % [M]	Group	1.14	1,27.0	0.29	0.29	C 67.4 ± 0.6; RMS 68.3 ± 0.6	C 67.4 ± 0.5; RMS 68.3 ± 0.5
	Session	5.64	1,172.0	0.019	NA	NA	NA
	Group×S	7.52	1,172.0	0.007	0.004	NA	NA
Incorrect-loss shift %	Group	1.88	1,44.0	0.18	0.18	C 63.0 ± 1; RMS 60.6 ± 1	NA
	Gender	6.48	1,44.0	0.015	0.015	F 60.0 ± 1.1; M 63.6 ± 0.9	NA
	Session	0.64	1,284.0	0.43	NA	NA	NA
	G×G	3.20	1,44.0	0.081	0.079	NA	NA
	Group×S	0.87	1,284.0	0.35	0.39	NA	NA
	Gender×S	0.20	1,284.0	0.66	0.68	NA	NA
	G×G×S	15.24	1,284.0	1e-04	0	NA	NA
Incorrect-loss shift % [F]	Group	5.08	1,17.0	0.038	0.040	C 62.4 ± 1.5; RMS 57.6 ± 1.5	C 62.4 ± 1.1; RMS 57.6 ± 1.5
	Session	0.81	1,112.0	0.37	NA	NA	NA
	Group×S	6.25	1,112.0	0.014	0.015	NA	NA
Incorrect-loss shift % [M]	Group	0.00	1,27.0	0.95	0.95	C 63.5 ± 1.2; RMS 63.6 ± 1.2	C 63.5 ± 1.1; RMS 63.6 ± 1.0
	Session	0.10	1,172.0	0.76	NA	NA	NA
	Group×S	9.86	1,172.0	0.002	0.003	NA	NA
Incorrect-win stay %	Group	0.46	1,44.0	0.50	0.51	C 70.5 ± 1.8; RMS 72.6 ± 1.8	NA
	Gender	11.59	1,44.0	0.001	0.001	F 67.3 ± 2.0; M 75.8 ± 1.6	NA

	Session	2.83	1,284.0	0.093	NA	NA	NA
	G×G	0.43	1,44.0	0.52	0.52	NA	NA
	Group×S	0.09	1,284.0	0.77	0.81	NA	NA
	Gender×S	1.71	1,284.0	0.19	0.30	NA	NA
	G×G×S	2.24	1,284.0	0.14	0.23	NA	NA
Latency to collect (ms)	Group	0.65	1,44.0	0.43	0.42	C 1194.8 ± 41.2; RMS 1248.6 ± 41.9	C 1194.9 ± 36.8; RMS 1248.8 ± 27.7
	Gender	0.34	1,44.0	0.56	0.56	F 1204.8 ± 45.7; M 1238.6 ± 36.9	NA
	Session	0.00	1,284.0	0.97	NA	NA	NA
	G×G	0.38	1,44.0	0.54	0.54	NA	NA
	Group×S	1.12	1,284.0	0.29	0.35	NA	NA
	Gender×S	1.86	1,284.0	0.17	0.22	NA	NA
	G×G×S	2.29	1,284.0	0.13	0.17	NA	NA
Latency to respond (ms)	Group	10.01	1,44.0	0.003	0.002	C 1202.2 ± 95.7; RMS 1653.9 ± 97.5	C 1201.9 ± 46.5; RMS 1654.0 ± 89.2
	Gender	0.07	1,44.0	0.80	0.80	F 1411.8 ± 106.2; M 1444.3 ± 85.9	NA
	Session	20.82	1,284.0	8e-06	NA	NA	NA
	G×G	1.04	1,44.0	0.31	0.31	NA	NA
	Group×S	0.18	1,284.0	0.67	0.74	NA	NA
	Gender×S	0.02	1,284.0	0.90	0.92	NA	NA
	G×G×S	0.26	1,284.0	0.61	0.69	NA	NA
Perseverations per reversal	Group	0.14	1,44.0	0.71	0.72	C 0.883 ± 0.086; RMS 0.967 ± 0.088	C 0.884 ± 0.060; RMS 0.969 ± 0.099
	Gender	0.82	1,44.2	0.37	0.38	F 0.983 ± 0.096; M 0.867 ± 0.077	NA
	Session	0.47	1,280.7	0.49	NA	NA	NA
	G×G	1.97	1,44.2	0.17	0.16	NA	NA
	Group×S	1.32	1,280.7	0.25	0.15	NA	NA
	Gender×S	0.00	1,280.9	0.97	0.97	NA	NA
	G×G×S	0.45	1,280.9	0.50	0.40	NA	NA
Reversal count	Group	0.33	1,44.0	0.57	0.58	C 3.71 ± 0.20; RMS 3.84 ± 0.21	C 3.71 ± 0.13; RMS 3.84 ± 0.19
	Gender	17.38	1,44.0	1e-04	2e-04	F 3.16 ± 0.23; M 4.38 ± 0.18	NA
	Session	7.83	1,284.0	0.005	NA	NA	NA
	G×G	0.34	1,44.0	0.56	0.56	NA	NA
	Group×S	0.76	1,284.0	0.38	0.41	NA	NA
	Gender×S	0.75	1,284.0	0.39	0.40	NA	NA
	G×G×S	0.02	1,284.0	0.89	0.90	NA	NA

Trial count	Group	0.13	1,44.0	0.72	0.72	C 160.7 ± 4.0; RMS 158.7 ± 4.1	NA
	Gender	15.24	1,44.0	3e-04	7e-04	F 148.6 ± 4.4; M 170.8 ± 3.6	NA
	Session	7.23	1,284.0	0.008	NA	NA	NA
	G×G	0.00	1,44.0	0.97	0.97	NA	NA
	Group×S	1.96	1,284.0	0.16	0.24	NA	NA
	Gender×S	0.65	1,284.0	0.42	0.51	NA	NA
	G×G×S	3.31	1,284.0	0.070	0.13	NA	NA

Abbreviations: TTA: touch training A; TTB: touch training B; DRL: deterministic reversal learning; PRL: probabilistic reversal learning; G×G: group × gender; S: session; SC: session count; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females; s: seconds; ms: milliseconds

7.1.1.7 Probabilistic reversal learning (post-stress)

Response	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
Correct-loss shift %	Group	0.10	1,40.9	0.75	0.75	C 59.9 ± 1.7; RMS 61.0 ± 1.8	C 59.7 ± 1.2; RMS 60.8 ± 1.7
	Gender	1.96	1,40.4	0.17	0.17	F 58.8 ± 1.9; M 62.1 ± 1.6	NA
	Session	3.33	1,86.0	0.072	NA	NA	NA
	Baseline	2.74	1,41.2	0.11	NA	NA	NA
	G×G	1.48	1,41.4	0.23	0.23	NA	NA
	Group×S	2.69	1,86.0	0.10	0.11	NA	NA
	Gender×S	0.25	1,85.7	0.62	0.63	NA	NA
	G×G×S	0.41	1,85.6	0.52	0.53	NA	NA
Correct-win stay %	Group	0.14	1,42.2	0.71	0.71	C 80.9 ± 0.9; RMS 80.3 ± 0.9	C 80.7 ± 0.7; RMS 80.2 ± 0.9
	Gender	0.01	1,42.7	0.93	0.92	F 80.5 ± 1.0; M 80.7 ± 0.9	NA
	Session	0.01	1,85.7	0.91	NA	NA	NA
	Baseline	25.63	1,45.7	7e-06	NA	NA	NA
	G×G	0.09	1,42.5	0.76	0.76	NA	NA
	Group×S	0.22	1,85.7	0.64	0.57	NA	NA
	Gender×S	0.04	1,85.3	0.84	0.80	NA	NA
	G×G×S	0.44	1,84.8	0.51	0.43	NA	NA
Correct touch %	Group	0.85	1,42.3	0.36	0.35	C 65.9 ± 0.6; RMS 64.9 ± 0.6	C 65.7 ± 0.5; RMS 65.0 ± 0.6

	Gender	4.30	1,42.9	0.044	0.040	F 64.4 ± 0.7; M 66.4 ± 0.6	NA
	Session	1.94	1,87.1	0.17	NA	NA	NA
	Baseline	9.90	1,51.4	0.003	NA	NA	NA
	G×G	2.85	1,42.4	0.098	0.10	NA	NA
	Group×S	6.82	1,88.4	0.011	0.003	NA	NA
	Gender×S	0.00	1,86.8	0.95	0.94	NA	NA
	G×G×S	0.13	1,87.4	0.72	0.69	NA	NA
Incorrect-loss shift %	Group	0.95	1,41.4	0.34	0.33	C 63.8 ± 1.1; RMS 62.3 ± 1.1	C 63.7 ± 0.9; RMS 62.6 ± 1.0
	Gender	6.52	1,41.1	0.014	0.015	F 61.0 ± 1.2; M 65.1 ± 1.0	NA
	Session	0.93	1,87.4	0.34	NA	NA	NA
	Baseline	3.82	1,44.9	0.057	NA	NA	NA
	G×G	0.00	1,40.9	0.96	0.96	NA	NA
	Group×S	3.24	1,87.9	0.075	0.055	NA	NA
	Gender×S	0.03	1,87.1	0.87	0.86	NA	NA
	G×G×S	0.37	1,87.4	0.54	0.52	NA	NA
Incorrect-win stay %	Group	1.53	1,42.1	0.22	0.22	C 76.3 ± 2.2; RMS 72.8 ± 2.3	C 75.7 ± 1.5; RMS 71.9 ± 2.2
	Gender	0.08	1,42.1	0.77	0.78	F 74.2 ± 2.5; M 74.9 ± 2.1	NA
	Session	0.88	1,84.9	0.35	NA	NA	NA
	Baseline	5.04	1,44.1	0.030	NA	NA	NA
	G×G	0.45	1,42.5	0.51	0.51	NA	NA
	Group×S	2.15	1,85.0	0.15	0.18	NA	NA
	Gender×S	1.21	1,84.7	0.27	0.31	NA	NA
	G×G×S	0.32	1,84.6	0.57	0.61	NA	NA
Latency to collect (ms)	Group	1.88	1,42.4	0.18	0.18	C 1281.8 ± 23.8; RMS 1322.4 ± 25.1	C 1278.5 ± 21.1; RMS 1315.0 ± 16.7
	Gender	6.65	1,42.0	0.014	0.011	F 1256.7 ± 26.1; M 1347.5 ± 22.5	NA
	Session	0.06	1,82.8	0.81	NA	NA	NA
	Baseline	150.62	1,41.1	2e-15	NA	NA	NA
	G×G	0.89	1,41.8	0.35	0.36	NA	NA
	Group×S	1.62	1,82.8	0.21	0.19	NA	NA
	Gender×S	0.48	1,82.7	0.49	0.47	NA	NA
	G×G×S	0.01	1,82.6	0.94	0.93	NA	NA
Latency to first reversal (s)	Group	0.14	1,40.6	0.71	0.71	C 626.0 ± 53.1;	C 633.5 ± 54.7;

						RMS 616.1 ± 58.2	RMS 617.6 ± 38.7
	Gender	0.67	1,39.9	0.42	0.42	F 656.6 ± 60.3; M 585.5 ± 50.1	NA
	Session	0.21	1,80.9	0.65	NA	NA	NA
	Baseline	0.23	1,40.6	0.64	NA	NA	NA
	G×G	1.13	1,39.2	0.29	0.30	NA	NA
	Group×S	0.00	1,80.8	0.99	0.99	NA	NA
	Gender×S	0.00	1,80.6	0.99	0.99	NA	NA
	G×G×S	0.50	1,79.8	0.48	0.36	NA	NA
Latency to respond (ms)	Group	8.83	1,41.7	0.005	0.004	C 1286.0 ± 100.3; RMS 1688.5 ± 106.4	C 1279.8 ± 66.6; RMS 1701.0 ± 98.8
	Gender	3.07	1,41.4	0.087	0.087	F 1357.7 ± 109.1; M 1616.8 ± 95.1	NA
	Session	0.06	1,85.9	0.81	NA	NA	NA
	Baseline	23.12	1,40.1	2e-05	NA	NA	NA
	G×G	0.59	1,41.2	0.45	0.45	NA	NA
	Group×S	0.00	1,85.9	0.95	0.95	NA	NA
	Gender×S	0.29	1,85.5	0.59	0.58	NA	NA
	G×G×S	1.61	1,85.5	0.21	0.18	NA	NA
Perseverations per reversal	Group	0.67	1,39.8	0.42	0.41	C 0.843 ± 0.100; RMS 0.665 ± 0.110	C 0.867 ± 0.123; RMS 0.679 ± 0.108
	Gender	8.73	1,38.9	0.005	0.004	F 0.958 ± 0.111; M 0.551 ± 0.093	NA
	Session	0.00	1,83.6	0.98	NA	NA	NA
	Baseline	1.30	1,39.7	0.26	NA	NA	NA
	G×G	1.75	1,38.6	0.19	0.19	NA	NA
	Group×S	0.63	1,83.3	0.43	0.42	NA	NA
	Gender×S	1.19	1,82.9	0.28	0.27	NA	NA
	G×G×S	0.91	1,82.8	0.34	0.34	NA	NA
Reversal count	Group	0.26	1,42.5	0.61	0.61	C 3.45 ± 0.24; RMS 3.23 ± 0.25	C 3.60 ± 0.24; RMS 3.32 ± 0.19
	Gender	0.65	1,42.8	0.43	0.42	F 3.48 ± 0.27; M 3.20 ± 0.23	NA
	Session	0.18	1,83.1	0.68	NA	NA	NA
	Baseline	10.17	1,45.3	0.003	NA	NA	NA
	G×G	0.31	1,42.6	0.58	0.58	NA	NA
	Group×S	1.44	1,83.3	0.23	0.22	NA	NA

Gender×S	0.27	1,82.9	0.61	0.60	NA	NA
G×G×S	0.97	1,83.0	0.33	0.31	NA	NA

Abbreviations: TTA: touch training A; TTB: touch training B; DRL: deterministic reversal learning; PRL: probabilistic reversal learning; G×G: group × gender; S: session; SC: session count; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females; s: seconds; ms: milliseconds

7.1.1.8 Probabilistic reversal learning trial-by-trial

Response	Predictor	Chisq	p (parametric)	EMMs (parametric)
P|(same choice as last trial)	Group	2.43	0.12	C 0.620 ± 0.007; RMS 0.637 ± 0.007
	Gender	9.03	0.003	F 0.612 ± 0.008; M 0.645 ± 0.006
	Session	2.98	0.084	NA
	Past outcome	9306.53	0	NA
	G×G	0.12	0.72	NA
	Group×S	1.76	0.18	NA
	Gender×S	4.21	0.040	NA
	Group×PO	0.01	0.94	NA
	Gender×PO	155.73	1e-35	NA
	Session×PO	18.80	1e-05	NA
	G×G×S	5.42	0.020	NA
	G×G×PO	6.78	0.009	NA
	Group×S×PO	1.39	0.24	NA
	Gender×S×PO	2.60	0.11	NA
	G×G×S×PO	5.61	0.018	NA
P|(same choice as 2 trials ago)	Group	1.64	0.20	C 0.551 ± 0.004; RMS 0.559 ± 0.004
	Gender	2.88	0.089	F 0.552 ± 0.005; M 0.558 ± 0.004
	Session	2.09	0.15	NA
	Past outcome	3783.91	0	NA
	G×G	0.32	0.57	NA
	Group×S	1.19	0.27	NA
	Gender×S	2.29	0.13	NA
	Group×PO	1.20	0.27	NA
	Gender×PO	53.07	3e-13	NA
	Session×PO	1.61	0.20	NA
	G×G×S	0.91	0.34	NA
	G×G×PO	2.00	0.16	NA
	Group×S×PO	0.30	0.58	NA
	Gender×S×PO	2.47	0.12	NA
	G×G×S×PO	0.18	0.67	NA
P|(same choice as 3 trials ago)	Group	0.72	0.40	C 0.539 ± 0.004; RMS 0.544 ± 0.004
	Gender	0.45	0.50	F 0.541 ± 0.004; M 0.541 ± 0.003

Session	0.01	0.93	NA
Past outcome	2120.98	0	NA
G×G	0.02	0.88	NA
Group×S	0.93	0.33	NA
Gender×S	0.07	0.79	NA
Group×PO	0.12	0.73	NA
Gender×PO	24.53	7e-07	NA
Session×PO	3.11	0.078	NA
G×G×S	2.26	0.13	NA
G×G×PO	1.01	0.31	NA
Group×S×PO	0.08	0.77	NA
Gender×S×PO	0.60	0.44	NA
G×G×S×PO	0.23	0.63	NA

Abbreviations: G×G: group × gender; S: session; PO: past outcome; SC: session count; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females; s: seconds; ms: milliseconds

7.1.1.9 Probabilistic reversal learning trial-by-trial post-hoc

Pairwise comparison	Z-score	p (parametric)	Estimates (parametric)
Slope between Groups within Female Punished	1.48	0.14	C 0.00151 ± 0.0179; RMS -0.0366 ± 0.0185
Slope between Groups within Male Punished	-3.46	5e-04	C -0.0425 ± 0.0145; RMS 0.0274 ± 0.0141
Slope between Groups within Female Rewarded	0.07	0.94	C 0.00892 ± 0.0175; RMS 0.00702 ± 0.0186
Slope between Groups within Male Rewarded	-0.28	0.78	C 0.0477 ± 0.0150; RMS 0.0537 ± 0.0149
EMMs between Groups within Female Punished	-2.02	0.043	C 0.387 ± 0.012; RMS 0.424 ± 0.013
EMMs between Groups within Female Rewarded	-0.28	0.78	C 0.783 ± 0.009; RMS 0.787 ± 0.009
EMMs between Groups within Male Rewarded	-1.63	0.10	C 0.838 ± 0.006; RMS 0.852 ± 0.005
EMMs between Groups within Male Punished Session 1	2.29	0.022	C 0.404 ± 0.015; RMS 0.358 ± 0.014
EMMs between Groups within Male Punished Session 7	-2.60	0.009	C 0.345 ± 0.014; RMS 0.397 ± 0.014

Abbreviations: EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.1.2 Cytokine analysis statistics

Response (analyte log conc.)	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
G-CSF	Group	0.13	1,35	0.72	NA	C 1.19 ± 0.05; RMS 1.22 ± 0.05	NA
	Gender	0.07	1,35	0.79	NA	F 1.22 ± 0.05; M 1.20 ± 0.05	NA
	G×G	0.02	1,35	0.90	NA	NA	NA
GM-CSF	Group	0.07	1,39.9	0.79	0.77	C 1.92 ± 0.06; RMS 1.95 ± 0.06	C 1.92 ± 0.05; RMS 1.95 ± 0.06
	Gender	1.38	1,40.0	0.25	0.20	F 1.99 ± 0.06; M 1.88 ± 0.06	NA
	Timepoint	58.14	1,39.6	3e-09	NA	NA	NA
	G×G	0.01	1,39.9	0.91	0.90	NA	NA
	Group×T	0.91	1,39.9	0.34	0.40	NA	NA
	Gender×T	2.05	1,40.0	0.16	0.20	NA	NA
	G×G×T	0.07	1,39.9	0.80	0.81	NA	NA
GRO-KC	Group	0.44	1,44.0	0.51	NA	C 1.94 ± 0.04; RMS 1.98 ± 0.04	NA
	Gender	0.32	1,44.2	0.57	NA	F 1.98 ± 0.04; M 1.94 ± 0.04	NA
	Timepoint	72.40	2,76.2	2e-18	NA	NA	NA
	G×G	0.46	1,44.0	0.50	NA	NA	NA
	Group×T	0.24	2,76.1	0.79	NA	NA	NA
	Gender×T	1.32	2,76.3	0.27	NA	NA	NA
	G×G×T	0.98	2,76.2	0.38	NA	NA	NA
IFN-g	Group	0.57	1,42.2	0.45	0.42	C 2.75 ± 0.05; RMS 2.82 ± 0.05	C 2.75 ± 0.04; RMS 2.82 ± 0.05
	Gender	3.70	1,42.6	0.061	0.042	F 2.85 ± 0.05; M 2.72 ± 0.05	NA
	Timepoint	23.15	2,70.5	2e-08	NA	NA	NA
	G×G	0.00	1,42.4	0.98	0.98	NA	NA
	Group×T	0.42	2,70.8	0.66	0.63	NA	NA
	Gender×T	0.93	2,71.3	0.40	0.40	NA	NA
	G×G×T	1.87	2,71.1	0.16	0.19	NA	NA
IL-1a	Group	0.45	1,44.8	0.51	0.45	C 2.28 ± 0.04; RMS 2.31 ± 0.03	C 2.28 ± 0.03; RMS 2.31 ± 0.03
	Gender	2.47	1,45.0	0.12	0.077	F 2.34 ± 0.04; M 2.25 ± 0.03	NA
	Timepoint	48.10	2,77.2	3e-14	NA	NA	NA
	G×G	0.05	1,44.9	0.82	0.80	NA	NA
	Group×T	0.07	2,77.4	0.94	0.94	NA	NA

	Gender×T	2.80	2,77.6	0.067	0.11	NA	NA
	G×G×T	0.72	2,77.6	0.49	0.52	NA	NA
IL-1b	Group	0.43	1,44.0	0.52	0.48	C 2.05 ± 0.03; RMS 2.09 ± 0.03	C 2.06 ± 0.03; RMS 2.09 ± 0.03
	Gender	4.32	1,44.2	0.044	0.028	F 2.12 ± 0.03; M 2.02 ± 0.03	NA
	Timepoint	43.72	2,75.4	2e-13	NA	NA	NA
	G×G	0.40	1,44.3	0.53	0.50	NA	NA
	Group×T	0.92	2,75.6	0.40	0.39	NA	NA
	Gender×T	1.85	2,75.9	0.16	0.21	NA	NA
	G×G×T	0.83	2,76.0	0.44	0.43	NA	NA
IL-2	Group	0.00	1,44.1	0.94	0.94	C 3.21 ± 0.04; RMS 3.21 ± 0.04	C 3.21 ± 0.04; RMS 3.21 ± 0.03
	Gender	2.86	1,44.4	0.098	0.070	F 3.26 ± 0.04; M 3.16 ± 0.04	NA
	Timepoint	45.71	2,74.7	1e-13	NA	NA	NA
	G×G	0.60	1,44.6	0.44	0.42	NA	NA
	Group×T	0.04	2,75.1	0.96	0.96	NA	NA
	Gender×T	2.07	2,75.4	0.13	0.16	NA	NA
	G×G×T	0.29	2,75.7	0.75	0.74	NA	NA
IL-4	Group	0.11	1,44.8	0.74	0.71	C 2.26 ± 0.04; RMS 2.28 ± 0.04	C 2.26 ± 0.03; RMS 2.28 ± 0.03
	Gender	2.42	1,45.0	0.13	0.087	F 2.32 ± 0.04; M 2.23 ± 0.04	NA
	Timepoint	27.01	2,76.7	1e-09	NA	NA	NA
	G×G	0.39	1,44.9	0.54	0.49	NA	NA
	Group×T	0.69	2,76.8	0.51	0.47	NA	NA
	Gender×T	2.04	2,77.1	0.14	0.18	NA	NA
	G×G×T	0.50	2,77.0	0.61	0.57	NA	NA
IL-5	Group	0.37	1,44.8	0.54	0.49	C 3.02 ± 0.02; RMS 3.04 ± 0.02	C 3.02 ± 0.02; RMS 3.04 ± 0.02
	Gender	4.12	1,45.0	0.048	0.021	F 3.06 ± 0.02; M 2.99 ± 0.02	NA
	Timepoint	68.25	2,77.2	8e-18	NA	NA	NA
	G×G	0.15	1,44.9	0.70	0.66	NA	NA
	Group×T	0.27	2,77.4	0.77	0.77	NA	NA
	Gender×T	1.34	2,77.6	0.27	0.30	NA	NA
	G×G×T	0.44	2,77.6	0.64	0.65	NA	NA
IL-6	Group	0.01	1,42.1	0.93	0.92	C 2.76 ± 0.05; RMS 2.76 ± 0.05	C 2.77 ± 0.03; RMS 2.76 ± 0.05
	Gender	3.30	1,42.5	0.076	0.052	F 2.83 ± 0.05; M 2.70 ± 0.05	NA
	Timepoint	20.99	2,67.9	8e-08	NA	NA	NA
	G×G	0.12	1,42.4	0.73	0.71	NA	NA

	Group×T	0.02	2,68.9	0.98	0.98	NA	NA
	Gender×T	1.44	2,69.3	0.24	0.27	NA	NA
	G×G×T	0.09	2,69.4	0.91	0.91	NA	NA
IL-7	Group	0.73	1,44.0	0.40	0.36	C 1.96 ± 0.04; RMS 2.01 ± 0.04	C 1.96 ± 0.04; RMS 2.02 ± 0.04
	Gender	2.29	1,44.1	0.14	0.11	F 2.04 ± 0.04; M 1.94 ± 0.04	NA
	Timepoint	38.63	2,73.9	3e-12	NA	NA	NA
	G×G	0.23	1,44.4	0.64	0.60	NA	NA
	Group×T	0.52	2,74.4	0.59	0.55	NA	NA
	Gender×T	2.56	2,74.6	0.084	0.13	NA	NA
	G×G×T	0.87	2,74.9	0.42	0.41	NA	NA
IL-10	Group	0.21	1,44.8	0.65	0.64	C 2.05 ± 0.03; RMS 2.07 ± 0.03	C 2.05 ± 0.03; RMS 2.07 ± 0.03
	Gender	3.49	1,45.0	0.068	0.052	F 2.11 ± 0.03; M 2.01 ± 0.03	NA
	Timepoint	40.05	2,77.2	1e-12	NA	NA	NA
	G×G	0.48	1,44.9	0.49	0.46	NA	NA
	Group×T	0.24	2,77.4	0.79	0.76	NA	NA
	Gender×T	2.03	2,77.6	0.14	0.17	NA	NA
	G×G×T	0.42	2,77.6	0.66	0.62	NA	NA
IL-12p70	Group	1.08	1,43.9	0.30	0.25	C 2.42 ± 0.04; RMS 2.48 ± 0.04	C 2.42 ± 0.04; RMS 2.48 ± 0.04
	Gender	1.59	1,44.1	0.21	0.16	F 2.50 ± 0.04; M 2.41 ± 0.04	NA
	Timepoint	31.81	2,74.0	1e-10	NA	NA	NA
	G×G	0.04	1,43.9	0.85	0.83	NA	NA
	Group×T	1.02	2,74.5	0.36	0.39	NA	NA
	Gender×T	3.07	2,74.7	0.052	0.11	NA	NA
	G×G×T	0.18	2,74.5	0.83	0.83	NA	NA
IL-13	Group	0.27	1,40.2	0.61	0.59	C 2.63 ± 0.07; RMS 2.59 ± 0.06	C 2.63 ± 0.05; RMS 2.59 ± 0.07
	Gender	2.07	1,40.5	0.16	0.14	F 2.68 ± 0.06; M 2.54 ± 0.06	NA
	Timepoint	28.38	1,39.6	4e-06	NA	NA	NA
	G×G	0.07	1,40.3	0.79	0.77	NA	NA
	Group×T	0.26	1,40.1	0.61	0.65	NA	NA
	Gender×T	1.58	1,40.4	0.22	0.26	NA	NA
	G×G×T	0.01	1,40.2	0.91	0.92	NA	NA
IL-17A	Group	0.10	1,42.7	0.75	0.72	C 1.66 ± 0.04; RMS 1.69 ± 0.04	C 1.66 ± 0.05; RMS 1.69 ± 0.04
	Gender	3.32	1,42.7	0.076	0.054	F 1.74 ± 0.04; M 1.61 ± 0.04	NA
	Timepoint	87.67	1,42.5	7e-12	NA	NA	NA

	G×G	0.00	1,42.7	0.95	0.95	NA	NA
	Group×T	1.72	1,42.7	0.20	0.24	NA	NA
	Gender×T	4.27	1,42.7	0.045	0.067	NA	NA
	G×G×T	0.18	1,42.7	0.67	0.70	NA	NA
IL-18	Group	0.18	1,43.8	0.68	0.59	C 3.46 ± 0.04; RMS 3.48 ± 0.04	C 3.46 ± 0.03; RMS 3.48 ± 0.03
	Gender	0.44	1,44.4	0.51	0.40	F 3.49 ± 0.04; M 3.45 ± 0.04	NA
	Timepoint	43.22	2,73.0	4e-13	NA	NA	NA
	G×G	1.25	1,45.0	0.27	0.17	NA	NA
	Group×T	0.98	2,73.7	0.38	0.42	NA	NA
	Gender×T	1.90	2,74.3	0.16	0.21	NA	NA
	G×G×T	0.69	2,74.9	0.50	0.54	NA	NA
M-CSF	Group	0.01	1,41.9	0.94	0.94	C 1.68 ± 0.02; RMS 1.68 ± 0.02	C 1.68 ± 0.02; RMS 1.68 ± 0.02
	Gender	1.83	1,43.0	0.18	0.11	F 1.70 ± 0.03; M 1.65 ± 0.02	NA
	Timepoint	359.05	1,41.8	4e-22	NA	NA	NA
	G×G	0.04	1,43.0	0.84	0.82	NA	NA
	Group×T	3.85	1,41.8	0.057	0.076	NA	NA
	Gender×T	0.21	1,42.9	0.65	0.67	NA	NA
	G×G×T	0.08	1,43.0	0.77	0.80	NA	NA
MCP-1	Group	1.29	1,44.2	0.26	0.24	C 2.99 ± 0.03; RMS 3.03 ± 0.03	C 2.99 ± 0.03; RMS 3.03 ± 0.03
	Gender	0.24	1,44.2	0.63	0.62	F 3.00 ± 0.0; M 3.02 ± 0.0	NA
	Timepoint	309.01	2,75.7	4e-37	NA	NA	NA
	G×G	0.08	1,44.2	0.78	0.77	NA	NA
	Group×T	0.93	2,76.0	0.40	0.36	NA	NA
	Gender×T	2.44	2,75.9	0.094	0.13	NA	NA
	G×G×T	1.37	2,76.0	0.26	0.26	NA	NA
MIP-1a	Group	0.03	1,44.3	0.87	0.87	C 1.62 ± 0.03; RMS 1.63 ± 0.03	C 1.62 ± 0.04; RMS 1.63 ± 0.03
	Gender	0.28	1,43.8	0.60	0.58	F 1.64 ± 0.03; M 1.61 ± 0.03	NA
	Timepoint	5.53	1,43.3	0.023	NA	NA	NA
	G×G	0.00	1,44.8	0.99	0.99	NA	NA
	Group×T	0.32	1,44.3	0.57	0.58	NA	NA
	Gender×T	1.24	1,43.8	0.27	0.27	NA	NA
	G×G×T	0.00	1,44.8	0.98	0.99	NA	NA
MIP-3a	Group	0.10	1,44.8	0.75	0.72	C 1.47 ± 0.03; RMS 1.48 ± 0.03	C 1.47 ± 0.03; RMS 1.48 ± 0.03
	Gender	0.09	1,45.0	0.76	0.72	F 1.49 ± 0.03; M 1.47 ± 0.03	NA

	Timepoint	35.11	2,77.2	1e-11	NA	NA	NA
	G×G	0.07	1,44.9	0.79	0.77	NA	NA
	Group×T	0.32	2,77.4	0.72	0.70	NA	NA
	Gender×T	4.21	2,77.6	0.018	0.049	NA	NA
	G×G×T	0.61	2,77.6	0.54	0.53	NA	NA
RANTES	Group	0.32	1,44.1	0.58	0.56	C 2.66 ± 0.03; RMS 2.69 ± 0.03	C 2.66 ± 0.03; RMS 2.69 ± 0.03
	Gender	0.06	1,44.2	0.81	0.81	F 2.67 ± 0.03; M 2.68 ± 0.03	NA
	Timepoint	19.49	1,44.3	6e-05	NA	NA	NA
	G×G	0.47	1,44.1	0.50	0.48	NA	NA
	Group×T	0.69	1,44.1	0.41	0.41	NA	NA
	Gender×T	1.65	1,44.2	0.21	0.22	NA	NA
	G×G×T	1.83	1,44.1	0.18	0.19	NA	NA
TNF-a	Group	0.03	1,44.8	0.86	0.86	C 2.98 ± 0.03; RMS 2.97 ± 0.03	C 2.98 ± 0.03; RMS 2.97 ± 0.03
	Gender	3.14	1,45.0	0.083	0.069	F 3.01 ± 0.03; M 2.93 ± 0.03	NA
	Timepoint	49.39	2,77.2	2e-14	NA	NA	NA
	G×G	1.42	1,44.9	0.24	0.22	NA	NA
	Group×T	0.14	2,77.4	0.87	0.86	NA	NA
	Gender×T	1.61	2,77.6	0.21	0.22	NA	NA
	G×G×T	0.64	2,77.6	0.53	0.51	NA	NA
VEGF	Group	0.69	1,43.8	0.41	0.35	C 2.26 ± 0.05; RMS 2.33 ± 0.05	C 2.26 ± 0.04; RMS 2.33 ± 0.05
	Gender	1.61	1,43.7	0.21	0.16	F 2.34 ± 0.05; M 2.24 ± 0.05	NA
	Timepoint	82.72	2,71.3	3e-19	NA	NA	NA
	G×G	0.39	1,44.0	0.54	0.49	NA	NA
	Group×T	0.52	2,72.4	0.60	0.60	NA	NA
	Gender×T	1.69	2,72.3	0.19	0.26	NA	NA
	G×G×T	0.02	2,72.7	0.98	0.98	NA	NA

Abbreviations: G×G: group × gender; ×T: × timepoint; EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females; G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; GRO-KC: growth-regulated oncogene - keratinocyte chemoattractant; IFN-g: interferon gamma, IL: interleukin; M-CSF: macrophage colony-stimulating factor, MCP-1: monocyte chemoattractant protein 1; MIP-1a: macrophage inflammatory protein 1 alpha; RANTES: regulated upon activation, normal T cell expressed and secreted; TNF-a: tumour necrosis factor alpha; VEGF: vascular endothelial growth factor

7.1.3 MRI analysis statistics

7.1.3.1 Regional brain volumes

Response (ROI volume in mm^3)	Predictor	F	Df	p (parametric)	p (permutation test)	EMMs (parametric)	EMMs (bootstrap)
Amygdala	Group	0.77	1,44.2	0.38	0.39	C 30.3 ± 0.1; RMS 30.4 ± 0.1	C 30.3 ± 0.1; RMS 30.4 ± 0.1
	Gender	15.96	1,75.5	1e-04	4e-04	F 29.9 ± 0.2; M 30.7 ± 0.1	NA
	Timepoint	1062.64	2,62.6	5e-49	NA	NA	NA
	TBV	118.01	1,80.6	2e-17	NA	NA	NA
	G×G	0.46	1,44.9	0.50	0.50	NA	NA
	Group×T	4.78	2,63.1	0.012	0.016	NA	NA
	Gender×T	2.16	2,73.3	0.12	0.14	NA	NA
	TBV×T	1.83	2,65.6	0.17	NA	NA	NA
	G×G×T	1.13	2,63.9	0.33	0.34	NA	NA
Cingulate cortex	Group	0.28	1,43.3	0.60	0.59	C 27.4 ± 0.1; RMS 27.6 ± 0.1	C 27.4 ± 0.1; RMS 27.6 ± 0.1
	Gender	0.66	1,69.5	0.42	0.42	F 27.7 ± 0.2; M 27.3 ± 0.2	NA
	Timepoint	293.88	2,65.0	3e-33	NA	NA	NA
	TBV	68.80	1,70.8	5e-12	NA	NA	NA
	G×G	0.05	1,44.2	0.82	0.83	NA	NA
	Group×T	0.22	2,65.6	0.80	0.81	NA	NA
	Gender×T	0.77	2,75.5	0.47	0.49	NA	NA
	TBV×T	1.30	2,69.4	0.28	NA	NA	NA
	G×G×T	0.51	2,66.6	0.60	0.62	NA	NA
Dorsal striatum	Group	0.22	1,45.1	0.64	0.65	C 66.9 ± 0.2; RMS 66.7 ± 0.2	C 66.9 ± 0.2; RMS 66.7 ± 0.2
	Gender	0.44	1,81.1	0.51	0.52	F 67.2 ± 0.3; M 66.4 ± 0.3	NA
	Timepoint	6715.52	2,59.7	5e-71	NA	NA	NA
	TBV	178.74	1,93.5	2e-23	NA	NA	NA
	G×G	0.12	1,45.7	0.73	0.73	NA	NA
	Group×T	2.34	2,60.2	0.10	0.11	NA	NA
	Gender×T	3.19	2,70.5	0.047	0.050	NA	NA
	TBV×T	1.94	2,61.5	0.15	NA	NA	NA
	G×G×T	0.13	2,60.8	0.88	0.89	NA	NA
Hippocampus	Group	0.21	1,44.7	0.65	0.66	C 83.0 ± 0.2; RMS 82.9 ±	C 83.0 ± 0.2; RMS 82.9 ± 0.2

						0.2	
	Gender	0.39	1,78.5	0.53	0.54	F 82.8 ± 0.3; M 83.1 ± 0.3	NA
	Timepoint	4961.62	2,61.2	2e-68	NA	NA	NA
	TBV	239.62	1,86.7	1e-26	NA	NA	NA
	G×G	0.00	1,45.3	0.98	0.98	NA	NA
	Group×T	0.06	2,61.7	0.94	0.95	NA	NA
	Gender×T	0.02	2,72.0	0.98	0.98	NA	NA
	TBV×T	0.25	2,63.6	0.78	NA	NA	NA
	G×G×T	0.11	2,62.4	0.90	0.90	NA	NA
Insula	Group	0.00	1,42.6	0.96	0.95	C 35.3 ± 0.1; RMS 35.4 ± 0.1	C 35.3 ± 0.1; RMS 35.4 ± 0.1
	Gender	0.25	1,65.7	0.62	0.62	F 35.2 ± 0.2; M 35.5 ± 0.2	NA
	Timepoint	818.06	2,66.5	2e-47	NA	NA	NA
	TBV	169.86	1,65.6	7e-20	NA	NA	NA
	G×G	0.18	1,43.6	0.67	0.67	NA	NA
	Group×T	0.53	2,67.2	0.59	0.57	NA	NA
	Gender×T	1.60	2,76.8	0.21	0.18	NA	NA
	TBV×T	2.83	2,72.0	0.066	NA	NA	NA
	G×G×T	0.83	2,68.2	0.44	0.40	NA	NA
Nucleus accumbens	Group	0.44	1,43.9	0.51	0.51	C 11.0 ± 0; RMS 11.1 ± 0	C 11.0 ± 0.0; RMS 11.1 ± 0.1
	Gender	0.38	1,73.3	0.54	0.54	F 11.0 ± 0; M 11.0 ± 0	NA
	Timepoint	1070.74	2,63.5	1e-49	NA	NA	NA
	TBV	70.56	1,76.7	2e-12	NA	NA	NA
	G×G	0.00	1,44.7	0.95	0.95	NA	NA
	Group×T	0.01	2,64.1	0.99	0.99	NA	NA
	Gender×T	1.31	2,74.1	0.28	0.33	NA	NA
	TBV×T	0.08	2,67.0	0.92	NA	NA	NA
	G×G×T	0.08	2,65.0	0.93	0.93	NA	NA

Abbreviations: G×G: group × gender; ×T: × timepoint; TBV: total brain volume (mean-centred within timepoint); EMMs: estimated marginal means; RMS: repeated maternal separation; C: controls; M: males; F: females

7.2 Supplementary figures

7.2.1 Behavioural analysis supplementary figures

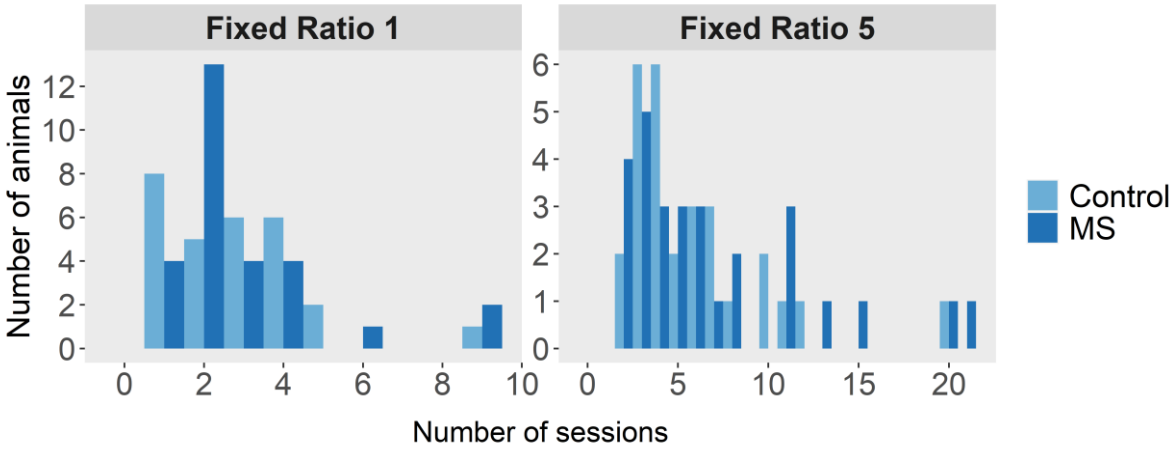

Figure S3.1. Repeated maternal separation (MS) did not affect sessions-to-criterion for either of the training stages for the progressive ratio (PR) task. Before being tested on the three progressive ratio (PR) schedules of reinforcement, animals were trained on fixed ratio 1 and 5 schedules, in which they had to respond on the target one and five times respectively to earn a reward. There were no differences in sessions-to-criterion between MS (n = 28) and control (n = 28) animals. Histogram bins are one session in width.

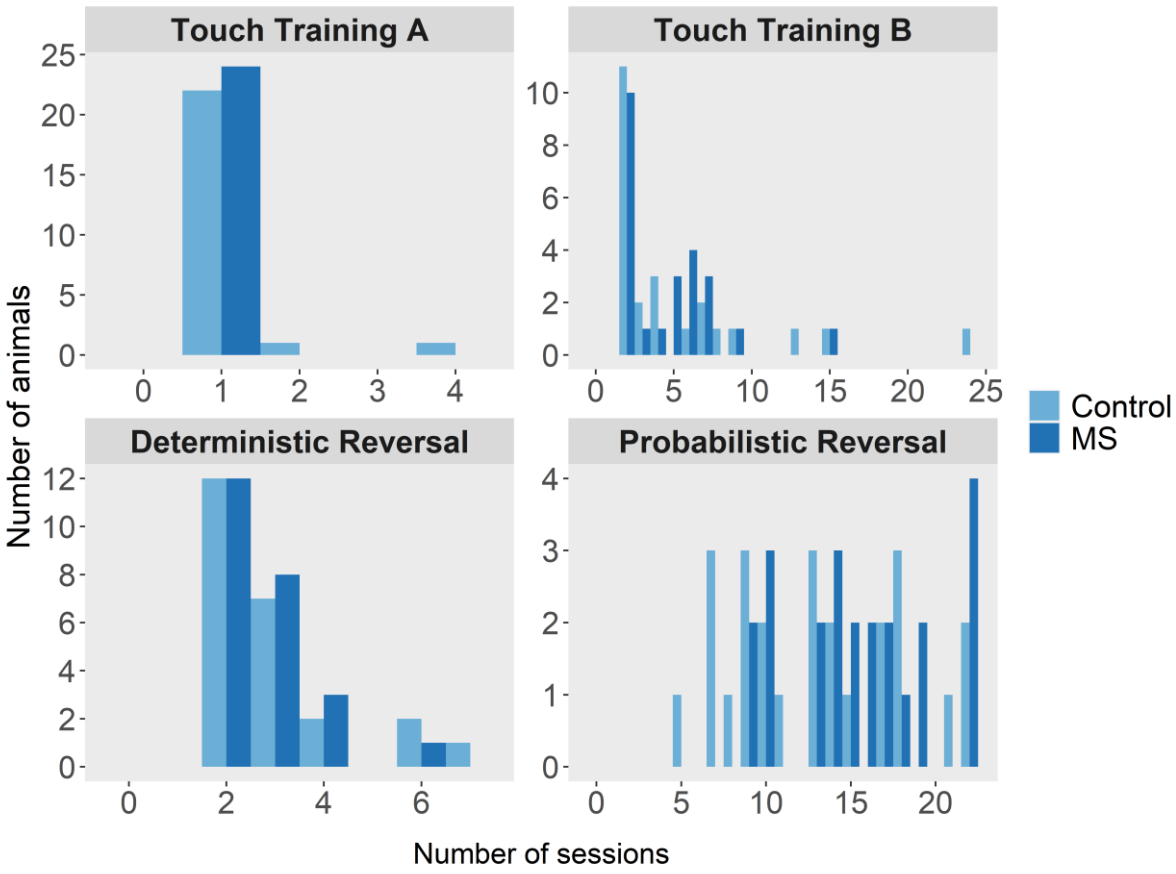

Figure S3.2. Repeated maternal separation (MS) did not affect sessions-to-criterion on any training stages for probabilistic reversal learning (PRL) or PRL session count before adult stress. Among animals included in the pre-adult stress analysis, there were no significant differences between repeated maternal separation (MS, n = 24) and control (n = 24) animals in sessions-to-criterion on the touch training A, touch training B, or deterministic reversal learning (DRL) training stages. Animals included in the post-stress analysis completed varying numbers of PRL sessions before being started on the adult stress (lower right panel; MS n = 25, control n = 23). Histogram bins are one session in width.

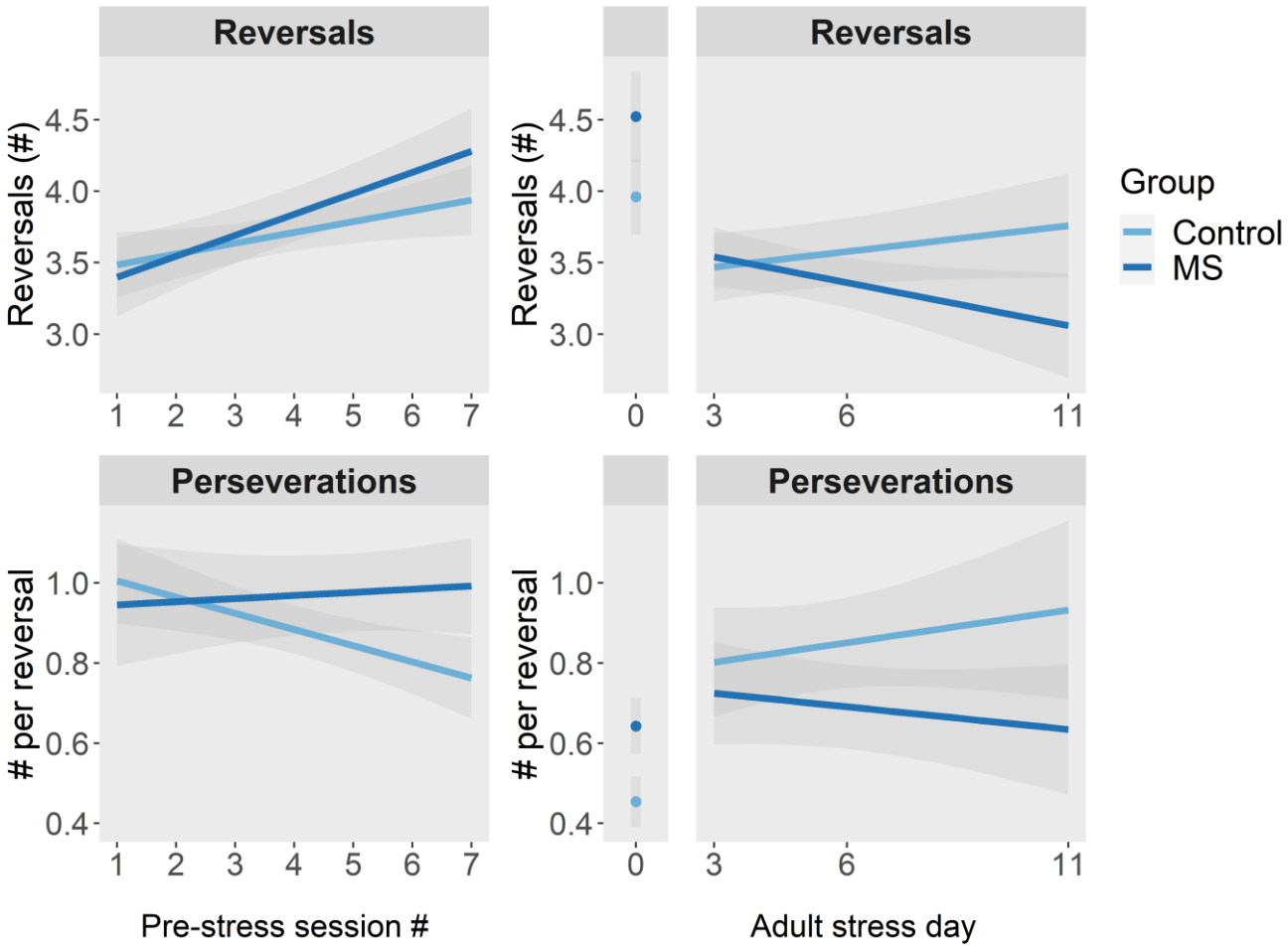

Figure S3.3. Repeated maternal separation (MS) had no effect on reversals attained or perseveration tendency on the probabilistic reversal learning (PRL) task. Across each animal's first seven PRL sessions, there were no differences between MS (n = 24) and control (n = 24) animals in the number of reversals completed or the mean number of perseverations following each reversal. Similarly, there was no difference between MS (n = 24-25 at stress days 3-6, n = 18 at stress day 11) and control (n = 23 at stress days 3-6, n = 16 at stress day 11) animals in the effect that stress had on their reversal count or perseveration tendency.

7.2.2 Systematic review supplementary figures

		Short-term		Long-term without stress		Long-term with stress	
		Protein	mRNA	Protein	mRNA	Protein	mRNA
Non-Blood	IL-1β	↔	↓	↔	↔	↑	↑
	TNF-α	↑	↑	↔	↔	↑ / ↔	↑
	IL-6	↑	↑	↔	↔	↑	↑
	IL-10	↑	↓	↔	↔	↓	↑ / ↓

Figure S4.1. Effects of repeated maternal separation (RMS) on cytokine expression in non-blood tissue, segregated by messenger RNA (mRNA) and protein. The most commonly reported outcomes (increase, decrease, or no change) are summarized for each cytokine for each stress condition, counting mRNA and protein results separately. Dark blue shading indicates a high level of confidence (three or more studies supporting each outcome), whereas light blue shading indicates low confidence.

Study	Species	Gender	Non-blood tissue assay substrate	Later-life stress
Amini-Khoei 2017	Mouse	Male	mRNA	Forced swim test and open field test
Amini-Khoei 2019	Mouse	Male	mRNA	Forced swim test, open field test, and elevated plus maze
Avitsur 2006	Mouse	Both	Protein (IL-6), mRNA (IL-1b, TNF-a, IL-6)	Influenza virus infection (inoculation nine days prior to sacrifice)
Avitsur 2013	Mouse	Both	N/A	Isolation housing for one day then a single IP injection of either saline, low-dose LPS, or high-dose LPS two hours before sacrifice
Baldy 2018	Rat	Both	N/A	N/A
Banqueri 2019	Rat	Male	mRNA	N/A
Barouei 2015	Rat	Both	N/A	Four days of thirty minutes daily restraint or isolation stress, immediately before sacrifice
Barreau 2004	Rat	Male	mRNA	N/A
Breivik 2015	Rat	Both	N/A	Three weeks of ligature-induced periodontitis and then an LPS injection two hours before sacrifice
Carboni 2010	Rat	Male	N/A	Forced swim test roughly a week before sacrifice in depression-prone rats
Dalle 2016	Rat	Male	mRNA	Intracerebral injection of the neurotoxin 6-hydroxydopamine to lesion the dopaminergic projections passing through the medial forebrain bundle
De Miguel 2018	Rat	Male	Protein	IP injection of either saline or low-dose LPS fourteen hours before sacrifice
Desbonnet 2010	Rat	Male	N/A	Forced swim test
Dimatelis 2012	Rat	Male	mRNA	N/A
Do Prado 2016	Rat	Both	N/A	Lack of enrichment other than one cage-mate for five weeks between weaning and sacrifice
Fuentes 2016	Mouse	Male	mRNA	One-hour water avoidance stress
Ganguly 2018	Rat	Both	N/A	N/A
Ganguly 2019	Rat	Both	Protein (TNF-a),	IP injection of saline four hours before

			mRNA (TNF-a)	sacrifice
Genty 2018	Rat	Male	mRNA	Compression trauma to a peripheral nerve
Giridharan 2019	Rat	Male	Protein	N/A
Grassi-Oliveira 2016	Rat	Both	N/A	N/A
Han 2019	Mouse	Male	Protein (IL-6), mRNA (IL-1b, TNF-a, IL-6)	Two weeks of IP saline injections followed in some animals by two weeks of two hours daily restraint
Kiank 2009	Mouse	Female	Protein	Five days of restraint and acoustic noise stress for two to four hours daily
Kruschinski 2008	Rat	Male	mRNA	Sensitization to ovalbumin by IP injection one and two weeks before sacrifice then tracheal instillation of ovalbumin a day before sacrifice
Lennon 2013	Mouse	Unclear	mRNA	N/A
Li 2017	Mouse	Unclear	mRNA	N/A
Majcher-Maslanka 2019	Rat	Both	mRNA	N/A
Mizoguchi 2019	Mouse	Male	N/A	Compression trauma to a peripheral nerve
Moya-Perez 2017	Mouse	Male	Protein	N/A
O'Mahony 2009	Rat	Male	N/A	Repeated brief open field testing
O'Malley 2011	Rat	Male	Protein	N/A
Pierce 2014	Mouse	Female	mRNA	Abdominal wall surgery and visceral organ distension to assess pain sensitivity, a week prior to sacrifice
Pierce 2016	Mouse	Female	mRNA	One-hour water avoidance stress and either sacrifice next day or sacrifice eight days later with three tail vein bleeds during that time
Pinheiro 2014	Rat	Male	Protein	Two weeks of daily IP saline injections then brief behavioural testing including open field test
Reus 2013	Rat	Male	Protein	Two weeks of daily IP saline injections with forced swim testing on the final two days, with sacrifice immediately after the final test

Riba 2017	Mouse	Unclear	Protein	N/A
Riba 2018	Mouse	Male	Protein	N/A
Roque 2016	Rat	Male	mRNA	N/A
Saavedra 2017	Rat	Male	Protein	N/A
Shao 2019	Rat	Male	mRNA	Abdominal wall electrode implantation five days prior to sacrifice and then testing for sensitivity to organ distension
Tang 2017	Mouse	Unclear	Protein	Colorectal distension for visual pain sensitivity scoring
Vig 2010	Mouse	Both	Protein	IP injection of ovalbumin nine and fourteen days prior to sacrifice, then intranasal inoculation of ovalbumin or saline one and three days prior to sacrifice
Viola 2019	Mouse	Male	mRNA	IP injection of poly-(I:C) five hours prior to sacrifice
Wang 2017	Mouse	Male	Protein	Three weeks of daily IP saline injections ending two weeks before sacrifice, followed by behavioural tests including elevated plus maze and open field test
Zajdel 2019	Mouse	Both	mRNA	N/A
Zhu 2017	Rat	Male	Protein	Two-hour sevoflurane anaesthetic three to five days prior to sacrifice

Figure S4.2. **Study characteristics.** The gender, species, and assay substrate are listed for each included study. For those included studies in which repeated maternal separation (RMS) was conducted followed by a procedure in late adolescence or adulthood that was likely to be stressful, the relevant procedure(s) applied in those studies are listed.

Study	Follow-up type	Microglial activation	Microglial density
Baldy 2018	Short-term	↑ (medulla)	↑ (medulla)
Majcher-Maślanka 2019	Short-term	N/A	↓ (prelimbic PFC)
Roque 2016	Short-term	↑ (hippocampus)	↔ (hippocampus)
Saavedra 2017	Short-term	↑ (hippocampus)	↓ (hippocampus)
Banqueri 2019	Long-term without stress	N/A	↑ (hippocampus, dorsal striatum, nucleus accumbens)
Ganguly 2018	Long-term without stress	↔ (prelimbic PFC)	↔ (prelimbic PFC)
Han 2019	Long-term with stress	↑ (hippocampus)	N/A
Mizoguchi 2019	Long-term with stress	N/A	↑ (spinal cord)

Figure S4.3. **Microglial outcomes.** The results of included studies measuring the effect of RMS on microglia activation or density are listed, as increase, decrease, or no change.

References

Abcam (2021). *Basic principles and types of ELISA*. Accessed 22-Dec-21, https://www.abcam.com/kits/elisa-principle

Abravanel, B. T., and Sinha, R. (2015). Emotion dysregulation mediates the relationship between lifetime cumulative adversity and depressive symptomatology. *Journal of Psychiatric Research* 61, 89–96. doi: 10.1016/j.jpsychires.2014.11.012

Adoue, C., Jaussent, I., Olié, E., Beziat, S., van den Eynde, F., Courtet, P., et al. (2015). A further assessment of decision-making in anorexia nervosa. *Eur Psychiatry* 30, 121–127. doi: 10.1016/j.eurpsy.2014.08.004

Agnew-Blais, J., and Danese, A. (2016). Childhood maltreatment and unfavourable clinical outcomes in bipolar disorder: a systematic review and meta-analysis. *Lancet Psychiatry* 3, 342–349. doi: 10.1016/S2215-0366(15)00544-1

Alfons, A. (2019). robustHD: Robust Methods for High-Dimensional Data, https://CRAN.R-project.org/package=robustHD

Alsiö, J., Phillips, B. U., Sala-Bayo, J., Nilsson, S. R. O., Calafat-Pla, T. C., Rizwand, A., et al. (2019). Dopamine D2-like receptor stimulation blocks negative feedback in visual and spatial reversal learning in the rat: behavioural and computational evidence. *Psychopharmacology (Berl)* 236, 2307–2323. doi: 10.1007/s00213-019-05296-y

Alves, N. D., Correia, J. S., Patrício, P., Mateus-Pinheiro, A., Machado-Santos, A. R., Loureiro-Campos, E., et al. (2017). Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression. *Transl Psychiatry* 7, e1058. doi: 10.1038/tp.2017.29

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders: DSM-5*. Arlington, Va. American Psychiatric Association.

Amini-Khoei, H., Haghani-Samani, E., Beigi, M., Soltani, A., Mobini, G. R., Balali-Dehkordi, S., et al. (2019). On the role of corticosterone in behavioral disorders, microbiota composition alteration and neuroimmune response in adult male mice subjected to maternal separation stress. *Int Immunopharmacol* 66, 242–250. doi: 10.1016/j.intimp.2018.11.037

Amini-Khoei, H., Mohammadi-Asl, A., Amiri, S., Hosseini, M.-J., Momeny, M., Hassanipour, M., et al. (2017). Oxytocin mitigated the depressive-like behaviors of maternal separation stress through modulating mitochondrial function and

neuroinflammation. *Prog Neuropsychopharmacol Biol Psychiatry* 76, 169–178. doi: 10.1016/j.pnpbp.2017.02.022

Amsterdam, J. D., Settle, R. G., Doty, R. L., Abelman, E., and Winokur, A. (1987). Taste and smell perception in depression. *Biol Psychiatry* 22, 1481–1485. doi: 10.1016/0006-3223(87)90108-9

Andersen, S. L. (2015). Exposure to early adversity: Points of cross-species translation that can lead to improved understanding of depression. *Dev Psychopathol* 27, 477–491. doi: 10.1017/S0954579415000103

Antoni, M. H., Lutgendorf, S. K., Blomberg, B., Carver, C. S., Lechner, S., Diaz, A., et al. (2012). Cognitive-behavioral stress management reverses anxiety-related leukocyte transcriptional dynamics. *Biol Psychiatry* 71, 366–372. doi: 10.1016/j.biopsych.2011.10.007

Antoniuk, S., Bijata, M., Ponimaskin, E., and Wlodarczyk, J. (2019). Chronic unpredictable mild stress for modeling depression in rodents: Meta-analysis of model reliability. *Neurosci Biobehav Rev* 99, 101–116. doi: 10.1016/j.neubiorev.2018.12.002

Arabameri, A., Sameni, H., and Bandegi, A. (2017). The effects of propolis extract on ovarian tissue and oxidative stress in rats with maternal separation stress. *IJRM* 15, 509–520. doi: 10.29252/ijrm.15.8.509

Armstrong, T., and Olatunji, B. O. (2012). Eye tracking of attention in the affective disorders: a meta-analytic review and synthesis. *Clin Psychol Rev* 32, 704–723. doi: 10.1016/j.cpr.2012.09.004

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci* 10, 410–422. doi: 10.1038/nrn2648

Audet, M.-C., Jacobson-Pick, S., Wann, B. P., and Anisman, H. (2011). Social defeat promotes specific cytokine variations within the prefrontal cortex upon subsequent aggressive or endotoxin challenges. *Brain Behav Immun* 25, 1197–1205. doi: 10.1016/j.bbi.2011.03.010

Avants, B. B., Epstein, C. L., Grossman, M., and Gee, J. C. (2008). Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Anal* 12, 26–41. doi: 10.1016/j.media.2007.06.004

Avants, B. B., Tustison, N. J., Song, G., Cook, P. A., Klein, A., and Gee, J. C. (2011). A reproducible evaluation of ANTs similarity metric performance in brain image registration. *NeuroImage* 54, 2033–2044. doi: 10.1016/j.neuroimage.2010.09.025

Avitsur, R., Hunzeker, J., and Sheridan, J. F. (2006). Role of early stress in the individual differences in host response to viral infection. *Brain Behav Immun* 20, 339–348. doi: 10.1016/j.bbi.2005.09.006

Avitsur, R., Maayan, R., and Weizman, A. (2013). Neonatal stress modulates sickness behavior: role for proinflammatory cytokines. *J Neuroimmunol* 257, 59–66. doi: 10.1016/j.jneuroim.2013.02.009

Avitsur, R., and Sheridan, J. F. (2009). Neonatal stress modulates sickness behavior. *Brain Behav Immun* 23, 977–985. doi: 10.1016/j.bbi.2009.05.056

Babson, K. A., Woodward, S. H., Schaer, M., Sephton, S. E., and Kaloupek, D. G. (2017). Salivary Cortisol and Regional Brain Volumes Among Veterans With and Without Posttraumatic Stress Disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging* 2, 372–379. doi: 10.1016/j.bpsc.2016.11.007

Bache, S. M., and Wickham, H. (2020). magrittr: A Forward-Pipe Operator for R, https://CRAN.R-project.org/package=magrittr

Baker, S., and Bielajew, C. (2007). Influence of housing on the consequences of chronic mild stress in female rats. *Stress* 10, 283–293. doi: 10.1080/10253890701265362

Bakic, J., Pourtois, G., Jepma, M., Duprat, R., Raedt, R. de, and Baeken, C. (2017). Spared internal but impaired external reward prediction error signals in major depressive disorder during reinforcement learning. *Depression and Anxiety* 34, 89–96. doi: 10.1002/da.22576

Baldy, C., Fournier, S., Boisjoly-Villeneuve, S., Tremblay, M.-È., and Kinkead, R. (2018). The influence of sex and neonatal stress on medullary microglia in rat pups. *Exp Physiol* 103, 1192–1199. doi: 10.1113/EP087088

Balodis, I. M., and Potenza, M. N. (2015). Anticipatory reward processing in addicted populations: a focus on the monetary incentive delay task. *Biol Psychiatry* 77, 434–444. doi: 10.1016/j.biopsych.2014.08.020

Banqueri, M., Méndez, M., Gómez-Lázaro, E., and Arias, J. L. (2019). Early life stress by repeated maternal separation induces long-term neuroinflammatory response in glial cells of male rats. *Stress* 22, 563–570. doi: 10.1080/10253890.2019.1604666

Bari, A., Theobald, D. E. H., Caprioli, D., Mar, A. C., Aidoo-Micah, A., Dalley, J. W., et al. (2010). Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats. *Neuropsychopharmacology* 35, 1290–1301. doi: 10.1038/npp.2009.233

Barman, S. M., and Yates, B. J. (2017). Deciphering the Neural Control of Sympathetic Nerve Activity: Status Report and Directions for Future Research. *Front Neurosci* 11, 730. doi: 10.3389/fnins.2017.00730

Barouei, J., Moussavi, M., and Hodgson, D. M. (2015). Perinatal maternal probiotic intervention impacts immune responses and ileal mucin gene expression in a rat model of irritable bowel syndrome. *Benef Microbes* 6, 83–95. doi: 10.3920/BM2013.0011

Barreau, F., Ferrier, L., Fioramonti, J., and Bueno, L. (2004). Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. *Gut* 53, 501–506. doi: 10.1136/gut.2003.024174

Barry, T. J., Murray, L., Fearon, P., Moutsiana, C., Johnstone, T., and Halligan, S. L. (2017). Amygdala volume and hypothalamic-pituitary-adrenal axis reactivity to social stress. *Psychoneuroendocrinology* 85, 96–99. doi: 10.1016/j.psyneuen.2017.07.487

Bassey, R. B., and Gondré-Lewis, M. C. (2019). Combined early life stressors: Prenatal nicotine and maternal deprivation interact to influence affective and drug seeking behavioral phenotypes in rats. *Behav Brain Res* 359, 814–822. doi: 10.1016/j.bbr.2018.07.022

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67, 1–48. doi: 10.18637/jss.v067.i01

Batten, S. V., Aslan, M., Maciejewski, P. K., and Mazure, C. M. (2004). Childhood maltreatment as a risk factor for adult cardiovascular disease and depression. *J Clin Psychiatry* 65, 249–254. doi: 10.4088/jcp.v65n0217

Batty, G. D., Deary, I. J., and Gottfredson, L. S. (2007). Premorbid (early life) IQ and later mortality risk: systematic review. *Ann Epidemiol* 17, 278–288. doi: 10.1016/j.annepidem.2006.07.010

Batty, G. D., Mortensen, E. L., and Osler, M. (2005). Childhood IQ in relation to later psychiatric disorder: evidence from a Danish birth cohort study. *Br J Psychiatry* 187, 180–181. doi: 10.1192/bjp.187.2.180

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C. M., and Mondelli, V. (2016). Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Mol Psychiatry* 21, 642–649. doi: 10.1038/mp.2015.67

Baur, V., Hänggi, J., and Jäncke, L. (2012). *Volumetric associations between uncinate fasciculus, amygdala, and trait anxiety*.

Behl, C., Lezoualc'h, F., Trapp, T., Widmann, M., Skutella, T., and Holsboer, F. (1997). Glucocorticoids enhance oxidative stress-induced cell death in hippocampal neurons in vitro. *Endocrinology* 138, 101–106. doi: 10.1210/endo.138.1.4835

Belin, D., Berson, N., Balado, E., Piazza, P. V., and Deroche-Gamonet, V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology* 36, 569–579. doi: 10.1038/npp.2010.188

Belin, D., Mar, A. C., Dalley, J. W., Robbins, T. W., and Everitt, B. J. (2008). High impulsivity predicts the switch to compulsive cocaine-taking. *Science* 320, 1352–1355. doi: 10.1126/science.1158136

Bengtsson, H. (2020). A Unifying Framework for Parallel and Distributed Processing in R using Futures.

Bergamini, G., Cathomas, F., Auer, S., Sigrist, H., Seifritz, E., Patterson, M., et al. (2016). Mouse psychosocial stress reduces motivation and cognitive function in operant reward tests: A model for reward pathology with effects of agomelatine. *Eur Neuropsychopharmacol* 26, 1448–1464. doi: 10.1016/j.euroneuro.2016.06.009

Berggren, N., and Derakshan, N. (2013). Attentional control deficits in trait anxiety: why you see them and why you don't. *Biol Psychol* 92, 440–446. doi: 10.1016/j.biopsycho.2012.03.007

Berlin, I., Givry-Steiner, L., Lecrubier, Y., and Puech, A. J. (1998). Measures of anhedonia and hedonic responses to sucrose in depressive and schizophrenic patients in comparison with healthy subjects. *Eur Psychiatry* 13, 303–309. doi: 10.1016/S0924-9338(98)80048-5

Berridge, K. C. (2007). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)* 191, 391–431. doi: 10.1007/s00213-006-0578-x

Bierhaus, A., Wolf, J., Andrassy, M., Rohleder, N., Humpert, P. M., Petrov, D., et al. (2003). A mechanism converting psychosocial stress into mononuclear cell activation. *Proc Natl Acad Sci U S A* 100, 1920–1925. doi: 10.1073/pnas.0438019100

Biggio, F., Pisu, M. G., Garau, A., Boero, G., Locci, V., Mostallino, M. C., et al. (2014). Maternal separation attenuates the effect of adolescent social isolation on HPA axis responsiveness in adult rats. *Eur Neuropsychopharmacol* 24, 1152–1161. doi: 10.1016/j.euroneuro.2014.03.009

Bishop, S. J. (2008). Neural mechanisms underlying selective attention to threat. *Ann N Y Acad Sci* 1129, 141–152. doi: 10.1196/annals.1417.016

Bishop, S. J. (2009). Trait anxiety and impoverished prefrontal control of attention. *Nat Neurosci* 12, 92–98. doi: 10.1038/nn.2242

Blackmon, K., Barr, W. B., Carlson, C., Devinsky, O., DuBois, J., Pogash, D., et al. (2011). Structural evidence for involvement of a left amygdala-orbitofrontal network in subclinical anxiety. *Psychiatry Research* 194, 296–303. doi: 10.1016/j.pscychresns.2011.05.007

Blair, H. T., Schafe, G. E., Bauer, E. P., Rodrigues, S. M., and LeDoux, J. E. (2001). Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn Mem* 8, 229–242. doi: 10.1101/lm.30901

Blumberg, M. S., Efimova, I. V., and Alberts, J. R. (1992). Ultrasonic vocalizations by rat pups: the primary importance of ambient temperature and the thermal significance of contact comfort. *Dev Psychobiol* 25, 229–250. doi: 10.1002/dev.420250402

Bobrovskaya, L., Beard, D., Bondarenko, E., Beig, M. I., Jobling, P., Walker, F. R., et al. (2013). Does exposure to chronic stress influence blood pressure in rats? *Auton Neurosci* 177, 217–223. doi: 10.1016/j.autneu.2013.05.001

Boecker, R., Holz, N. E., Buchmann, A. F., Blomeyer, D., Plichta, M. M., Wolf, I., et al. (2014). Impact of early life adversity on reward processing in young adults: EEG-fMRI results from a prospective study over 25 years. *PLoS ONE* 9, e104185. doi: 10.1371/journal.pone.0104185

Bonapersona, V., Kentrop, J., van Lissa, C. J., van der Veen, R., Joëls, M., and Sarabdjitsingh, R. A. (2019). The behavioral phenotype of early life adversity: A 3-level meta-analysis of rodent studies. *Neurosci Biobehav Rev* 102, 299–307. doi: 10.1016/j.neubiorev.2019.04.021

Borges-Aguiar, A. C., Schauffer, L. Z., Kloet, E. R. de, and Schenberg, L. C. (2018). Daily maternal separations during stress hyporesponsive period decrease the thresholds of panic-like behaviors to electrical stimulation of the dorsal periaqueductal gray of the adult rat. *Behav Brain Res* 344, 132–144. doi: 10.1016/j.bbr.2018.02.020

Bosy-Westphal, A., Reinecke, U., Schlörke, T., Illner, K., Kutzner, D., Heller, M., et al. (2004). Effect of organ and tissue masses on resting energy expenditure in underweight, normal weight and obese adults. *Int J Obes Relat Metab Disord* 28, 72–79. doi: 10.1038/sj.ijo.0802526

Bou-Dargham, M. J., Khamis, Z. I., Cognetta, A. B., and Sang, Q.-X. A. (2017). The Role of Interleukin-1 in Inflammatory and Malignant Human Skin Diseases and the Rationale for Targeting Interleukin-1 Alpha. *Med Res Rev* 37, 180–216. doi: 10.1002/med.21406

Boullier, M., and Blair, M. (2018). Adverse childhood experiences. *Paediatrics and Child Health* 28, 132–137. doi: 10.1016/j.paed.2017.12.008

Bourgin, J., Cachia, A., Boumezbeur, F., Djemaï, B., Bottlaender, M., Duchesnay, E., et al. (2015). Hyper-responsivity to stress in rats is associated with a large increase in amygdala volume. A 7T MRI study. *Eur Neuropsychopharmacol* 25, 828–835. doi: 10.1016/j.euroneuro.2015.02.010

Breivik, T., Gundersen, Y., Murison, R., Turner, J. D., Muller, C. P., Gjermo, P., et al. (2015). Maternal Deprivation of Lewis Rat Pups Increases the Severity of Experi-mental Periodontitis in Adulthood. *Open Dent J* 9, 65–78. doi: 10.2174/1874210601509010065

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., and Charney, D. S. (2000). Hippocampal volume reduction in major depression. *AJP* 157, 115–118. doi: 10.1176/ajp.157.1.115

Brenes Sáenz, J. C., Villagra, O. R., and Fornaguera Trías, J. (2006). Factor analysis of Forced Swimming test, Sucrose Preference test and Open Field test on enriched, social and isolated reared rats. *Behav Brain Res* 169, 57–65. doi: 10.1016/j.bbr.2005.12.001

Brown, D. W., Anda, R. F., Felitti, V. J., Edwards, V. J., Malarcher, A. M., Croft, J. B., et al. (2010). Adverse childhood experiences are associated with the risk of lung cancer: a prospective cohort study. *BMC Public Health* 10, 20. doi: 10.1186/1471-2458-10-20

Brown, E. S., Woolston, D. J., and Frol, A. B. (2008). Amygdala volume in patients receiving chronic corticosteroid therapy. *Biol Psychiatry* 63, 705–709. doi: 10.1016/j.biopsych.2007.09.014

Buckner, J. D., Joiner, T. E., Pettit, J. W., Lewinsohn, P. M., and Schmidt, N. B. (2008). Implications of the DSM's emphasis on sadness and anhedonia in major depressive disorder. *Psychiatry Research* 159, 25–30. doi: 10.1016/j.psychres.2007.05.010

Bufalino, C., Hepgul, N., Aguglia, E., and Pariante, C. M. (2013). The role of immune genes in the association between depression and inflammation: a review of recent clinical studies. *Brain Behav Immun* 31, 31–47. doi: 10.1016/j.bbi.2012.04.009

Burenkova, O. V., Averkina, A. A., Aleksandrova, E. A., and Zarayskaya, I. Y. (2020). Brief but enough: 45-min maternal separation elicits behavioral and physiological responses in neonatal mice and changes in dam maternal behavior. *Physiol Behav* 222, 112877. doi: 10.1016/j.physbeh.2020.112877

Burke, A. R., McCormick, C. M., Pellis, S. M., and Lukkes, J. L. (2017). Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. *Neurosci Biobehav Rev* 76, 280–300. doi: 10.1016/j.neubiorev.2017.01.018

Buskirk, T. D., Willoughby, L. M., and Tomazic, T. J. (2013). "Nonparametric Statistical Techniques," in *The Oxford handbook of quantitative methods*, ed. T. D. Little (New York: Oxford University Press).

Buss, C., Davis, E. P., Shahbaba, B., Pruessner, J. C., Head, K., and Sandman, C. A. (2012). Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *Proc Natl Acad Sci U S A* 109, E1312-9. doi: 10.1073/pnas.1201295109

Cacciaglia, R., Pohlack, S. T., Flor, H., and Nees, F. (2015). Dissociable roles for hippocampal and amygdalar volume in human fear conditioning. *Brain Struct Funct* 220, 2575–2586. doi: 10.1007/s00429-014-0807-8

Calabrese, E., Badea, A., Watson, C., and Johnson, G. A. (2013a). A quantitative magnetic resonance histology atlas of postnatal rat brain development with regional estimates of growth and variability. *NeuroImage* 71, 196–206. doi: 10.1016/j.neuroimage.2013.01.017

Calabrese, E., Johnson, G. A., and Watson, C. (2013b). An ontology-based segmentation scheme for tracking postnatal changes in the developing rodent brain with MRI. *NeuroImage* 67, 375–384. doi: 10.1016/j.neuroimage.2012.11.037

Campbell, E. J., Mitchell, C. S., Adams, C. D., Yeoh, J. W., Hodgson, D. M., Graham, B. A., et al. (2017). Chemogenetic activation of the lateral hypothalamus reverses early life stress-induced deficits in motivational drive. *Eur J Neurosci* 46, 2285–2296. doi: 10.1111/ejn.13674

Carboni, L., Becchi, S., Piubelli, C., Mallei, A., Giambelli, R., Razzoli, M., et al. (2010). Early-life stress and antidepressants modulate peripheral biomarkers in a gene-environment rat model of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 34, 1037–1048. doi: 10.1016/j.pnpbp.2010.05.019

Carlyle, B. C., Duque, A., Kitchen, R. R., Bordner, K. A., Coman, D., Doolittle, E., et al. (2012). Maternal separation with early weaning: a rodent model providing novel insights into neglect associated developmental deficits. *Dev Psychopathol* 24, 1401–1416. doi: 10.1017/S095457941200079X

Carpenter, L. L., Carvalho, J. P., Tyrka, A. R., Wier, L. M., Mello, A. F., Mello, M. F., et al. (2007). Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. *Biol Psychiatry* 62, 1080–1087. doi: 10.1016/j.biopsych.2007.05.002

Carpenter, L. L., Gawuga, C. E., Tyrka, A. R., Lee, J. K., Anderson, G. M., and Price, L. H. (2010). Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology* 35, 2617–2623. doi: 10.1038/npp.2010.159

Carpenter, L. L., Tyrka, A. R., Ross, N. S., Khoury, L., Anderson, G. M., and Price, L. H. (2009). Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. *Biol Psychiatry* 66, 69–75. doi: 10.1016/j.biopsych.2009.02.030

Chatterjee, P., Chiasson, V. L., Bounds, K. R., and Mitchell, B. M. (2014). Regulation of the Anti-Inflammatory Cytokines Interleukin-4 and Interleukin-10 during Pregnancy. *Front Immunol* 5, 253. doi: 10.3389/fimmu.2014.00253

Chavanne, A. V., and Robinson, O. J. (2021). The Overlapping Neurobiology of Induced and Pathological Anxiety: A Meta-Analysis of Functional Neural Activation. *Am J Psychiatry* 178, 156–164. doi: 10.1176/appi.ajp.2020.19111153

Chawla, N., and Ostafin, B. (2007). Experiential avoidance as a functional dimensional approach to psychopathology: an empirical review. *J Clin Psychol* 63, 871–890. doi: 10.1002/jclp.20400

Children's Services Statistics Team (2021). *Characteristics of children in need, Reporting Year 2021*. Accessed 25-Nov-21, https://explore-education-statistics.service.gov.uk/find-statistics/characteristics-of-children-in-need

Chocyk, A., Majcher-Maślanka, I., Dudys, D., Przyborowska, A., and Wędzony, K. (2013). Impact of early-life stress on the medial prefrontal cortex functions - a search for the pathomechanisms of anxiety and mood disorders. *Pharmacological Reports* 65, 1462–1470. doi: 10.1016/S1734-1140(13)71506-8

Chocyk, A., Majcher-Maślanka, I., Przyborowska, A., Maćkowiak, M., and Wędzony, K. (2015). Early-life stress increases the survival of midbrain neurons during postnatal development and enhances reward-related and anxiolytic-like behaviors in a sex-dependent fashion. *Int J Dev Neurosci* 44, 33–47. doi: 10.1016/j.ijdevneu.2015.05.002

Choi, J., Jeong, B., Polcari, A., Rohan, M. L., and Teicher, M. H. (2012). Reduced fractional anisotropy in the visual limbic pathway of young adults witnessing domestic violence in childhood. *NeuroImage* 59, 1071–1079. doi: 10.1016/j.neuroimage.2011.09.033

Choi, J., Jeong, B., Rohan, M. L., Polcari, A. M., and Teicher, M. H. (2009). Preliminary evidence for white matter tract abnormalities in young adults exposed to parental verbal abuse. *Biol Psychiatry* 65, 227–234. doi: 10.1016/j.biopsych.2008.06.022

Cisler, J. M., and Koster, E. H. W. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. *Clin Psychol Rev* 30, 203–216. doi: 10.1016/j.cpr.2009.11.003

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale N.J. L. Erlbaum Associates.

Corral-Frías, N. S., Nikolova, Y. S., Michalski, L. J., Baranger, D. A. A., Hariri, A. R., and Bogdan, R. (2015). Stress-related anhedonia is associated with ventral striatum reactivity to reward and transdiagnostic psychiatric symptomatology. *Psychological Medicine* 45, 2605–2617. doi: 10.1017/S0033291715000525

Cosquer, B., Galani, R., Kuster, N., and Cassel, J.-C. (2005). Whole-body exposure to 2.45 GHz electromagnetic fields does not alter anxiety responses in rats: a plus-maze study including test validation. *Behav Brain Res* 156, 65–74. doi: 10.1016/j.bbr.2004.05.007

Couto, F. S. d., Batalha, V. L., Valadas, J. S., Data-Franca, J., Ribeiro, J. A., and Lopes, L. V. (2012). Escitalopram improves memory deficits induced by maternal separation in the rat. *Eur J Pharmacol* 695, 71–75. doi: 10.1016/j.ejphar.2012.08.020

Creswell, J. D., Irwin, M. R., Burklund, L. J., Lieberman, M. D., Arevalo, J. M. G., Ma, J., et al. (2012). Mindfulness-Based Stress Reduction training reduces loneliness and pro-inflammatory gene expression in older adults: a small randomized controlled trial. *Brain Behav Immun* 26, 1095–1101. doi: 10.1016/j.bbi.2012.07.006

Crouch, J. L., Shelton, C. R., Bardeen, J. R., Hiraoka, R., Milner, J. S., and Skowronski, J. J. (2012). Does Attentional Control Mediate the Association Between Adverse Early Experiences and Child Physical Abuse Risk? *J Fam Viol* 27, 97–103. doi: 10.1007/s10896-011-9406-0

Cruz, F. C., Quadros, I. M., Planeta, C. d. S., and Miczek, K. A. (2008). Maternal separation stress in male mice: long-term increases in alcohol intake. *Psychopharmacology (Berl)* 201, 459–468. doi: 10.1007/s00213-008-1307-4

Cryan, J. F., and Sweeney, F. F. (2011). The age of anxiety: role of animal models of anxiolytic action in drug discovery. *Br J Pharmacol* 164, 1129–1161. doi: 10.1111/j.1476-5381.2011.01362.x

Cutajar, M. C., Mullen, P. E., Ogloff, J. R. P., Thomas, S. D., Wells, D. L., and Spataro, J. (2010). Psychopathology in a large cohort of sexually abused children followed up to 43 years. *Child Abuse Negl* 34, 813–822. doi: 10.1016/j.chiabu.2010.04.004

Dagytė, G., Trentani, A., Postema, F., Luiten, P. G., Den Boer, J. A., Gabriel, C., et al. (2010). The novel antidepressant agomelatine normalizes hippocampal neuronal activity

and promotes neurogenesis in chronically stressed rats. *CNS Neurosci Ther* 16, 195–207. doi: 10.1111/j.1755-5949.2009.00125.x

Dalgleish, T., Taghavi, R., Neshat-Doost, H., Moradi, A., Canterbury, R., and Yule, W. (2003). Patterns of processing bias for emotional information across clinical disorders: a comparison of attention, memory, and prospective cognition in children and adolescents with depression, generalized anxiety, and posttraumatic stress disorder. *J Clin Child Adolesc Psychol* 32, 10–21. doi: 10.1207/S15374424JCCP3201_02

Dallé, E., Daniels, W. M. U., and Mabandla, M. V. (2017). Fluvoxamine maleate normalizes striatal neuronal inflammatory cytokine activity in a Parkinsonian rat model associated with depression. *Behav Brain Res* 316, 189–196. doi: 10.1016/j.bbr.2016.08.005

Dallé, E., Daniels, W. M. U., and Mabandla, M. V. (2020). Long-Term Treatment with Fluvoxamine Decreases Nonmotor Symptoms and Dopamine Depletion in a Postnatal Stress Rat Model of Parkinson's Disease. *Oxid Med Cell Longev* 2020, 1941480. doi: 10.1155/2020/1941480

Danese, A., Moffitt, T. E., Harrington, H., Milne, B. J., Polanczyk, G., Pariante, C. M., et al. (2009). Adverse childhood experiences and adult risk factors for age-related disease: depression, inflammation, and clustering of metabolic risk markers. *Arch Pediatr Adolesc Med* 163, 1135–1143. doi: 10.1001/archpediatrics.2009.214

D'Aquila, P., Monleon, S., Borsini, F., Brain, P., and Willner, P. (1997). Anti-anhedonic actions of the novel serotonergic agent flibanserin, a potential rapidly-acting antidepressant. *Eur J Pharmacol* 340, 121–132. doi: 10.1016/s0014-2999(97)01412-x

Daskalakis, N. P., Bagot, R. C., Parker, K. J., Vinkers, C. H., and Kloet, E. R. de (2013). The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* 38, 1858–1873. doi: 10.1016/j.psyneuen.2013.06.008

Daskalakis, N. P., Claessens, S. E. F., Laboyrie, J. J. L., Enthoven, L., Oitzl, M. S., Champagne, D. L., et al. (2011). The newborn rat's stress system readily habituates to repeated and prolonged maternal separation, while continuing to respond to stressors in context dependent fashion. *Horm Behav* 60, 165–176. doi: 10.1016/j.yhbeh.2011.04.003

de Bellis, M. D., Casey, B. J., Dahl, R. E., Birmaher, B., Williamson, D. E., Thomas, K. M., et al. (2000). A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biol Psychiatry* 48, 51–57. doi: 10.1016/s0006-3223(00)00835-0

De Bellis, M. D., and Zisk, A. (2014). The biological effects of childhood trauma. *Child Adolesc Psychiatr Clin N Am* 23, 185-222, vii. doi: 10.1016/j.chc.2014.01.002

De Miguel, C., Obi, I. E., Ho, D. H., Loria, A. S., and Pollock, J. S. (2018). Early life stress induces immune priming in kidneys of adult male rats. *Am J Physiol Renal Physiol* 314, F343-F355. doi: 10.1152/ajprenal.00590.2016

Deak, T., Bordner, K. A., McElderry, N. K., Barnum, C. J., Blandino, P., Deak, M. M., et al. (2005). Stress-induced increases in hypothalamic IL-1: a systematic analysis of multiple stressor paradigms. *Brain Res Bull* 64, 541–556. doi: 10.1016/j.brainresbull.2004.11.003

DePrince, A. P., Weinzierl, K. M., and Combs, M. D. (2009). Executive function performance and trauma exposure in a community sample of children. *Child Abuse Negl* 33, 353–361. doi: 10.1016/j.chiabu.2008.08.002

Derakshan, N., and Eysenck, M. W. (2009). Anxiety, Processing Efficiency, and Cognitive Performance. *European Psychologist* 14, 168–176. doi: 10.1027/1016-9040.14.2.168

Der-Avakian, A., and Markou, A. (2010). Neonatal maternal separation exacerbates the reward-enhancing effect of acute amphetamine administration and the anhedonic effect of repeated social defeat in adult rats. *Neuroscience* 170, 1189–1198. doi: 10.1016/j.neuroscience.2010.08.002

Der-Avakian, A., and Markou, A. (2012). The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci* 35, 68–77. doi: 10.1016/j.tins.2011.11.005

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., and Dinan, T. G. (2010). Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. *Neuroscience* 170, 1179–1188. doi: 10.1016/j.neuroscience.2010.08.005

Deshmane, S. L., Kremlev, S., Amini, S., and Sawaya, B. E. (2009). Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* 29, 313–326. doi: 10.1089/jir.2008.0027

Dichter, G. S., Smoski, M. J., Kampov-Polevoy, A. B., Gallop, R., and Garbutt, J. C. (2010). Unipolar depression does not moderate responses to the Sweet Taste Test. *Depression and Anxiety* 27, 859–863. doi: 10.1002/da.20690

Dickstein, D. P., Finger, E. C., Brotman, M. A., Rich, B. A., Pine, D. S., Blair, J. R., et al. (2010). Impaired probabilistic reversal learning in youths with mood and anxiety disorders. *Psychological Medicine* 40, 1089–1100. doi: 10.1017/S0033291709991462

Dieterich, A., Srivastava, P., Sharif, A., Stech, K., Floeder, J., Yohn, S. E., et al. (2019). Chronic corticosterone administration induces negative valence and impairs positive valence behaviors in mice. *Transl Psychiatry* 9, 337. doi: 10.1038/s41398-019-0674-4

Dieu-Nosjean, M. C., Massacrier, C., Homey, B., Vanbervliet, B., Pin, J. J., Vicari, A., et al. (2000). Macrophage inflammatory protein 3alpha is expressed at inflamed epithelial

surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. *J Exp Med* 192, 705–718. doi: 10.1084/jem.192.5.705

Dimatelis, J. J., Pillay, N. S., Mutyaba, A. K., Russell, V. A., Daniels, W. M. U., and Stein, D. J. (2012). Early maternal separation leads to down-regulation of cytokine gene expression. *Metab Brain Dis* 27, 393–397. doi: 10.1007/s11011-012-9304-z

Dimatelis, J. J., Vermeulen, I. M., Bugarith, K., Stein, D. J., and Russell, V. A. (2016). Female rats are resistant to developing the depressive phenotype induced by maternal separation stress. *Metab Brain Dis* 31, 109–119. doi: 10.1007/s11011-015-9723-8

Dinarello, C. A., Novick, D., Kim, S., and Kaplanski, G. (2013). Interleukin-18 and IL-18 binding protein. *Front Immunol* 4, 289. doi: 10.3389/fimmu.2013.00289

Do Prado, C. H., Narahari, T., Holland, F. H., Lee, H.-N., Murthy, S. K., and Brenhouse, H. C. (2016). Effects of early adolescent environmental enrichment on cognitive dysfunction, prefrontal cortex development, and inflammatory cytokines after early life stress. *Dev Psychobiol* 58, 482–491. doi: 10.1002/dev.21390

Dombrovski, A. Y., Clark, L., Siegle, G. J., Butters, M. A., Ichikawa, N., Sahakian, B. J., et al. (2010). Reward/Punishment reversal learning in older suicide attempters. *Am J Psychiatry* 167, 699–707. doi: 10.1176/appi.ajp.2009.09030407

Dombrovski, A. Y., Szanto, K., Clark, L., Aizenstein, H. J., Chase, H. W., Reynolds, C. F., et al. (2015). Corticostriatothalamic reward prediction error signals and executive control in late-life depression. *Psychological Medicine* 45, 1413–1424. doi: 10.1017/S0033291714002517

Driessen, M., Herrmann, J., Stahl, K., Zwaan, M., Meier, S., Hill, A., et al. (2000). Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization. *Archives of General Psychiatry* 57, 1115–1122. doi: 10.1001/archpsyc.57.12.1115

Dutcher, E. G., Pama, E. A. C., Lynall, M.-E., Khan, S., Clatworthy, M. R., Robbins, T. W., et al. (2020). Early-life stress and inflammation: A systematic review of a key experimental approach in rodents. *Brain Neurosci Adv* 4, 2398212820978049. doi: 10.1177/2398212820978049

Elizalde, N., Gil-Bea, F. J., Ramírez, M. J., Aisa, B., Lasheras, B., Del Rio, J., et al. (2008). Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: effect of antidepressant treatment. *Psychopharmacology (Berl)* 199, 1–14. doi: 10.1007/s00213-007-1035-1

Ellenbroek, B. A., and Riva, M. A. (2003). Early maternal deprivation as an animal model for schizophrenia. *Clinical Neuroscience Research* 3, 297–302. doi: 10.1016/S1566-2772(03)00090-2

Elzinga, B. M., Roelofs, K., Tollenaar, M. S., Bakvis, P., van Pelt, J., and Spinhoven, P. (2008). Diminished cortisol responses to psychosocial stress associated with lifetime adverse events a study among healthy young subjects. *Psychoneuroendocrinology* 33, 227–237. doi: 10.1016/j.psyneuen.2007.11.004

Ennis, G. E., Quintin, E.-M., Saelzler, U., Kennedy, K. M., Hertzog, C., and Moffat, S. D. (2019). Cortisol relates to regional limbic system structure in older but not younger adults. *Psychoneuroendocrinology* 101, 111–120. doi: 10.1016/j.psyneuen.2018.09.014

Enthoven, L., Oitzl, M. S., Koning, N., van der Mark, M., and Kloet, E. R. de (2008). Hypothalamic-pituitary-adrenal axis activity of newborn mice rapidly desensitizes to repeated maternal absence but becomes highly responsive to novelty. *Endocrinology* 149, 6366–6377. doi: 10.1210/en.2008-0238

Erburu, M., Cajaleon, L., Guruceaga, E., Venzala, E., Muñoz-Cobo, I., Beltrán, E., et al. (2015). Chronic mild stress and imipramine treatment elicit opposite changes in behavior and in gene expression in the mouse prefrontal cortex. *Pharmacol Biochem Behav* 135, 227–236. doi: 10.1016/j.pbb.2015.06.001

Estes, M. L., and McAllister, A. K. (2016). Maternal immune activation: Implications for neuropsychiatric disorders. *Science* 353, 772–777. doi: 10.1126/science.aag3194

Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., and Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Archives of General Psychiatry* 66, 1361–1372. doi: 10.1001/archgenpsychiatry.2009.104

Everaert, J., Koster, E. H. W., and Derakshan, N. (2012). The combined cognitive bias hypothesis in depression. *Clin Psychol Rev* 32, 413–424. doi: 10.1016/j.cpr.2012.04.003

Eysenck, M. W. (1979). Anxiety, learning, and memory: A reconceptualization. *Journal of Research in Personality* 13, 363–385. doi: 10.1016/0092-6566(79)90001-1

Eysenck, M. W., and Derakshan, N. (2011). New perspectives in attentional control theory. *Personality and Individual Differences* 50, 955–960. doi: 10.1016/j.paid.2010.08.019

Eysenck, M. W., Derakshan, N., Santos, R., and Calvo, M. G. (2007). Anxiety and cognitive performance: attentional control theory. *Emotion* 7, 336–353. doi: 10.1037/1528-3542.7.2.336

Feger, J., and Drew, Z. (2021). *PD weighted spin-echo images*. Accessed 28-Dec-21, https://doi.org/10.53347/rID-60744

Feldcamp, L., Doucet, J.-S., Pawling, J., Fadel, M. P., Fletcher, P. J., Maunder, R., et al. (2016). Mgat5 modulates the effect of early life stress on adult behavior and physical health in mice. *Behav Brain Res* 312, 253–264. doi: 10.1016/j.bbr.2016.06.033

Felger, J. C., Li, Z., Haroon, E., Woolwine, B. J., Jung, M. Y., Hu, X., et al. (2016). Inflammation is associated with decreased functional connectivity within corticostriatal reward circuitry in depression. *Mol Psychiatry* 21, 1358–1365. doi: 10.1038/mp.2015.168

Fernández-Teruel, A. (2021). Conflict between Threat Sensitivity and Sensation Seeking in the Adolescent Brain: Role of the Hippocampus, and Neurobehavioural Plasticity Induced by Pleasurable Early Enriched Experience. *Brain Sci* 11. doi: 10.3390/brainsci11020268

Ferrara, N., Gerber, H.-P., and LeCouter, J. (2003). The biology of VEGF and its receptors. *Nat Med* 9, 669–676. doi: 10.1038/nm0603-669

Fields, A., Bloom, P. A., VanTieghem, M., Harmon, C., Choy, T., Camacho, N. L., et al. (2021). Adaptation in the face of adversity: Decrements and enhancements in children's cognitive control behavior following early caregiving instability. *Dev Sci* 24, e13133. doi: 10.1111/desc.13133

Filippo, K. de, Dudeck, A., Hasenberg, M., Nye, E., van Rooijen, N., Hartmann, K., et al. (2013). Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. *Blood* 121, 4930–4937. doi: 10.1182/blood-2013-02-486217

Fishbein, D., Warner, T., Krebs, C., Trevarthen, N., Flannery, B., and Hammond, J. (2009). Differential relationships between personal and community stressors and children's neurocognitive functioning. *Child Maltreat* 14, 299–315. doi: 10.1177/1077559508326355

Fjell, A. M., and Walhovd, K. B. (2010). Structural brain changes in aging: courses, causes and cognitive consequences. *Reviews in the Neurosciences* 21, 187–221. doi: 10.1515/revneuro.2010.21.3.187

Fleshner, M. (2013). Stress-evoked sterile inflammation, danger associated molecular patterns (DAMPs), microbial associated molecular patterns (MAMPs) and the inflammasome. *Brain Behav Immun* 27, 1–7. doi: 10.1016/j.bbi.2012.08.012

Fleshner, M., and Crane, C. R. (2017). Exosomes, DAMPs and miRNA: Features of Stress Physiology and Immune Homeostasis. *Trends Immunol* 38, 768–776. doi: 10.1016/j.it.2017.08.002

Fowler, C. H., Bogdan, R., and Gaffrey, M. S. (2021). Stress-induced cortisol response is associated with right amygdala volume in early childhood. *Neurobiol Stress* 14, 100329. doi: 10.1016/j.ynstr.2021.100329

Fox, J., and Weisberg, S. (2019). *An R Companion to Applied Regression*. Thousand Oaks CA: Sage.

Francisco-Cruz, A., Aguilar-Santelises, M., Ramos-Espinosa, O., Mata-Espinosa, D., Marquina-Castillo, B., Barrios-Payan, J., et al. (2014). Granulocyte-macrophage colony-stimulating factor: not just another haematopoietic growth factor. *Med Oncol* 31, 774. doi: 10.1007/s12032-013-0774-6

Frank, M. G., Hershman, S. A., Weber, M. D., Watkins, L. R., and Maier, S. F. (2014). Chronic exposure to exogenous glucocorticoids primes microglia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus. *Psychoneuroendocrinology* 40, 191–200. doi: 10.1016/j.psyneuen.2013.11.006

Frodl, T., Carballedo, A., Hughes, M. M., Saleh, K., Fagan, A., Skokauskas, N., et al. (2012). Reduced expression of glucocorticoid-inducible genes GILZ and SGK-1: high IL-6 levels are associated with reduced hippocampal volumes in major depressive disorder. *Transl Psychiatry* 2, e88. doi: 10.1038/tp.2012.14

Fuentes, I. M., Walker, N. K., Pierce, A. N., Holt, B. R., Di Silvestro, E. R., and Christianson, J. A. (2016). Neonatal maternal separation increases susceptibility to experimental colitis and acute stress exposure in male mice. *IBRO Rep* 1, 10–18. doi: 10.1016/j.ibror.2016.07.001

Ganguly, P., Honeycutt, J. A., Rowe, J. R., Demaestri, C., and Brenhouse, H. C. (2019). Effects of early life stress on cocaine conditioning and AMPA receptor composition are sex-specific and driven by TNF. *Brain Behav Immun* 78, 41–51. doi: 10.1016/j.bbi.2019.01.006

Ganguly, P., Thompson, V., Gildawie, K., and Brenhouse, H. C. (2018). Adolescent food restriction in rats alters prefrontal cortex microglia in an experience-dependent manner. *Stress* 21, 162–168. doi: 10.1080/10253890.2017.1423054

Gao, Y., Ma, J., Tang, J., Liang, X., Huang, C.-X., Wang, S.-R., et al. (2017). White matter atrophy and myelinated fiber disruption in a rat model of depression. *J Comp Neurol* 525, 1922–1933. doi: 10.1002/cne.24178

García-Díaz, D. F., Campion, J., Milagro, F. I., Lomba, A., Marzo, F., and Martínez, J. A. (2007). Chronic mild stress induces variations in locomotive behavior and metabolic rates in high fat fed rats. *J Physiol Biochem* 63, 337–346. doi: 10.1007/BF03165765

Genty, J., Tetsi Nomigni, M., Anton, F., and Hanesch, U. (2018). Maternal separation stress leads to resilience against neuropathic pain in adulthood. *Neurobiol Stress* 8, 21–32. doi: 10.1016/j.ynstr.2017.11.002

Gerrits, M., Grootkarijn, A., Bekkering, B. F., Bruinsma, M., Den Boer, J. A., and Ter Horst, G. J. (2005). Cyclic estradiol replacement attenuates stress-induced c-Fos expression in the PVN of ovariectomized rats. *Brain Res Bull* 67, 147–155. doi: 10.1016/j.brainresbull.2005.06.021

Geyer, M. A., and Markou, A. (1995). "Animal Models of Psychiatric Disorders," in *Psychopharmacology: The fourth generation of progress / editors-in-chief Floyd E. Bloom, David J. Kupfer*, eds. F. E. Bloom, and D. J. Kupfer (New York: Raven Press).

Giridharan, V. V., Réus, G. Z., Selvaraj, S., Scaini, G., Barichello, T., and Quevedo, J. (2019). Maternal deprivation increases microglial activation and neuroinflammatory markers in the prefrontal cortex and hippocampus of infant rats. *Journal of Psychiatric Research* 115, 13–20. doi: 10.1016/j.jpsychires.2019.05.001

Gjerstad, J. K., Lightman, S. L., and Spiga, F. (2018). Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. *Stress* 21, 403–416. doi: 10.1080/10253890.2018.1470238

Gläscher, J., and Adolphs, R. (2003). Processing of the arousal of subliminal and supraliminal emotional stimuli by the human amygdala. *J. Neurosci.* 23, 10274–10282. doi: 10.1523/JNEUROSCI.23-32-10274.2003

Gold, P. W. (2015). The organization of the stress system and its dysregulation in depressive illness. *Mol Psychiatry* 20, 32–47. doi: 10.1038/mp.2014.163

Gondré-Lewis, M. C., Darius, P. J., Wang, H., and Allard, J. S. (2016). Stereological analyses of reward system nuclei in maternally deprived/separated alcohol drinking rats. *J Chem Neuroanat* 76, 122–132. doi: 10.1016/j.jchemneu.2016.02.004

Gongvatana, A., Correia, S., Dunsiger, S., Gauthier, L., Devlin, K. N., Ross, S., et al. (2014). Plasma cytokine levels are related to brain volumes in HIV-infected individuals. *J Neuroimmune Pharmacol* 9, 740–750. doi: 10.1007/s11481-014-9567-8

González-Pardo, H., Arias, J. L., Vallejo, G., and Conejo, N. M. (2019). Influence of environmental enrichment on the volume of brain regions sensitive to early life stress by maternal separation in rats. *Psicothema* 31, 46–52. doi: 10.7334/psicothema2018.290

Goode, K., McClernon, K., Zhao, J., Zhang, Y., and Huo, Y. (2021). redres: Residuals and Diagnostic Plots for Mixed Models, https://github.com/goodekat/redres.git

Goodwin, R. D., and Stein, M. B. (2004). Association between childhood trauma and physical disorders among adults in the United States. *Psychological Medicine* 34, 509–520. doi: 10.1017/s003329170300134x

Gotink, R. A., Vernooij, M. W., Ikram, M. A., Niessen, W. J., Krestin, G. P., Hofman, A., et al. (2018). Meditation and yoga practice are associated with smaller right amygdala volume: the Rotterdam study. *Brain Imaging Behav* 12, 1631–1639. doi: 10.1007/s11682-018-9826-z

Gotlib, I. H., and Joormann, J. (2010). Cognition and depression: current status and future directions. *Annu Rev Clin Psychol* 6, 285–312. doi: 10.1146/annurev.clinpsy.121208.131305

Gottschalk, M. G., Mortas, P., Haman, M., Ozcan, S., Biemans, B., and Bahn, S. (2018). Fluoxetine, not donepezil, reverses anhedonia, cognitive dysfunctions and hippocampal proteome changes during repeated social defeat exposure. *Eur Neuropsychopharmacol* 28, 195–210. doi: 10.1016/j.euroneuro.2017.11.002

Gouin, J.-P., Glaser, R., Malarkey, W. B., Beversdorf, D., and Kiecolt-Glaser, J. (2012a). Chronic stress, daily stressors, and circulating inflammatory markers. *Health Psychol* 31, 264–268. doi: 10.1037/a0025536

Gouin, J.-P., Glaser, R., Malarkey, W. B., Beversdorf, D., and Kiecolt-Glaser, J. K. (2012b). Childhood abuse and inflammatory responses to daily stressors. *Ann Behav Med* 44, 287–292. doi: 10.1007/s12160-012-9386-1

Gourley, S. L., Swanson, A. M., and Koleske, A. J. (2013). Corticosteroid-induced neural remodeling predicts behavioral vulnerability and resilience. *J Neurosci* 33, 3107–3112. doi: 10.1523/JNEUROSCI.2138-12.2013

Gracia-Rubio, I., Moscoso-Castro, M., Pozo, O. J., Marcos, J., Nadal, R., and Valverde, O. (2016). Maternal separation induces neuroinflammation and long-lasting emotional alterations in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 65, 104–117. doi: 10.1016/j.pnpbp.2015.09.003

Graeber, M. B. (2010). Changing face of microglia. *Science* 330, 783–788. doi: 10.1126/science.1190929

Graham, A. M., Rasmussen, J. M., Rudolph, M. D., Heim, C. M., Gilmore, J. H., Styner, M., et al. (2018). Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age. *Biol Psychiatry* 83, 109–119. doi: 10.1016/j.biopsych.2017.05.027

Granata, L., Valentine, A., Hirsch, J. L., Honeycutt, J., and Brenhouse, H. (2021). Trajectories of Mother-Infant Communication: An Experiential Measure of the Impacts of Early Life Adversity. *Front Hum Neurosci* 15, 632702. doi: 10.3389/fnhum.2021.632702

Grassi-Oliveira, R., Honeycutt, J. A., Holland, F. H., Ganguly, P., and Brenhouse, H. C. (2016). Cognitive impairment effects of early life stress in adolescents can be predicted with early biomarkers: Impacts of sex, experience, and cytokines. *Psychoneuroendocrinology* 71, 19–30. doi: 10.1016/j.psyneuen.2016.04.016

Groenewold, N. A., Opmeer, E. M., Jonge, P. de, Aleman, A., and Costafreda, S. G. (2013). Emotional valence modulates brain functional abnormalities in depression: evidence from a meta-analysis of fMRI studies. *Neurosci Biobehav Rev* 37, 152–163. doi: 10.1016/j.neubiorev.2012.11.015

Grolemund, G., and Wickham, H. (2011). Dates and Times Made Easy with lubridate. *Journal of Statistical Software* 40, 1–25.

Guan, J., Ding, Y., Rong, Y., Geng, Y., Lai, L., Qi, D., et al. (2020). Early Life Stress Increases Brain Glutamate and Induces Neurobehavioral Manifestations in Rats. *ACS Chem Neurosci* 11, 4169–4178. doi: 10.1021/acschemneuro.0c00454

Hailwood, J. M., Heath, C. J., Robbins, T. W., Saksida, L. M., and Bussey, T. J. (2018). Validation and optimisation of a touchscreen progressive ratio test of motivation in male rats. *Psychopharmacology (Berl)* 235, 2739–2753. doi: 10.1007/s00213-018-4969-6

Hamilton, J. P., Siemer, M., and Gotlib, I. H. (2008). Amygdala volume in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Mol Psychiatry* 13, 993–1000. doi: 10.1038/mp.2008.57

Han, Y., Zhang, L., Wang, Q., Zhang, D., Zhao, Q., Zhang, J., et al. (2019). Minocycline inhibits microglial activation and alleviates depressive-like behaviors in male adolescent mice subjected to maternal separation. *Psychoneuroendocrinology* 107, 37–45. doi: 10.1016/j.psyneuen.2019.04.021

Hanson, J. L., Albert, D., Iselin, A.-M. R., Carré, J. M., Dodge, K. A., and Hariri, A. R. (2016). Cumulative stress in childhood is associated with blunted reward-related brain activity in adulthood. *Soc Cogn Affect Neurosci* 11, 405–412. doi: 10.1093/scan/nsv124

Hanson, J. L., Hariri, A. R., and Williamson, D. E. (2015). Blunted Ventral Striatum Development in Adolescence Reflects Emotional Neglect and Predicts Depressive Symptoms. *Biol Psychiatry* 78, 598–605. doi: 10.1016/j.biopsych.2015.05.010

Harmer, C. J., O'Sullivan, U., Favaron, E., Massey-Chase, R., Ayres, R., Reinecke, A., et al. (2009). Effect of acute antidepressant administration on negative affective bias in depressed patients. *AJP* 166, 1178–1184. doi: 10.1176/appi.ajp.2009.09020149

Harris, R. B. S. (2015). Chronic and acute effects of stress on energy balance: are there appropriate animal models? *Am J Physiol Regul Integr Comp Physiol* 308, R250-65. doi: 10.1152/ajpregu.00361.2014

Harris, R. B. S., Mitchell, T. D., Simpson, J., Redmann, S. M., Youngblood, B. D., and Ryan, D. H. (2002). Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status. *Am J Physiol Regul Integr Comp Physiol* 282, R77-88. doi: 10.1152/ajpregu.2002.282.1.R77

Harrison, E. L., Jaehne, E. J., Jawahar, M. C., Corrigan, F., and Baune, B. T. (2014). Maternal separation modifies behavioural and neuroendocrine responses to stress in CCR7 deficient mice. *Behav Brain Res* 263, 169–175. doi: 10.1016/j.bbr.2014.01.036

Hartig, F. (2021). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models, https://CRAN.R-project.org/package=DHARMa

Hastings, R. S., Parsey, R. V., Oquendo, M. A., Arango, V., and Mann, J. J. (2004). Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression. *Neuropsychopharmacology* 29, 952–959. doi: 10.1038/sj.npp.1300371

Hegde, A., Suresh, S., and Mitra, R. (2020). Early-life short-term environmental enrichment counteracts the effects of stress on anxiety-like behavior, brain-derived neurotrophic factor and nuclear translocation of glucocorticoid receptors in the basolateral amygdala. *Sci Rep* 10, 14053. doi: 10.1038/s41598-020-70875-5

Heikkinen, N., Niskanen, E., Könönen, M., Tolmunen, T., Kekkonen, V., Kivimäki, P., et al. (2017). Alcohol consumption during adolescence is associated with reduced grey matter volumes. *Addiction* 112, 604–613. doi: 10.1111/add.13697

Heim, C. M., Mayberg, H. S., Mletzko, T., Nemeroff, C. B., and Pruessner, J. C. (2013). Decreased cortical representation of genital somatosensory field after childhood sexual abuse. *Am J Psychiatry* 170, 616–623. doi: 10.1176/appi.ajp.2013.12070950

Helwig, N. E. (2017). *Bootstrap Confidence Intervals*. Accessed 24-Jun-21, http://users.stat.umn.edu/~helwig/notes/bootci-Notes.pdf

Hemmingsson, T., Melin, B., Allebeck, P., and Lundberg, I. (2006). The association between cognitive ability measured at ages 18-20 and mortality during 30 years of follow-up--a prospective observational study among Swedish males born 1949-51. *Int J Epidemiol* 35, 665–670. doi: 10.1093/ije/dyi321

Henkelman, R. M., Stanisz, G. J., and Graham, S. J. (2001). Magnetization transfer in MRI: a review. *NMR Biomed* 14, 57–64. doi: 10.1002/nbm.683

Hennessey, T., Andari, E., and Rainnie, D. G. (2018). RDoC-based categorization of amygdala functions and its implications in autism. *Neurosci Biobehav Rev* 90, 115–129. doi: 10.1016/j.neubiorev.2018.04.007

Henry, L., and Wickham, H. (2020). purrr: Functional Programming Tools, https://CRAN.R-project.org/package=purrr

Herpfer, I., Hezel, H., Reichardt, W., Clark, K., Geiger, J., Gross, C. M., et al. (2012). Early life stress differentially modulates distinct forms of brain plasticity in young and adult mice. *PLoS ONE* 7, e46004. doi: 10.1371/journal.pone.0046004

Hershenberg, R., Satterthwaite, T. D., Daldal, A., Katchmar, N., Moore, T. M., Kable, J. W., et al. (2016). Diminished effort on a progressive ratio task in both unipolar and bipolar depression. *Journal of Affective Disorders* 196, 97–100. doi: 10.1016/j.jad.2016.02.003

Herts, K. L., McLaughlin, K. A., and Hatzenbuehler, M. L. (2012). Emotion dysregulation as a mechanism linking stress exposure to adolescent aggressive behavior. *J Abnorm Child Psychol* 40, 1111–1122. doi: 10.1007/s10802-012-9629-4

Hesterberg, T. (2014). *What Teachers Should Know about the Bootstrap: Resampling in the Undergraduate Statistics Curriculum*.

Hesterberg, T. (2015). resample: Resampling Functions, https://CRAN.R-project.org/package=resample

Hettema, J. M., Kettenmann, B., Ahluwalia, V., McCarthy, C., Kates, W. R., Schmitt, J. E., et al. (2012). Pilot multimodal twin imaging study of generalized anxiety disorder. *Depression and Anxiety* 29, 202–209. doi: 10.1002/da.20901

Hilbert, K., Lueken, U., and Beesdo-Baum, K. (2014). Neural structures, functioning and connectivity in Generalized Anxiety Disorder and interaction with neuroendocrine systems: a systematic review. *Journal of Affective Disorders* 158, 114–126. doi: 10.1016/j.jad.2014.01.022

Hill, M. N., Hillard, C. J., and McEwen, B. S. (2011). Alterations in corticolimbic dendritic morphology and emotional behavior in cannabinoid CB1 receptor-deficient mice parallel the effects of chronic stress. *Cereb Cortex* 21, 2056–2064. doi: 10.1093/cercor/bhq280

Hill, R. A., Klug, M., Kiss Von Soly, S., Binder, M. D., Hannan, A. J., and van den Buuse, M. (2014). Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. *Hippocampus* 24, 1197–1211. doi: 10.1002/hipo.22302

Hodel, A. S., Hunt, R. H., Cowell, R. A., van den Heuvel, S. E., Gunnar, M. R., and Thomas, K. M. (2015). Duration of early adversity and structural brain development in post-institutionalized adolescents. *NeuroImage* 105, 112–119. doi: 10.1016/j.neuroimage.2014.10.020

Hodes, G. E., Kana, V., Menard, C., Merad, M., and Russo, S. J. (2015). Neuroimmune mechanisms of depression. *Nat Neurosci* 18, 1386–1393. doi: 10.1038/nn.4113

Hölzel, B. K., Carmody, J., Evans, K. C., Hoge, E. A., Dusek, J. A., Morgan, L., et al. (2010). Stress reduction correlates with structural changes in the amygdala. *Soc Cogn Affect Neurosci* 5, 11–17. doi: 10.1093/scan/nsp034

Horii-Hayashi, N., Sasagawa, T., Matsunaga, W., Matsusue, Y., Azuma, C., and Nishi, M. (2013). Developmental changes in desensitisation of c-Fos expression induced by repeated maternal separation in pre-weaned mice. *J Neuroendocrinol* 25, 158–167. doi: 10.1111/j.1365-2826.2012.02377.x

Huang, J., Shen, C., Ye, R., Shi, Y., and Li, W. (2021). The Effect of Early Maternal Separation Combined With Adolescent Chronic Unpredictable Mild Stress on Behavior and Synaptic Plasticity in Adult Female Rats. *Front Psychiatry* 12, 539299. doi: 10.3389/fpsyt.2021.539299

Hughes, J. R., Pleasants, C. N., and Pickens, R. W. (1985). Measurement of reinforcement in depression: a pilot study. *J Behav Ther Exp Psychiatry* 16, 231–236. doi: 10.1016/0005-7916(85)90068-0

Hui, J., Zhang, Z., Liu, S., Xi, G., Zhang, X., Teng, G., et al. (2010). Adolescent escitalopram administration modifies neurochemical alterations in the hippocampus of maternally separated rats. *Eur Neuropsychopharmacol* 20, 875–883. doi: 10.1016/j.euroneuro.2010.08.010

Hui, J.-J., Zhang, Z.-J., Liu, S.-S., Xi, G.-J., Zhang, X.-R., Teng, G.-J., et al. (2011). Hippocampal neurochemistry is involved in the behavioural effects of neonatal maternal separation and their reversal by post-weaning environmental enrichment: a magnetic resonance study. *Behav Brain Res* 217, 122–127. doi: 10.1016/j.bbr.2010.10.014

Humphreys, K. L., Kircanski, K., Colich, N. L., and Gotlib, I. H. (2016). Attentional avoidance of fearful facial expressions following early life stress is associated with impaired social functioning. *J Child Psychol Psychiatry* 57, 1174–1182. doi: 10.1111/jcpp.12607

Huot, R. L., Thrivikraman, K. V., Meaney, M. J., and Plotsky, P. M. (2001). Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in

Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology (Berl)* 158, 366–373. doi: 10.1007/s002130100701

Iglewicz, B., and Hoaglin, D. C. (1993). *How to detect and handle outliers*. Milwaukee, Wis. ASQC.

Iguchi, Y., Kosugi, S., Lin, Z., Nishikawa, H., Minabe, Y., and Toda, S. (2015). Pre-stress performance in an instrumental training predicts post-stress behavioral alterations in chronically stressed rats. *Front Behav Neurosci* 9, 119. doi: 10.3389/fnbeh.2015.00119

Ironside, M., Admon, R., Maddox, S. A., Mehta, M., Douglas, S., Olson, D. P., et al. (2020). Inflammation and depressive phenotypes: evidence from medical records from over 12 000 patients and brain morphology. *Psychological Medicine* 50, 2790–2798. doi: 10.1017/S0033291719002940

Irwin, M. R., and Cole, S. W. (2011). Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol* 11, 625–632. doi: 10.1038/nri3042

Ivy, A. S., Brunson, K. L., Sandman, C., and Baram, T. Z. (2008). Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience* 154, 1132–1142. doi: 10.1016/j.neuroscience.2008.04.019

Iwakura, Y., Nakae, S., Saijo, S., and Ishigame, H. (2008). The roles of IL-17A in inflammatory immune responses and host defense against pathogens. *Immunological Reviews* 226, 57–79. doi: 10.1111/j.1600-065X.2008.00699.x

Izquierdo, A., Brigman, J. L., Radke, A. K., Rudebeck, P. H., and Holmes, A. (2017). The neural basis of reversal learning: An updated perspective. *Neuroscience* 345, 12–26. doi: 10.1016/j.neuroscience.2016.03.021

Jacobson, M. L., Kim, L. A., Patro, R., Rosati, B., and McKinnon, D. (2018). Common and differential transcriptional responses to different models of traumatic stress exposure in rats. *Transl Psychiatry* 8, 1–12. doi: 10.1038/s41398-018-0223-6

Janusek, L. W., Tell, D., Gaylord-Harden, N., and Mathews, H. L. (2017). Relationship of childhood adversity and neighborhood violence to a proinflammatory phenotype in emerging adult African American men: An epigenetic link. *Brain Behav Immun* 60, 126–135. doi: 10.1016/j.bbi.2016.10.006

Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., and Smith, S. M. (2012). FSL. *NeuroImage* 62, 782–790. doi: 10.1016/j.neuroimage.2011.09.015

Jenkinson, M., and Chappell, M. (2018). *Introduction to neuroimaging analysis*. New York, NY: Oxford University Press.

Jeong, J. Y., Lee, D. H., and Kang, S. S. (2013). Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. *Endocrinol Metab (Seoul)* 28, 288–296. doi: 10.3803/EnM.2013.28.4.288

Juster, R.-P., Sindi, S., Marin, M.-F., Perna, A., Hashemi, A., Pruessner, J. C., et al. (2011). A clinical allostatic load index is associated with burnout symptoms and hypocortisolemic profiles in healthy workers. *Psychoneuroendocrinology* 36, 797–805. doi: 10.1016/j.psyneuen.2010.11.001

Kaidbey, J. H., Ranger, M., Myers, M. M., Anwar, M., Ludwig, R. J., Schulz, A. M., et al. (2019). Early Life Maternal Separation and Maternal Behaviour Modulate Acoustic Characteristics of Rat Pup Ultrasonic Vocalizations. *Sci Rep* 9, 19012. doi: 10.1038/s41598-019-54800-z

Kambe, Y., and Miyata, A. (2015). Potential involvement of the mitochondrial unfolded protein response in depressive-like symptoms in mice. *Neurosci Lett* 588, 166–171. doi: 10.1016/j.neulet.2015.01.006

Kaneko, W. M., Riley, E. P., and Ehlers, C. L. (1994). Behavioral and electrophysiological effects of early repeated maternal separation. *Depression* 2, 43–53. doi: 10.1002/depr.3050020106

Kang, J. in, Mueller, S. G., Wu, G. W. Y., Lin, J., Ng, P., Yehuda, R., et al. (2020). Effect of Combat Exposure and Posttraumatic Stress Disorder on Telomere Length and Amygdala Volume. *Biol Psychiatry Cogn Neurosci Neuroimaging* 5, 678–687. doi: 10.1016/j.bpsc.2020.03.007

Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., and Jones, P. B. (2014). Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. *JAMA Psychiatry* 71, 1121–1128. doi: 10.1001/jamapsychiatry.2014.1332

Kiank, C., Mundt, A., and Schuett, C. (2009). Mild postnatal separation stress reduces repeated stress-induced immunosuppression in adult BALB/c mice. *Neuro Endocrinol Lett* 30, 761–768.

Kim, J., and Cicchetti, D. (2010). Longitudinal pathways linking child maltreatment, emotion regulation, peer relations, and psychopathology. *J Child Psychol Psychiatry* 51, 706–716. doi: 10.1111/j.1469-7610.2009.02202.x

Kim, T.-K., Kim, J.-E., Choi, J., Park, J.-Y., Lee, J.-E., Lee, E.-H., et al. (2017). Local Interleukin-18 System in the Basolateral Amygdala Regulates Susceptibility to Chronic Stress. *Mol Neurobiol* 54, 5347–5358. doi: 10.1007/s12035-016-0052-7

Kinn Rød, A. M., Milde, A. M., Grønli, J., Jellestad, F. K., Sundberg, H., and Murison, R. (2012). Long-term effects of footshock and social defeat on anxiety-like behaviours in rats: relationships to pre-stressor plasma corticosterone concentration. *Stress* 15, 658–670. doi: 10.3109/10253890.2012.663836

Kirkham, E. J., and Levita, L. (2020). Early life stress is associated with reduced avoidance of threatening facial expressions. *Dev Psychopathol* 32, 1059–1067. doi: 10.1017/S095457941900110X

Klimes-Dougan, B., Eberly, L. E., Westlund Schreiner, M., Kurkiewicz, P., Houri, A., Schlesinger, A., et al. (2014). Multilevel assessment of the neurobiological threat system in depressed adolescents: interplay between the limbic system and hypothalamic-pituitary-adrenal axis. *Dev Psychopathol* 26, 1321–1335. doi: 10.1017/S0954579414001059

Knuesel, I., Chicha, L., Britschgi, M., Schobel, S. A., Bodmer, M., Hellings, J. A., et al. (2014). Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol* 10, 643–660. doi: 10.1038/nrneurol.2014.187

Koe, A. S., Ashokan, A., and Mitra, R. (2016). Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation. *Transl Psychiatry* 6, e729. doi: 10.1038/tp.2015.217

Koenen, K. C., Moffitt, T. E., Roberts, A. L., Martin, L. T., Kubzansky, L., Harrington, H., et al. (2009). Childhood IQ and adult mental disorders: a test of the cognitive reserve hypothesis. *AJP* 166, 50–57. doi: 10.1176/appi.ajp.2008.08030343

Köhler, C. A., Freitas, T. H., Maes, M., Andrade, N. Q. de, Liu, C. S., Fernandes, B. S., et al. (2017). Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatrica Scandinavica* 135, 373–387. doi: 10.1111/acps.12698

Kolesar, T. A., Bilevicius, E., Wilson, A. D., and Kornelsen, J. (2019). Systematic review and meta-analyses of neural structural and functional differences in generalized anxiety disorder and healthy controls using magnetic resonance imaging. *Neuroimage Clin* 24, 102016. doi: 10.1016/j.nicl.2019.102016

Kosten, T. A., Zhang, X. Y., and Kehoe, P. (2006). Heightened cocaine and food self-administration in female rats with neonatal isolation experience. *Neuropsychopharmacology* 31, 70–76. doi: 10.1038/sj.npp.1300779

Kruschinski, C., Skripuletz, T., Bedoui, S., Raber, K., Straub, R. H., Hoffmann, T., et al. (2008). Postnatal life events affect the severity of asthmatic airway inflammation in the adult rat. *J Immunol* 180, 3919–3925. doi: 10.4049/jimmunol.180.6.3919

Kühn, S., Schubert, F., and Gallinat, J. (2011). Structural correlates of trait anxiety: reduced thickness in medial orbitofrontal cortex accompanied by volume increase in nucleus accumbens. *Journal of Affective Disorders* 134, 315–319. doi: 10.1016/j.jad.2011.06.003

Kumar, V., Abbas, A. K., Aster, J. C., Cotran, R. S., and Robbins, S. L. (2015). *Robbins and Cotran Pathologic Basis of Disease*. Philadelphia, PA: Elsevier/Saunders.

Kundakovic, M., Lim, S., Gudsnuk, K., and Champagne, F. A. (2013). Sex-specific and strain-dependent effects of early life adversity on behavioral and epigenetic outcomes. *Front Psychiatry* 4, 78. doi: 10.3389/fpsyt.2013.00078

LaFleur, B. J., and Greevy, R. A. (2009). Introduction to permutation and resampling-based hypothesis tests. *J Clin Child Adolesc Psychol* 38, 286–294. doi: 10.1080/15374410902740411

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol* 4, 863. doi: 10.3389/fpsyg.2013.00863

Lam, J. C. W., Shields, G. S., Trainor, B. C., Slavich, G. M., and Yonelinas, A. P. (2019). Greater lifetime stress exposure predicts blunted cortisol but heightened DHEA responses to acute stress. *Stress Health* 35, 15–26. doi: 10.1002/smi.2835

Landau, S., Emsley, R., and Dunn, G. (2018). Beyond total treatment effects in randomised controlled trials: Baseline measurement of intermediate outcomes needed to reduce confounding in mediation investigations. *Clin Trials* 15, 247–256. doi: 10.1177/1740774518760300

Lange, C., and Irle, E. (2004). Enlarged amygdala volume and reduced hippocampal volume in young women with major depression. *Psychological Medicine* 34, 1059–1064. doi: 10.1017/s0033291703001806

Langston, R. F., Ainge, J. A., Couey, J. J., Canto, C. B., Bjerknes, T. L., Witter, M. P., et al. (2010). Development of the spatial representation system in the rat. *Science* 328, 1576–1580. doi: 10.1126/science.1188210

Lansford, J. E., Dodge, K. A., Pettit, G. S., Bates, J. E., Crozier, J., and Kaplow, J. (2002). A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence. *Arch Pediatr Adolesc Med* 156, 824–830. doi: 10.1001/archpedi.156.8.824

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol* 23, 727–738. doi: 10.1023/a:1025048802629

LeDoux, J., and Daw, N. D. (2018). Surviving threats: neural circuit and computational implications of a new taxonomy of defensive behaviour. *Nat Rev Neurosci* 19, 269–282. doi: 10.1038/nrn.2018.22

Lee, Y. J., Koe, A. S., Ashokan, A., and Mitra, R. (2020). Female rats are resilient to the behavioral effects of maternal separation stress and exhibit stress-induced neurogenesis. *Heliyon* 6, e04753. doi: 10.1016/j.heliyon.2020.e04753

Lennon, E. M., Maharshak, N., Elloumi, H., Borst, L., Plevy, S. E., and Moeser, A. J. (2013). Early life stress triggers persistent colonic barrier dysfunction and exacerbates colitis in adult IL-10-/- mice. *Inflamm Bowel Dis* 19, 712–719. doi: 10.1097/MIB.0b013e3182802a4e

Lenth, R. V. (2021). emmeans: Estimated Marginal Means, aka Least-Squares Means, https://CRAN.R-project.org/package=emmeans

Leppänen, J. M. (2006). Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. *Curr Opin Psychiatry* 19, 34–39. doi: 10.1097/01.yco.0000191500.46411.00

Levine, S., Huchton, D. M., Wiener, S. G., and Rosenfeld, P. (1991). Time course of the effect of maternal deprivation on the hypothalamic-pituitary-adrenal axis in the infant rat. *Dev Psychobiol* 24, 547–558. doi: 10.1002/dev.420240803

Levy, J. A. (2009). The unexpected pleiotropic activities of RANTES. *J Immunol* 182, 3945–3946. doi: 10.4049/jimmunol.0990015

Leys, C., Ley, C., Klein, O., Bernard, P., and Licata, L. (2013). Detecting outliers: Do not use standard deviation around the mean, use absolute deviation around the median. *Journal of Experimental Social Psychology* 49, 764–766. doi: 10.1016/j.jesp.2013.03.013

Li, B., Lee, C., Martin, Z., Li, X., Koike, Y., Hock, A., et al. (2017). Intestinal epithelial injury induced by maternal separation is protected by hydrogen sulfide. *J Pediatr Surg* 52, 40–44. doi: 10.1016/j.jpedsurg.2016.10.013

Li, H., Buisman-Pijlman, F. T. A., Nunez-Salces, M., Christie, S., Frisby, C. L., Inserra, A., et al. (2019). Chronic stress induces hypersensitivity of murine gastric vagal afferents. *Neurogastroenterol Motil* 31, e13669. doi: 10.1111/nmo.13669

Li, X.-L., Yuan, Y.-G., Xu, H., Di Wu, Gong, W.-G., Geng, L.-Y., et al. (2015). Changed Synaptic Plasticity in Neural Circuits of Depressive-Like and Escitalopram-Treated Rats. *Int J Neuropsychopharmacol* 18, pyv046. doi: 10.1093/ijnp/pyv046

Liang, X., Hsu, L.-M., Lu, H., Sumiyoshi, A., He, Y., and Yang, Y. (2018). The Rich-Club Organization in Rat Functional Brain Network to Balance Between Communication Cost and Efficiency. *Cereb Cortex* 28, 924–935. doi: 10.1093/cercor/bhw416

Liu, M.-Y., Yin, C.-Y., Zhu, L.-J., Zhu, X.-H., Xu, C., Luo, C.-X., et al. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nat Protoc* 13, 1686–1698. doi: 10.1038/s41596-018-0011-z

Loria, A. S., Brands, M. W., Pollock, D. M., and Pollock, J. S. (2013). Early life stress sensitizes the renal and systemic sympathetic system in rats. *Am J Physiol Renal Physiol* 305, F390-5. doi: 10.1152/ajprenal.00008.2013

Ludbrook, J., and Dudley, H. (1998). Why Permutation Tests are Superior to t and F Tests in Biomedical Research. *The American Statistician* 52, 127–132. doi: 10.1080/00031305.1998.10480551

Luke, S. G. (2017). Evaluating significance in linear mixed-effects models in R. *Behav Res Methods* 49, 1494–1502. doi: 10.3758/s13428-016-0809-y

Luminex Corporation (2021). *MAGPIX System*. Accessed 21-Dec-21, https://www.luminexcorp.com/magpix-system/

Lupien, S. J., McEwen, B. S., Gunnar, M. R., and Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 10, 434–445. doi: 10.1038/nrn2639

Lupien, S. J., Parent, S., Evans, A. C., Tremblay, R. E., Zelazo, P. D., Corbo, V., et al. (2011). Larger amygdala but no change in hippocampal volume in 10-year-old children exposed to maternal depressive symptomatology since birth. *Proc Natl Acad Sci U S A* 108, 14324–14329. doi: 10.1073/pnas.1105371108

Luzina, I. G., Keegan, A. D., Heller, N. M., Rook, G. A. W., Shea-Donohue, T., and Atamas, S. P. (2012). Regulation of inflammation by interleukin-4: a review of "alternatives". *J Leukoc Biol* 92, 753–764. doi: 10.1189/jlb.0412214

Maccari, S., Krugers, H. J., Morley-Fletcher, S., Szyf, M., and Brunton, P. J. (2014). The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. *J Neuroendocrinol* 26, 707–723. doi: 10.1111/jne.12175

Maes, M. (1999). Major depression and activation of the inflammatory response system. *Adv Exp Med Biol* 461, 25–46. doi: 10.1007/978-0-585-37970-8_2

Majcher-Maślanka, I., Solarz, A., and Chocyk, A. (2019). Maternal separation disturbs postnatal development of the medial prefrontal cortex and affects the number of neurons

and glial cells in adolescent rats. *Neuroscience* 423, 131–147. doi: 10.1016/j.neuroscience.2019.10.033

Makovac, E., Meeten, F., Watson, D. R., Garfinkel, S. N., Critchley, H. D., and Ottaviani, C. (2016). Neurostructural abnormalities associated with axes of emotion dysregulation in generalized anxiety. *Neuroimage Clin* 10, 172–181. doi: 10.1016/j.nicl.2015.11.022

Malek, T. R. (2008). The biology of interleukin-2. *Annu Rev Immunol* 26, 453–479. doi: 10.1146/annurev.immunol.26.021607.090357

Malter Cohen, M., Jing, D., Yang, R. R., Tottenham, N., Lee, F. S., and Casey, B. J. (2013). Early-life stress has persistent effects on amygdala function and development in mice and humans. *Proc Natl Acad Sci U S A* 110, 18274–18278. doi: 10.1073/pnas.1310163110

Malykhin, N. V., Carter, R., Hegadoren, K. M., Seres, P., and Coupland, N. J. (2012). Fronto-limbic volumetric changes in major depressive disorder. *Journal of Affective Disorders* 136, 1104–1113. doi: 10.1016/j.jad.2011.10.038

Maniam, J., and Morris, M. J. (2010a). Long-term postpartum anxiety and depression-like behavior in mother rats subjected to maternal separation are ameliorated by palatable high fat diet. *Behav Brain Res* 208, 72–79. doi: 10.1016/j.bbr.2009.11.005

Maniam, J., and Morris, M. J. (2010b). Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. *Psychoneuroendocrinology* 35, 717–728. doi: 10.1016/j.psyneuen.2009.10.013

Marco, E. M., Adriani, W., Llorente, R., Laviola, G., and Viveros, M.-P. (2009). Detrimental psychophysiological effects of early maternal deprivation in adolescent and adult rodents: altered responses to cannabinoid exposure. *Neurosci Biobehav Rev* 33, 498–507. doi: 10.1016/j.neubiorev.2008.03.008

Martí, O., Harbuz, M. S., Andrés, R., Lightman, S. L., and Armario, A. (1999). Activation of the hypothalamic-pituitary axis in adrenalectomised rats: potentiation by chronic stress. *Brain Research* 821, 1–7. doi: 10.1016/s0006-8993(98)01212-8

Martí, O., Martí, J., and Armario, A. (1994). Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol Behav* 55, 747–753. doi: 10.1016/0031-9384(94)90055-8

Maslanik, T., Bernstein-Hanley, I., Helwig, B., and Fleshner, M. (2012). The impact of acute-stressor exposure on splenic innate immunity: a gene expression analysis. *Brain Behav Immun* 26, 142–149. doi: 10.1016/j.bbi.2011.08.006

Maslanik, T., Mahaffey, L., Tannura, K., Beninson, L. A., Greenwood, B. N., and Fleshner, M. (2013). The inflammasome and danger associated molecular patterns (DAMPs) are

implicated in cytokine and chemokine responses following stressor exposure. *Brain Behav Immun* 28, 54–62. doi: 10.1016/j.bbi.2012.10.014

Mathersul, D. C., Eising, C. M., DeSouza, D. D., Spiegel, D., and Bayley, P. J. (2020). Brain and Physiological Markers of Autonomic Function Are Associated With Treatment-Related Improvements in Self-Reported Autonomic Dysfunction in Veterans With Gulf War Illness: An Exploratory Pilot Study. *Glob Adv Health Med* 9, 2164956120922812. doi: 10.1177/2164956120922812

Matthews, K., Dalley, J. W., Matthews, C., Tsai, T. H., and Robbins, T. W. (2001). Periodic maternal separation of neonatal rats produces region- and gender-specific effects on biogenic amine content in postmortem adult brain. *Synapse* 40, 1–10.

Matthews, K., Forbes, N., and Reid, I. C. (1995). Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav* 57, 241–248. doi: 10.1016/0031-9384(94)00286-e

Matthews, K., Hall, F. S., Wilkinson, L. S., and Robbins, T. W. (1996a). Retarded acquisition and reduced expression of conditioned locomotor activity in adult rats following repeated early maternal separation: effects of prefeeding, d-amphetamine, dopamine antagonists and clonidine. *Psychopharmacology (Berl)* 126, 75–84. doi: 10.1007/BF02246414

Matthews, K., Robbins, T. W., Everitt, B. J., and Caine, S. B. (1999). Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. *Psychopharmacology (Berl)* 141, 123–134. doi: 10.1007/s002130050816

Matthews, K., Wilkinson, L. S., and Robbins, T. W. (1996b). Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. *Physiol Behav* 59, 99–107.

Maughan, A., and Cicchetti, D. (2002). Impact of child maltreatment and interadult violence on children's emotion regulation abilities and socioemotional adjustment. *Child Dev* 73, 1525–1542. doi: 10.1111/1467-8624.00488

Maurer, M., and Stebut, E. von (2004). Macrophage inflammatory protein-1. *Int J Biochem Cell Biol* 36, 1882–1886. doi: 10.1016/j.biocel.2003.10.019

Maxwell, M. A., and Cole, D. A. (2009). Weight change and appetite disturbance as symptoms of adolescent depression: toward an integrative biopsychosocial model. *Clin Psychol Rev* 29, 260–273. doi: 10.1016/j.cpr.2009.01.007

McArthur, R., and Borsini, F. (2006). Animal models of depression in drug discovery: a historical perspective. *Pharmacol Biochem Behav* 84, 436–452. doi: 10.1016/j.pbb.2006.06.005

McEwen, B. S. (2012). Brain on stress: how the social environment gets under the skin. *Proc Natl Acad Sci U S A* 109 Suppl 2, 17180–17185. doi: 10.1073/pnas.1121254109

Meagher, M. W., Sieve, A. N., Johnson, R. R., Satterlee, D., Belyavskyi, M., Mi, W., et al. (2010). Neonatal maternal separation alters immune, endocrine, and behavioral responses to acute Theiler's virus infection in adult mice. *Behav Genet* 40, 233–249. doi: 10.1007/s10519-010-9333-5

Mehta, M. A., Golembo, N. I., Nosarti, C., Colvert, E., Mota, A., Williams, S. C. R., et al. (2009). Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot. *J Child Psychol Psychiatry* 50, 943–951. doi: 10.1111/j.1469-7610.2009.02084.x

Meng, X., Shen, F., Li, C., Li, Y., and Wang, X. (2016). Depression-like behaviors in tree shrews and comparison of the effects of treatment with fluoxetine and carbetocin. *Pharmacol Biochem Behav* 145, 1–8. doi: 10.1016/j.pbb.2016.03.006

Merali, Z., Graitson, S., Mackay, J. C., and Kent, P. (2013). Stress and eating: a dual role for bombesin-like peptides. *Front Neurosci* 7, 193. doi: 10.3389/fnins.2013.00193

Merke, D. P., Fields, J. D., Keil, M. F., Vaituzis, A. C., Chrousos, G. P., and Giedd, J. N. (2003). Children with classic congenital adrenal hyperplasia have decreased amygdala volume: potential prenatal and postnatal hormonal effects. *J Clin Endocrinol Metab* 88, 1760–1765. doi: 10.1210/jc.2002-021730

Merke, D. P., Giedd, J. N., Keil, M. F., Mehlinger, S. L., Wiggs, E. A., Holzer, S., et al. (2005). Children experience cognitive decline despite reversal of brain atrophy one year after resolution of Cushing syndrome. *J Clin Endocrinol Metab* 90, 2531–2536. doi: 10.1210/jc.2004-2488

Michel, M.-L., Pang, D. J., Haque, S. F. Y., Potocnik, A. J., Pennington, D. J., and Hayday, A. C. (2012). Interleukin 7 (IL-7) selectively promotes mouse and human IL-17-producing γδ cells. *Proc Natl Acad Sci U S A* 109, 17549–17554. doi: 10.1073/pnas.1204327109

Miczek, K. A., Nikulina, E. M., Shimamoto, A., and Covington, H. E. (2011). Escalated or suppressed cocaine reward, tegmental BDNF, and accumbal dopamine caused by episodic versus continuous social stress in rats. *J Neurosci* 31, 9848–9857. doi: 10.1523/JNEUROSCI.0637-11.2011

Miller, A. H., and Raison, C. L. (2015). Are Anti-inflammatory Therapies Viable Treatments for Psychiatric Disorders? Where the Rubber Meets the Road. *JAMA Psychiatry* 72, 527–528. doi: 10.1001/jamapsychiatry.2015.22

Miller, A. H., and Raison, C. L. (2016). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol* 16, 22–34. doi: 10.1038/nri.2015.5

Miller, G. E., Chen, E., and Parker, K. J. (2011). Psychological stress in childhood and susceptibility to the chronic diseases of aging: moving toward a model of behavioral and biological mechanisms. *Psychol Bull* 137, 959–997. doi: 10.1037/a0024768

Mills, R., Alati, R., O'Callaghan, M., Najman, J. M., Williams, G. M., Bor, W., et al. (2011). Child abuse and neglect and cognitive function at 14 years of age: findings from a birth cohort. *Pediatrics* 127, 4–10. doi: 10.1542/peds.2009-3479

Minami, S., Satoyoshi, H., Ide, S., Inoue, T., Yoshioka, M., and Minami, M. (2017). Suppression of reward-induced dopamine release in the nucleus accumbens in animal models of depression: Differential responses to drug treatment. *Neurosci Lett* 650, 72–76. doi: 10.1016/j.neulet.2017.04.028

Mitra, R., and Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. *Proc Natl Acad Sci USA* 105, 5573–5578. doi: 10.1073/pnas.0705615105

Mizoguchi, H., Fukumoto, K., Sakamoto, G., Jin, S., Toyama, A., Wang, T., et al. (2019). Maternal separation as a risk factor for aggravation of neuropathic pain in later life in mice. *Behav Brain Res* 359, 942–949. doi: 10.1016/j.bbr.2018.06.015

Mogg, K., Millar, N., and Bradley, B. P. (2000). Biases in eye movements to threatening facial expressions in generalized anxiety disorder and depressive disorder. *J Abnorm Psychol* 109, 695–704. doi: 10.1037//0021-843x.109.4.695

Mohlman, J., Price, R. B., Eldreth, D. A., Chazin, D., Glover, D. M., and Kates, W. R. (2009). The relation of worry to prefrontal cortex volume in older adults with and without generalized anxiety disorder. *Psychiatry Research* 173, 121–127. doi: 10.1016/j.pscychresns.2008.09.010

Monsey, M. S., Boyle, L. M., Zhang, M. L., Nguyen, C. P., Kronman, H. G., Ota, K. T., et al. (2014). Chronic corticosterone exposure persistently elevates the expression of memory-related genes in the lateral amygdala and enhances the consolidation of a Pavlovian fear memory. *PLoS ONE* 9, e91530. doi: 10.1371/journal.pone.0091530

Moreau, J.-L., Borgulya, J., Jenck, F., and Martin, J. R. (1994). Tolcapone: a potential new antidepressant detected in a novel animal model of depression. *Behav Pharmacol* 5, 344–350.

Morgan, N., Irwin, M. R., Chung, M., and Wang, C. (2014). The effects of mind-body therapies on the immune system: meta-analysis. *PLoS ONE* 9, e100903. doi: 10.1371/journal.pone.0100903

Morin, E. L., Howell, B. R., Feczko, E., Earl, E., Pincus, M., Reding, K., et al. (2020). Developmental outcomes of early adverse care on amygdala functional connectivity in nonhuman primates. *Dev Psychopathol* 32, 1579–1596. doi: 10.1017/S0954579420001133

Morin, E. L., Howell, B. R., Meyer, J. S., and Sanchez, M. M. (2019). Effects of early maternal care on adolescent attention bias to threat in nonhuman primates. *Dev Cogn Neurosci* 38, 100643. doi: 10.1016/j.dcn.2019.100643

Morton, D. B., Jennings, M., Buckwell, A., Ewbank, R., Godfrey, C., Holgate, B., et al. (2001). Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare. *Lab Anim* 35, 1–41. doi: 10.1258/0023677011911345

Mostafavi, S., Battle, A., Zhu, X., Potash, J. B., Weissman, M. M., Shi, J., et al. (2014). Type I interferon signaling genes in recurrent major depression: increased expression detected by whole-blood RNA sequencing. *Mol Psychiatry* 19, 1267–1274. doi: 10.1038/mp.2013.161

Moya-Pérez, A., Perez-Villalba, A., Benítez-Páez, A., Campillo, I., and Sanz, Y. (2017). Bifidobacterium CECT 7765 modulates early stress-induced immune, neuroendocrine and behavioral alterations in mice. *Brain Behav Immun* 65, 43–56. doi: 10.1016/j.bbi.2017.05.011

Mrdalj, J., Murison, R., Soulé, J., Kinn Rød, A. M., Milde, A. M., Pallesen, S., et al. (2016). Mild daily stressors in adulthood may counteract behavioural effects after constant presence of mother during early life. *Physiol Behav* 165, 313–321. doi: 10.1016/j.physbeh.2016.08.018

Mukherjee, D., Filipowicz, A. L. S., Vo, K., Satterthwaite, T. D., and Kable, J. W. (2020). Reward and punishment reversal-learning in major depressive disorder. *J Abnorm Psychol* 129, 810–823. doi: 10.1037/abn0000641

Murphy, F. C., Michael, A., Robbins, T. W., and Sahakian, B. J. (2003). Neuropsychological impairment in patients with major depressive disorder: the effects of feedback on task performance. *Psychological Medicine* 33, 455–467. doi: 10.1017/s0033291702007018

Murphy, K., and Weaver, C. (2016). *Janeway's immunobiology*. New York NY: Garland Science/Taylor & Francis Group LLC.

Myers-Schulz, B., and Koenigs, M. (2012). Functional anatomy of ventromedial prefrontal cortex: implications for mood and anxiety disorders. *Mol Psychiatry* 17, 132–141. doi: 10.1038/mp.2011.88

Nakamura, T., Walker, A. K., Sominsky, L., Allen, T., Rosengren, S., and Hodgson, D. M. (2011). Maternal separation in early life impairs tumor immunity in adulthood in the F344 rat. *Stress* 14, 335–343. doi: 10.3109/10253890.2010.548014

Nakayasu, T., and Ishii, K. (2008). Effects of pair-housing after social defeat experience on elevated plus-maze behavior in rats. *Behav Processes* 78, 477–480. doi: 10.1016/j.beproc.2008.02.007

Nanni, V., Uher, R., and Danese, A. (2012). Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *AJP* 169, 141–151. doi: 10.1176/appi.ajp.2011.11020335

Nelson, E. C., Heath, A. C., Lynskey, M. T., Bucholz, K. K., Madden, P. A. F., Statham, D. J., et al. (2006). Childhood sexual abuse and risks for licit and illicit drug-related outcomes: a twin study. *Psychological Medicine* 36, 1473–1483. doi: 10.1017/S0033291706008397

Nestler, E. J., and Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nat Neurosci* 13, 1161–1169. doi: 10.1038/nn.2647

Newman, M. G., Llera, S. J., Erickson, T. M., Przeworski, A., and Castonguay, L. G. (2013). Worry and generalized anxiety disorder: a review and theoretical synthesis of evidence on nature, etiology, mechanisms, and treatment. *Annu Rev Clin Psychol* 9, 275–297. doi: 10.1146/annurev-clinpsy-050212-185544

Nikolova, Y. S., Misquitta, K. A., Rocco, B. R., Prevot, T. D., Knodt, A. R., Ellegood, J., et al. (2018). Shifting priorities: highly conserved behavioral and brain network adaptations to chronic stress across species. *Transl Psychiatry* 8, 26. doi: 10.1038/s41398-017-0083-5

Noll, J. G., Shenk, C. E., Yeh, M. T., Ji, J., Putnam, F. W., and Trickett, P. K. (2010). Receptive language and educational attainment for sexually abused females. *Pediatrics* 126, e615-22. doi: 10.1542/peds.2010-0496

Nylander, I., and Roman, E. (2013). Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? *Psychopharmacology (Berl)* 229, 555–569. doi: 10.1007/s00213-013-3217-3

O'Rand, A. M., Hamil-Luker, J., and Elman, C. (2009). Childhood adversity, educational trajectories, and self-reported health in later life among U.S. women and men at the turn of the century. *Z Erziehungswiss* 12, 409–436. doi: 10.1007/s11618-009-0081-3

Odeon, M. M., and Acosta, G. B. (2019). Repeated maternal separation: Alcohol consumption, anxious behavior and corticosterone were reversed by a non-pharmacological treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 95, 109726. doi: 10.1016/j.pnpbp.2019.109726

Ogishima, H., Maeda, S., Tanaka, Y., and Shimada, H. (2020). Effects of Depressive Symptoms, Feelings, and Interoception on Reward-Based Decision-Making: Investigation Using Reinforcement Learning Model. *Brain Sci* 10. doi: 10.3390/brainsci10080508

Ognibene, E., Adriani, W., Macrì, S., and Laviola, G. (2007). Neurobehavioural disorders in the infant reeler mouse model: interaction of genetic vulnerability and consequences of maternal separation. *Behav Brain Res* 177, 142–149. doi: 10.1016/j.bbr.2006.10.027

Oines, E., Murison, R., Mrdalj, J., Grønli, J., and Milde, A. M. (2012). Neonatal maternal separation in male rats increases intestinal permeability and affects behavior after chronic social stress. *Physiol Behav* 105, 1058–1066. doi: 10.1016/j.physbeh.2011.11.024

Olausson, P., Kiraly, D. D., Gourley, S. L., and Taylor, J. R. (2013). Persistent effects of prior chronic exposure to corticosterone on reward-related learning and motivation in rodents. *Psychopharmacology (Berl)* 225, 569–577. doi: 10.1007/s00213-012-2844-4

Olsavsky, A. K., Stoddard, J., Erhart, A., Tribble, R., and Kim, P. (2021). Reported maternal childhood maltreatment experiences, amygdala activation and functional connectivity to infant cry. *Soc Cogn Affect Neurosci* 16, 418–427. doi: 10.1093/scan/nsab005

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., et al. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry* 65, 263–267. doi: 10.1016/j.biopsych.2008.06.026

O'Malley, D., Liston, M., Hyland, N. P., Dinan, T. G., and Cryan, J. F. (2011). Colonic soluble mediators from the maternal separation model of irritable bowel syndrome activate submucosal neurons via an interleukin-6-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 300, G241-52. doi: 10.1152/ajpgi.00385.2010

Onaka, T. (2020). Stress adaptation by increasing the area of the cell membrane. *J Physiol* 598, 2541–2542. doi: 10.1113/JP280033

Opp, M. R., ed (2016). *Primer of psychoneuroimmunology research*. Los Angeles CA: Psychoneuroimmunology Research Society.

Orsetti, M., Colella, L., Dellarole, A., Canonico, P. L., Ferri, S., and Ghi, P. (2006). Effects of chronic administration of olanzapine, amitriptyline, haloperidol or sodium valproate in naive and anhedonic rats. *Int J Neuropsychopharmacol* 9, 427–436. doi: 10.1017/S146114570500564X

Orso, R., Creutzberg, K. C., Wearick-Silva, L. E., Wendt Viola, T., Tractenberg, S. G., Benetti, F., et al. (2019). How Early Life Stress Impact Maternal Care: A Systematic Review of Rodent Studies. *Front Behav Neurosci* 13, 197. doi: 10.3389/fnbeh.2019.00197

Otte, C., Gold, S. M., Penninx, B. W. J. H., Pariante, C. M., Etkin, A., Fava, M., et al. (2016). Major depressive disorder. *Nat Rev Dis Primers* 2, 16065. doi: 10.1038/nrdp.2016.65

Pace, T. W. W., Mletzko, T. C., Alagbe, O., Musselman, D. L., Nemeroff, C. B., Miller, A. H., et al. (2006). Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *AJP* 163, 1630–1633. doi: 10.1176/ajp.2006.163.9.1630

Pace, T. W. W., Negi, L. T., Adame, D. D., Cole, S. P., Sivilli, T. I., Brown, T. D., et al. (2009). Effect of compassion meditation on neuroendocrine, innate immune and behavioral responses to psychosocial stress. *Psychoneuroendocrinology* 34, 87–98. doi: 10.1016/j.psyneuen.2008.08.011

Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., et al. (2014). Stress-system genes and life stress predict cortisol levels and amygdala and hippocampal volumes in children. *Neuropsychopharmacology* 39, 1245–1253. doi: 10.1038/npp.2013.327

Papp, E. A., Leergaard, T. B., Calabrese, E., Johnson, G. A., and Bjaalie, J. G. (2014). Waxholm Space atlas of the Sprague Dawley rat brain. *NeuroImage* 97, 374–386. doi: 10.1016/j.neuroimage.2014.04.001

Papp, M., Gruca, P., Lason-Tyburkiewicz, M., and Willner, P. (2016). Antidepressant, anxiolytic and procognitive effects of rivastigmine and donepezil in the chronic mild stress model in rats. *Psychopharmacology (Berl)* 233, 1235–1243. doi: 10.1007/s00213-016-4206-0

Park, E. S., Freeborn, J., Venna, V. R., Roos, S., Rhoads, J. M., and Liu, Y. (2021). Lactobacillus reuteri effects on maternal separation stress in newborn mice. *Pediatr Res*. doi: 10.1038/s41390-021-01374-0

Park, S. E., Park, D., Song, K.-I., Seong, J.-K., Chung, S., and Youn, I. (2017). Differential heart rate variability and physiological responses associated with accumulated short- and long-term stress in rodents. *Physiol Behav* 171, 21–31. doi: 10.1016/j.physbeh.2016.12.036

Pascoe, M. C., Thompson, D. R., and Ski, C. F. (2017). Yoga, mindfulness-based stress reduction and stress-related physiological measures: A meta-analysis. *Psychoneuroendocrinology* 86, 152–168. doi: 10.1016/j.psyneuen.2017.08.008

Pastor-Ciurana, J., Rabasa, C., Ortega-Sánchez, J. A., Sanchís-Ollè, M., Gabriel-Salazar, M., Ginesta, M., et al. (2014). Prior exposure to repeated immobilization or chronic unpredictable stress protects from some negative sequels of an acute immobilization. *Behav Brain Res* 265, 155–162. doi: 10.1016/j.bbr.2014.02.028

Paternain, L., Martisova, E., Milagro, F. I., Ramírez, M. J., Martínez, J. A., and Campión, J. (2012). Postnatal maternal separation modifies the response to an obesogenic diet in adulthood in rats. *Dis Model Mech* 5, 691–697. doi: 10.1242/dmm.009043

Paxinos, G., and Watson, C. (1998). *The rat brain in stereotaxic coordinates*. San Diego, London: Academic.

Paxinos, G., and Watson, C. (2007). *The rat brain in stereotaxic coordinates*. Amsterdam: Elsevier.

Pechtel, P., Lyons-Ruth, K., Anderson, C. M., and Teicher, M. H. (2014). Sensitive periods of amygdala development: the role of maltreatment in preadolescence. *NeuroImage* 97, 236–244. doi: 10.1016/j.neuroimage.2014.04.025

Pedersen, T. L. (2020). patchwork: The Composer of Plots, https://CRAN.R-project.org/package=patchwork

Pellow, S., Chopin, P., File, S. E., and Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Proc Natl Acad Sci U S A* 14, 149–167.

Pestka, S., Krause, C. D., Sarkar, D., Walter, M. R., Shi, Y., and Fisher, P. B. (2004). Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol* 22, 929–979. doi: 10.1146/annurev.immunol.22.012703.104622

Peverill, M., Sheridan, M. A., Busso, D. S., and McLaughlin, K. A. (2019). Atypical Prefrontal-Amygdala Circuitry Following Childhood Exposure to Abuse: Links With Adolescent Psychopathology. *Child Maltreat* 24, 411–423. doi: 10.1177/1077559519852676

Phillips, B. U., Dewan, S., Nilsson, S. R. O., Robbins, T. W., Heath, C. J., Saksida, L. M., et al. (2018). Selective effects of 5-HT2C receptor modulation on performance of a novel valence-probe visual discrimination task and probabilistic reversal learning in mice. *Psychopharmacology (Berl)* 235, 2101–2111. doi: 10.1007/s00213-018-4907-7

Piazza, P. V., Deminière, J. M., Le Moal, M., and Simon, H. (1989). Factors that predict individual vulnerability to amphetamine self-administration. *Science* 245, 1511–1513.

Picard, K., Bisht, K., Poggini, S., Garofalo, S., Golia, M. T., Basilico, B., et al. (2021). Microglial-glucocorticoid receptor depletion alters the response of hippocampal microglia and neurons in a chronic unpredictable mild stress paradigm in female mice. *Brain Behav Immun* 97, 423–439. doi: 10.1016/j.bbi.2021.07.022

Pierce, A. N., Di Silvestro, E. R., Eller, O. C., Wang, R., Ryals, J. M., and Christianson, J. A. (2016). Urinary bladder hypersensitivity and dysfunction in female mice following early life and adult stress. *Brain Research* 1639, 58–73. doi: 10.1016/j.brainres.2016.02.039

Pierce, A. N., Ryals, J. M., Wang, R., and Christianson, J. A. (2014). Vaginal hypersensitivity and hypothalamic-pituitary-adrenal axis dysfunction as a result of neonatal maternal separation in female mice. *Neuroscience* 263, 216–230. doi: 10.1016/j.neuroscience.2014.01.022

Pine, D. S., Mogg, K., Bradley, B. P., Montgomery, L., Monk, C. S., McClure, E., et al. (2005). Attention bias to threat in maltreated children: implications for vulnerability to stress-related psychopathology. *AJP* 162, 291–296. doi: 10.1176/appi.ajp.162.2.291

Pinheiro, R. M. C., Lima, M. N. M. de, Portal, B. C. D., Busato, S. B., Falavigna, L., Ferreira, R. D. P., et al. (2015). Long-lasting recognition memory impairment and alterations in brain levels of cytokines and BDNF induced by maternal deprivation: effects of valproic acid and topiramate. *J Neural Transm (Vienna)* 122, 709–719. doi: 10.1007/s00702-014-1303-2

Pirko, I., Johnson, A. J., Chen, Y., Lindquist, D. M., Lohrey, A. K., Ying, J., et al. (2011). Brain atrophy correlates with functional outcome in a murine model of multiple sclerosis. *NeuroImage* 54, 802–806. doi: 10.1016/j.neuroimage.2010.08.055

Pizzagalli, D. A. (2014). Depression, stress, and anhedonia: toward a synthesis and integrated model. *Annu Rev Clin Psychol* 10, 393–423. doi: 10.1146/annurev-clinpsy-050212-185606

Pizzagalli, D. A., Holmes, A. J., Dillon, D. G., Goetz, E. L., Birk, J. L., Bogdan, R., et al. (2009). Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *Am J Psychiatry* 166, 702–710. doi: 10.1176/appi.ajp.2008.08081201

Plewes, D. B., and Kucharczyk, W. (2012). Physics of MRI: a primer. *J Magn Reson Imaging* 35, 1038–1054. doi: 10.1002/jmri.23642

Powell, T. R., Fernandes, C., and Schalkwyk, L. C. (2012). Depression-Related Behavioral Tests. *Curr Protoc Mouse Biol* 2, 119–127. doi: 10.1002/9780470942390.mo110176

Pryce, C. R., and Feldon, J. (2003). Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. *Neurosci Biobehav Rev* 27, 57–71. doi: 10.1016/S0149-7634(03)00009-5

Qiao, H., Li, M.-X., Xu, C., Chen, H.-B., An, S.-C., and Ma, X.-M. (2016). Dendritic Spines in Depression: What We Learned from Animal Models. *Neural Plast* 2016, 8056370. doi: 10.1155/2016/8056370

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

R&D Systems, Inc. (2021). *Luminex Assay Principle*. Accessed 21-Dec-21, https://www.rndsystems.com/resources/technical/luminex-assay-principle

Raison, C. L., and Miller, A. H. (2013). The evolutionary significance of depression in Pathogen Host Defense (PATHOS-D). *Mol Psychiatry* 18, 15–37. doi: 10.1038/mp.2012.2

Raison, C. L., Rutherford, R. E., Woolwine, B. J., Shuo, C., Schettler, P., Drake, D. F., et al. (2013). A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry* 70, 31–41. doi: 10.1001/2013.jamapsychiatry.4

Ramaker, M. J., and Dulawa, S. C. (2017). Identifying fast-onset antidepressants using rodent models. *Mol Psychiatry* 22, 656–665.

Raymond, C., Marin, M.-F., Wolosianski, V., Journault, A.-A., Longpré, C., and Lupien, S. J. (2021). Adult Women First Exposed to Early Adversity After 8 Years Old Show Attentional Bias to Threat. *Front Behav Neurosci* 15, 628099. doi: 10.3389/fnbeh.2021.628099

Rees, S. L., Steiner, M., and Fleming, A. S. (2006). Early deprivation, but not maternal separation, attenuates rise in corticosterone levels after exposure to a novel environment in both juvenile and adult female rats. *Behav Brain Res* 175, 383–391. doi: 10.1016/j.bbr.2006.09.013

Remijnse, P. L., Nielen, M. M. A., van Balkom, A. J. L. M., Hendriks, G.-J., Hoogendijk, W. J., Uylings, H. B. M., et al. (2009). Differential frontal-striatal and paralimbic activity during reversal learning in major depressive disorder and obsessive-compulsive disorder. *Psychological Medicine* 39, 1503–1518. doi: 10.1017/S0033291708005072

Reshetnikov, V. V., Ayriyants, K. A., Ryabushkina, Y. A., Sozonov, N. G., and Bondar, N. P. (2021). Sex-specific behavioral and structural alterations caused by early-life stress in C57BL/6 and BTBR mice. *Behav Brain Res* 414, 113489. doi: 10.1016/j.bbr.2021.113489

Réus, G. Z., Dos Santos, M. A. B., Abelaira, H. M., Ribeiro, K. F., Petronilho, F., Vuolo, F., et al. (2013). Imipramine reverses alterations in cytokines and BDNF levels induced by maternal deprivation in adult rats. *Behav Brain Res* 242, 40–46. doi: 10.1016/j.bbr.2012.11.044

Riba, A., Olier, M., Lacroix-Lamandé, S., Lencina, C., Bacquié, V., Harkat, C., et al. (2017). Paneth Cell Defects Induce Microbiota Dysbiosis in Mice and Promote Visceral Hypersensitivity. *Gastroenterology* 153, 1594-1606.e2. doi: 10.1053/j.gastro.2017.08.044

Riba, A., Olier, M., Lacroix-Lamandé, S., Lencina, C., Bacquié, V., Harkat, C., et al. (2018). Early life stress in mice is a suitable model for Irritable Bowel Syndrome but does not predispose to colitis nor increase susceptibility to enteric infections. *Brain Behav Immun* 73, 403–415. doi: 10.1016/j.bbi.2018.05.024

Rice, C. J., Sandman, C. A., Lenjavi, M. R., and Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology* 149, 4892–4900. doi: 10.1210/en.2008-0633

Roberts, A. W. (2005). G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors* 23, 33–41. doi: 10.1080/08977190500055836

Roberts, C., Sahakian, B. J., and Robbins, T. W. (2020). Psychological mechanisms and functions of 5-HT and SSRIs in potential therapeutic change: Lessons from the serotonergic modulation of action selection, learning, affect, and social cognition. *Neurosci Biobehav Rev* 119, 138–167. doi: 10.1016/j.neubiorev.2020.09.001

Roos, L. G., Levens, S. M., and Bennett, J. M. (2018). Stressful life events, relationship stressors, and cortisol reactivity: The moderating role of suppression. *Psychoneuroendocrinology* 89, 69–77. doi: 10.1016/j.psyneuen.2017.12.026

Roozendaal, B., McEwen, B. S., and Chattarji, S. (2009). Stress, memory and the amygdala. *Nat Rev Neurosci* 10, 423–433. doi: 10.1038/nrn2651

Roque, A., Ochoa-Zarzosa, A., and Torner, L. (2016). Maternal separation activates microglial cells and induces an inflammatory response in the hippocampus of male rat pups, independently of hypothalamic and peripheral cytokine levels. *Brain Behav Immun* 55, 39–48. doi: 10.1016/j.bbi.2015.09.017

Roque, S., Mesquita, A. R., Palha, J. A., Sousa, N., and Correia-Neves, M. (2014). The behavioral and immunological impact of maternal separation: a matter of timing. *Front Behav Neurosci* 8, 192. doi: 10.3389/fnbeh.2014.00192

Rorden, C., and Brett, M. (2000). Stereotaxic display of brain lesions. *Behav Neurol* 12, 191–200. doi: 10.1155/2000/421719

Rosenkranz, J. A., Moore, H., and Grace, A. A. (2003). The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *J. Neurosci.* 23, 11054–11064.

Rüedi-Bettschen, D., Pedersen, E.-M., Feldon, J., and Pryce, C. R. (2005). Early deprivation under specific conditions leads to reduced interest in reward in adulthood in Wistar rats. *Behav Brain Res* 156, 297–310. doi: 10.1016/j.bbr.2004.06.001

Rüedi-Bettschen, D., Zhang, W., Russig, H., Ferger, B., Weston, A., Pedersen, E.-M., et al. (2006). Early deprivation leads to altered behavioural, autonomic and endocrine responses to environmental challenge in adult Fischer rats. *Eur J Neurosci* 24, 2879–2893. doi: 10.1111/j.1460-9568.2006.05158.x

Rygula, R., Clarke, H. F., Cardinal, R. N., Cockcroft, G. J., Xia, J., Dalley, J. W., et al. (2015). Role of Central Serotonin in Anticipation of Rewarding and Punishing Outcomes: Effects of Selective Amygdala or Orbitofrontal 5-HT Depletion. *Cereb Cortex* 25, 3064–3076. doi: 10.1093/cercor/bhu102

Saavedra, L. M., Fenton Navarro, B., and Torner, L. (2017). Early Life Stress Activates Glial Cells in the Hippocampus but Attenuates Cytokine Secretion in Response to an Immune Challenge in Rat Pups. *Neuroimmunomodulation* 24, 242–255. doi: 10.1159/000485383

Sabat, R., Grütz, G., Warszawska, K., Kirsch, S., Witte, E., Wolk, K., et al. (2010). Biology of interleukin-10. *Cytokine Growth Factor Rev* 21, 331–344. doi: 10.1016/j.cytogfr.2010.09.002

Salamone, J. D., Yohn, S. E., López-Cruz, L., San Miguel, N., and Correa, M. (2016). Activational and effort-related aspects of motivation: neural mechanisms and implications for psychopathology. *Brain* 139, 1325–1347. doi: 10.1093/brain/aww050

Salzberg, M., Kumar, G., Supit, L., Jones, N. C., Morris, M. J., Rees, S., et al. (2007). Early postnatal stress confers enduring vulnerability to limbic epileptogenesis. *Epilepsia* 48, 2079–2085. doi: 10.1111/j.1528-1167.2007.01246.x

Sanders, B. J., and Anticevic, A. (2007). Maternal separation enhances neuronal activation and cardiovascular responses to acute stress in borderline hypertensive rats. *Behav Brain Res* 183, 25–30. doi: 10.1016/j.bbr.2007.05.020

Sanz, H., Aponte, J. J., Harezlak, J., Dong, Y., Ayestaran, A., Nhabomba, A., et al. (2017). drLumi: An open-source package to manage data, calibrate, and conduct quality control of multiplex bead-based immunoassays data analysis. *PLoS ONE* 12, e0187901. doi: 10.1371/journal.pone.0187901

Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev* 7, 284–301. doi: 10.1210/edrv-7-3-284

Sarkar, A., and Kabbaj, M. (2016). Sex Differences in Effects of Ketamine on Behavior, Spine Density, and Synaptic Proteins in Socially Isolated Rats. *Biol Psychiatry* 80, 448–456. doi: 10.1016/j.biopsych.2015.12.025

Scheggi, S., Pelliccia, T., Gambarana, C., and De Montis, M. G. (2018). Aripiprazole relieves motivational anhedonia in rats. *Journal of Affective Disorders* 227, 192–197. doi: 10.1016/j.jad.2017.10.032

Schienle, A., Ebner, F., and Schäfer, A. (2011). Localized gray matter volume abnormalities in generalized anxiety disorder. *Eur Arch Psychiatry Clin Neurosci* 261, 303–307. doi: 10.1007/s00406-010-0147-5

Schmaal, L., Veltman, D. J., van Erp, T. G. M., Sämann, P. G., Frodl, T., Jahanshad, N., et al. (2016). Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* 21, 806–812. doi: 10.1038/mp.2015.69

Schmahl, C. G., Vermetten, E., Elzinga, B. M., and Douglas Bremner, J. (2003). Magnetic resonance imaging of hippocampal and amygdala volume in women with childhood abuse and borderline personality disorder. *Psychiatry Research: Neuroimaging* 122, 193–198. doi: 10.1016/S0925-4927(03)00023-4

Schmalbach, I., Herhaus, B., Pässler, S., Runst, S., Berth, H., Wolff-Stephan, S., et al. (2020). Cortisol reactivity in patients with anorexia nervosa after stress induction. *Transl Psychiatry* 10, 275. doi: 10.1038/s41398-020-00955-7

Schmidt, M., Enthoven, L., van Woezik, J. H. G., Levine, S., Kloet, E. R. de, and Oitzl, M. S. (2004). The dynamics of the hypothalamic-pituitary-adrenal axis during maternal deprivation. *J Neuroendocrinol* 16, 52–57. doi: 10.1111/j.1365-2826.2004.01123.x

Schmidt, M. V., Levine, S., Alam, S., Harbich, D., Sterlemann, V., Ganea, K., et al. (2006). Metabolic signals modulate hypothalamic-pituitary-adrenal axis activation during maternal separation of the neonatal mouse. *J Neuroendocrinol* 18, 865–874. doi: 10.1111/j.1365-2826.2006.01482.x

Schmidt, M. V., Wang, X.-D., and Meijer, O. C. (2011). Early life stress paradigms in rodents: potential animal models of depression? *Psychopharmacology (Berl)* 214, 131–140. doi: 10.1007/s00213-010-2096-0

Schwarz, A. J., Danckaert, A., Reese, T., Gozzi, A., Paxinos, G., Watson, C., et al. (2006). A stereotaxic MRI template set for the rat brain with tissue class distribution maps and co-registered anatomical atlas: application to pharmacological MRI. *NeuroImage* 32, 538–550. doi: 10.1016/j.neuroimage.2006.04.214

Sedlak, A. J., Mettenburg, J., Basena, M., Petta, I., McPherson, K., Greene, A., et al. (2010). *Fourth National Incidence Study of Child Abuse and Neglect (NIS–4): Report to Congress*.

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., and Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol* 106-107, 1–16. doi: 10.1016/j.pneurobio.2013.04.001

Senn, S. (2006). Change from baseline and analysis of covariance revisited. *Stat Med* 25, 4334–4344. doi: 10.1002/sim.2682

Shalev, U., and Kafkafi, N. (2002). Repeated maternal separation does not alter sucrose-reinforced and open-field behaviors. *Pharmacol Biochem Behav* 73, 115–122.

Shao, L., Liu, Y., Xiao, J., Wang, Q., Liu, F., and Ding, J. (2019). Activating metabotropic glutamate receptor-7 attenuates visceral hypersensitivity in neonatal maternally separated rats. *Int J Mol Med* 43, 761–770. doi: 10.3892/ijmm.2018.4022

Shekhar, A., Truitt, W., Rainnie, D., and Sajdyk, T. (2005). Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. *Stress* 8, 209–219. doi: 10.1080/10253890500504557

Sheline, Y. I., Price, J. L., Yan, Z., and Mintun, M. A. (2010). Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. *Proc Natl Acad Sci U S A* 107, 11020–11025. doi: 10.1073/pnas.1000446107

Shi, R., Sharpe, L., and Abbott, M. (2019). A meta-analysis of the relationship between anxiety and attentional control. *Clin Psychol Rev* 72, 101754. doi: 10.1016/j.cpr.2019.101754

Shu, C., Xiao, L., Tang, J., Wang, G., Zhang, X., and Wang, X. (2015). Blunted behavioral and molecular responses to chronic mild stress in adult rats with experience of infancy maternal separation. *Tohoku J Exp Med* 235, 81–87. doi: 10.1620/tjem.235.81

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., et al. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage* 23 Suppl 1, S208-19. doi: 10.1016/j.neuroimage.2004.07.051

Sonino, N., Fallo, F., and Fava, G. A. (2010). Psychosomatic aspects of Cushing's syndrome. *Rev Endocr Metab Disord* 11, 95–104. doi: 10.1007/s11154-009-9123-7

Spampinato, M. V., Wood, J. N., Simone, V. de, and Grafman, J. (2009). Neural correlates of anxiety in healthy volunteers: a voxel-based morphometry study. *J Neuropsychiatry Clin Neurosci* 21, 199–205. doi: 10.1176/jnp.2009.21.2.199

Spierling, S. R., Mattock, M., and Zorrilla, E. P. (2017). Modeling hypohedonia following repeated social defeat: Individual vulnerability and dopaminergic involvement. *Physiol Behav* 177, 99–106. doi: 10.1016/j.physbeh.2017.04.016

Spinhoven, P., Elzinga, B. M., Hovens, J. G. F. M., Roelofs, K., Zitman, F. G., van Oppen, P., et al. (2010). The specificity of childhood adversities and negative life events across the life span to anxiety and depressive disorders. *Journal of Affective Disorders* 126, 103–112. doi: 10.1016/j.jad.2010.02.132

Stedenfeld, K. A., Clinton, S. M., Kerman, I. A., Akil, H., Watson, S. J., and Sved, A. F. (2011). Novelty-seeking behavior predicts vulnerability in a rodent model of depression. *Physiol Behav* 103, 210–216. doi: 10.1016/j.physbeh.2011.02.001

Steptoe, A., and Kivimäki, M. (2012). Stress and cardiovascular disease. *Nat Rev Cardiol* 9, 360–370. doi: 10.1038/nrcardio.2012.45

Steuwe, C., Daniels, J. K., Frewen, P. A., Densmore, M., Theberge, J., and Lanius, R. A. (2015). Effect of direct eye contact in women with PTSD related to interpersonal trauma: Psychophysiological interaction analysis of connectivity of an innate alarm system. *Psychiatry Research* 232, 162–167. doi: 10.1016/j.pscychresns.2015.02.010

Stikkelbroek, Y., Bodden, D. H. M., Kleinjan, M., Reijnders, M., and van Baar, A. L. (2016). Adolescent Depression and Negative Life Events, the Mediating Role of Cognitive Emotion Regulation. *PLoS ONE* 11, e0161062. doi: 10.1371/journal.pone.0161062

Strowig, T., Henao-Mejia, J., Elinav, E., and Flavell, R. (2012). Inflammasomes in health and disease. *Nature* 481, 278–286. doi: 10.1038/nature10759

Stuart, S. A., Hinchcliffe, J. K., and Robinson, E. S. J. (2019). Evidence that neuropsychological deficits following early life adversity may underlie vulnerability to depression. *Neuropsychopharmacology* 44, 1623–1630. doi: 10.1038/s41386-019-0388-6

Suor, J. H., Jimmy, J., Monk, C. S., Phan, K. L., and Burkhouse, K. L. (2020). Parsing differences in amygdala volume among individuals with and without social and generalized anxiety disorders across the lifespan. *Journal of Psychiatric Research* 128, 83–89. doi: 10.1016/j.jpsychires.2020.05.027

Swiecicki, L., Zatorski, P., Bzinkowska, D., Sienkiewicz-Jarosz, H., Szyndler, J., and Scinska, A. (2009). Gustatory and olfactory function in patients with unipolar and bipolar depression. *Prog Neuropsychopharmacol Biol Psychiatry* 33, 827–834. doi: 10.1016/j.pnpbp.2009.03.030

Szabó, C., Németh, A., and Kéri, S. (2013). Ethical sensitivity in obsessive-compulsive disorder and generalized anxiety disorder: the role of reversal learning. *J Behav Ther Exp Psychiatry* 44, 404–410. doi: 10.1016/j.jbtep.2013.04.001

Tait Wojno, E. D., Hunter, C. A., and Stumhofer, J. S. (2019). The Immunobiology of the Interleukin-12 Family: Room for Discovery. *Immunity* 50, 851–870. doi: 10.1016/j.immuni.2019.03.011

Takiguchi, S., Fujisawa, T. X., Mizushima, S., Saito, D. N., Okamoto, Y., Shimada, K., et al. (2015). Ventral striatum dysfunction in children and adolescents with reactive attachment disorder: functional MRI study. *BJPsych Open* 1, 121–128. doi: 10.1192/bjpo.bp.115.001586

Tang, H.-L., Zhang, G., Ji, N.-N., Du, L., Chen, B.-B., Hua, R., et al. (2017). Toll-Like Receptor 4 in Paraventricular Nucleus Mediates Visceral Hypersensitivity Induced by Maternal Separation. *Front Pharmacol* 8, 309. doi: 10.3389/fphar.2017.00309

Taylor Tavares, J. V., Clark, L., Furey, M. L., Williams, G. B., Sahakian, B. J., and Drevets, W. C. (2008). Neural basis of abnormal response to negative feedback in unmedicated mood disorders. *NeuroImage* 42, 1118–1126. doi: 10.1016/j.neuroimage.2008.05.049

Teicher, M. H., and Samson, J. A. (2013). Childhood maltreatment and psychopathology: A case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. *AJP* 170, 1114–1133. doi: 10.1176/appi.ajp.2013.12070957

Teicher, M. H., and Samson, J. A. (2016). Annual Research Review: Enduring neurobiological effects of childhood abuse and neglect. *J Child Psychol Psychiatry* 57, 241–266. doi: 10.1111/jcpp.12507

Teicher, M. H., Samson, J. A., Anderson, C. M., and Ohashi, K. (2016). The effects of childhood maltreatment on brain structure, function and connectivity. *Nat Rev Neurosci* 17, 652–666. doi: 10.1038/nrn.2016.111

Thomas, A. W., Delevich, K., Chang, I., and Wilbrecht, L. (2020). Variation in early life maternal care predicts later long range frontal cortex synapse development in mice. *Dev Cogn Neurosci* 41, 100737. doi: 10.1016/j.dcn.2019.100737

Thornton, J. L., Everett, N. A., Webb, P., Turner, A. J., Cornish, J. L., and Baracz, S. J. (2021). Adolescent oxytocin administration reduces depression-like behaviour induced by

early life stress in adult male and female rats. *Prog Neuropsychopharmacol Biol Psychiatry*, 110279. doi: 10.1016/j.pnpbp.2021.110279

Tomoda, A., Navalta, C. P., Polcari, A., Sadato, N., and Teicher, M. H. (2009). Childhood sexual abuse is associated with reduced gray matter volume in visual cortex of young women. *Biol Psychiatry* 66, 642–648. doi: 10.1016/j.biopsych.2009.04.021

Tomoda, A., Polcari, A., Anderson, C. M., and Teicher, M. H. (2012). Reduced visual cortex gray matter volume and thickness in young adults who witnessed domestic violence during childhood. *PLoS ONE* 7, e52528. doi: 10.1371/journal.pone.0052528

Tottenham, N., Hare, T. A., Quinn, B. T., McCarry, T. W., Nurse, M., Gilhooly, T., et al. (2010). Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci* 13, 46–61. doi: 10.1111/j.1467-7687.2009.00852.x

Tournier, J.-D., Smith, R., Raffelt, D., Tabbara, R., Dhollander, T., Pietsch, M., et al. (2019). MRtrix3: A fast, flexible and open software framework for medical image processing and visualisation. *NeuroImage* 202, 116137. doi: 10.1016/j.neuroimage.2019.116137

Tractenberg, S. G., Levandowski, M. L., Azeredo, L. A. de, Orso, R., Roithmann, L. G., Hoffmann, E. S., et al. (2016). An overview of maternal separation effects on behavioural outcomes in mice: Evidence from a four-stage methodological systematic review. *Neurosci Biobehav Rev* 68, 489–503. doi: 10.1016/j.neubiorev.2016.06.021

Treadway, M. T., Bossaller, N. A., Shelton, R. C., and Zald, D. H. (2012). Effort-based decision-making in major depressive disorder: a translational model of motivational anhedonia. *J Abnorm Psychol* 121, 553–558. doi: 10.1037/a0028813

Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E., and Zald, D. H. (2009). Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS ONE* 4, e6598. doi: 10.1371/journal.pone.0006598

Treadway, M. T., Cooper, J. A., and Miller, A. H. (2019). Can't or Won't? Immunometabolic Constraints on Dopaminergic Drive. *Trends in Cognitive Sciences* 23, 435–448. doi: 10.1016/j.tics.2019.03.003

Treadway, M. T., and Zald, D. H. (2011). Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neurosci Biobehav Rev* 35, 537–555. doi: 10.1016/j.neubiorev.2010.06.006

Trent, L., Stander, V., Thomsen, C., and Merrill, L. (2007). Alcohol abuse among U.S. Navy recruits who were maltreated in childhood. *Alcohol Alcohol* 42, 370–375. doi: 10.1093/alcalc/agm036

Trew, J. L. (2011). Exploring the roles of approach and avoidance in depression: an integrative model. *Clin Psychol Rev* 31, 1156–1168. doi: 10.1016/j.cpr.2011.07.007

Tucker, L. B., and McCabe, J. T. (2017). Behavior of Male and Female C57BL/6J Mice Is More Consistent with Repeated Trials in the Elevated Zero Maze than in the Elevated Plus Maze. *Front Behav Neurosci* 11, 13. doi: 10.3389/fnbeh.2017.00013

Turner, P. V., Brabb, T., Pekow, C., and Vasbinder, M. A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. *J Am Assoc Lab Anim Sci* 50, 600–613.

Tustison, N. J., Avants, B. B., Cook, P. A., Zheng, Y., Egan, A., Yushkevich, P. A., et al. (2010). N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging* 29, 1310–1320. doi: 10.1109/TMI.2010.2046908

Tustison, N. J., Cook, P. A., Klein, A., Song, G., Das, S. R., Duda, J. T., et al. (2014). Large-scale evaluation of ANTs and FreeSurfer cortical thickness measurements. *NeuroImage* 99, 166–179. doi: 10.1016/j.neuroimage.2014.05.044

U.S. Department of Health and Human Services (2021). *Child Maltreatment 2019*.

Ulrich-Lai, Y. M., and Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* 10, 397–409. doi: 10.1038/nrn2647

Vachon, D. D., Krueger, R. F., Rogosch, F. A., and Cicchetti, D. (2015). Assessment of the Harmful Psychiatric and Behavioral Effects of Different Forms of Child Maltreatment. *JAMA Psychiatry* 72, 1135–1142. doi: 10.1001/jamapsychiatry.2015.1792

Valassi, E., Crespo, I., Santos, A., and Webb, S. M. (2012). Clinical consequences of Cushing's syndrome. *Pituitary* 15, 319–329. doi: 10.1007/s11102-012-0394-8

Valdés-Hernández, P. A., Sumiyoshi, A., Nonaka, H., Haga, R., Aubert-Vásquez, E., Ogawa, T., et al. (2011). An in vivo MRI Template Set for Morphometry, Tissue Segmentation, and fMRI Localization in Rats. *Front Neuroinform* 5, 26. doi: 10.3389/fninf.2011.00026

Van Bodegom, M., Homberg, J. R., and Henckens, M. J. A. G. (2017). Modulation of the Hypothalamic-Pituitary-Adrenal Axis by Early Life Stress Exposure. *Front Cell Neurosci* 11, 87. doi: 10.3389/fncel.2017.00087

van Dam, N. T., Rando, K., Potenza, M. N., Tuit, K., and Sinha, R. (2014). Childhood maltreatment, altered limbic neurobiology, and substance use relapse severity via trauma-

specific reductions in limbic gray matter volume. *JAMA Psychiatry* 71, 917–925. doi: 10.1001/jamapsychiatry.2014.680

van Eijndhoven, P., van Wingen, G., van Oijen, K., Rijpkema, M., Goraj, B., Jan Verkes, R., et al. (2009). Amygdala volume marks the acute state in the early course of depression. *Biol Psychiatry* 65, 812–818. doi: 10.1016/j.biopsych.2008.10.027

van Harmelen, A.-L., van Tol, M.-J., van der Wee, N. J. A., Veltman, D. J., Aleman, A., Spinhoven, P., et al. (2010). Reduced medial prefrontal cortex volume in adults reporting childhood emotional maltreatment. *Biol Psychiatry* 68, 832–838. doi: 10.1016/j.biopsych.2010.06.011

van Rooij, S. J. H., Smith, R. D., Stenson, A. F., Ely, T. D., Yang, X., Tottenham, N., et al. (2020). Increased activation of the fear neurocircuitry in children exposed to violence. *Depression and Anxiety* 37, 303–312. doi: 10.1002/da.22994

Vannan, A., Powell, G. L., Scott, S. N., Pagni, B. A., and Neisewander, J. L. (2018). Animal Models of the Impact of Social Stress on Cocaine Use Disorders. *Int Rev Neurobiol* 140, 131–169. doi: 10.1016/bs.irn.2018.07.005

Varga, J., Domokos, A., Barna, I., Jankord, R., Bagdy, G., and Zelena, D. (2011). Lack of vasopressin does not prevent the behavioural and endocrine changes induced by chronic unpredictable stress. *Brain Res Bull* 84, 45–52. doi: 10.1016/j.brainresbull.2010.09.014

Vasconcelos, M., Stein, D. J., and Almeida, R. M. M. de (2015). Social defeat protocol and relevant biomarkers, implications for stress response physiology, drug abuse, mood disorders and individual stress vulnerability: a systematic review of the last decade. *Trends Psychiatry Psychother* 37, 51–66. doi: 10.1590/2237-6089-2014-0034

Vaughan, D., and Dancho, M. (2021). furrr: Apply Mapping Functions in Parallel using Futures, https://CRAN.R-project.org/package=furrr

Veena, J., Srikumar, B. N., Raju, T. R., and Shankaranarayana Rao, B. S. (2009). Exposure to enriched environment restores the survival and differentiation of new born cells in the hippocampus and ameliorates depressive symptoms in chronically stressed rats. *Neurosci Lett* 455, 178–182. doi: 10.1016/j.neulet.2009.03.059

Veenema, A. H., Reber, S. O., Selch, S., Obermeier, F., and Neumann, I. D. (2008). Early life stress enhances the vulnerability to chronic psychosocial stress and experimental colitis in adult mice. *Endocrinology* 149, 2727–2736. doi: 10.1210/en.2007-1469

Veer, I. M., Beckmann, C. F., van Tol, M.-J., Ferrarini, L., Milles, J., Veltman, D. J., et al. (2010). Whole brain resting-state analysis reveals decreased functional connectivity in major depression. *Front Syst Neurosci* 4. doi: 10.3389/fnsys.2010.00041

Vermetten, E., Schmahl, C., Lindner, S., Loewenstein, R. J., and Bremner, J. D. (2006). Hippocampal and amygdalar volumes in dissociative identity disorder. *AJP* 163, 630–636. doi: 10.1176/ajp.2006.163.4.630

Vig, R., Gordon, J. R., Thébaud, B., Befus, A. D., and Vliagoftis, H. (2010). The effect of early-life stress on airway inflammation in adult mice. *Neuroimmunomodulation* 17, 229–239. doi: 10.1159/000290039

Viola, T. W., Creutzberg, K. C., Zaparte, A., Kestering-Ferreira, É., Tractenberg, S. G., Centeno-Silva, A., et al. (2019). Acute neuroinflammation elicited by TLR-3 systemic activation combined with early life stress induces working memory impairments in male adolescent mice. *Behav Brain Res* 376, 112221. doi: 10.1016/j.bbr.2019.112221

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., and Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci* 22, 6810–6818.

Walf, A. A., and Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2, 322–328. doi: 10.1038/nprot.2007.44

Wallace, D. L., Han, M.-H., Graham, D. L., Green, T. A., Vialou, V., Iñiguez, S. D., et al. (2009). CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. *Nat Neurosci* 12, 200–209. doi: 10.1038/nn.2257

Wan, F. (2021). Statistical analysis of two arm randomized pre-post designs with one post-treatment measurement. *BMC Med Res Methodol* 21, 150. doi: 10.1186/s12874-021-01323-9

Wang, Q., Dong, X., Wang, Y., Liu, M., Sun, A., Li, N., et al. (2017). Adolescent escitalopram prevents the effects of maternal separation on depression- and anxiety-like behaviours and regulates the levels of inflammatory cytokines in adult male mice. *Int J Dev Neurosci* 62, 37–45. doi: 10.1016/j.ijdevneu.2017.07.007

Waraczynski, M. (2016). Toward a systems-oriented approach to the role of the extended amygdala in adaptive responding. *Neurosci Biobehav Rev* 68, 177–194. doi: 10.1016/j.neubiorev.2016.05.015

Weber, M. D., Frank, M. G., Tracey, K. J., Watkins, L. R., and Maier, S. F. (2015). Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male Sprague Dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome. *J Neurosci* 35, 316–324. doi: 10.1523/JNEUROSCI.3561-14.2015

Weber, M. D., Godbout, J. P., and Sheridan, J. F. (2017). Repeated Social Defeat, Neuroinflammation, and Behavior: Monocytes Carry the Signal. *Neuropsychopharmacology* 42, 46–61. doi: 10.1038/npp.2016.102

Wegman, H. L., and Stetler, C. (2009). A meta-analytic review of the effects of childhood abuse on medical outcomes in adulthood. *Psychosom Med* 71, 805–812. doi: 10.1097/PSY.0b013e3181bb2b46

Wei, T., and Simko, V. (2017). R package "corrplot": Visualization of a Correlation Matrix, https://github.com/taiyun/corrplot

Wei, Y., Wang, G., Wang, H., He, J., Zhang, N., Wu, Z., et al. (2018). Sex-dependent impact of different degrees of maternal separation experience on OFT behavioral performances after adult chronic unpredictable mild stress exposure in rats. *Physiol Behav* 194, 153–161. doi: 10.1016/j.physbeh.2018.04.034

Weissman, D. G., Bitran, D., Miller, A. B., Schaefer, J. D., Sheridan, M. A., and McLaughlin, K. A. (2019). Difficulties with emotion regulation as a transdiagnostic mechanism linking child maltreatment with the emergence of psychopathology. *Dev Psychopathol* 31, 899–915. doi: 10.1017/S0954579419000348

Westenbroek, C., Snijders, T. A. B., Den Boer, J. A., Gerrits, M., Fokkema, D. S., and Ter Horst, G. J. (2005). Pair-housing of male and female rats during chronic stress exposure results in gender-specific behavioral responses. *Horm Behav* 47, 620–628. doi: 10.1016/j.yhbeh.2005.01.004

Whittle, S., Dennison, M., Vijayakumar, N., Simmons, J. G., Yücel, M., Lubman, D. I., et al. (2013). Childhood maltreatment and psychopathology affect brain development during adolescence. *J Am Acad Child Adolesc Psychiatry* 52, 940-952.e1. doi: 10.1016/j.jaac.2013.06.007

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wickham, H. (2019). stringr: Simple, Consistent Wrappers for Common String Operations, https://CRAN.R-project.org/package=stringr

Wickham, H. (2021). tidyr: Tidy Messy Data, https://CRAN.R-project.org/package=tidyr

Wickham, H., François, R., Henry, L., and Müller, K. (2021). dplyr: A Grammar of Data Manipulation, https://CRAN.R-project.org/package=dplyr

Wieck, A., Andersen, S. L., and Brenhouse, H. C. (2013). Evidence for a neuroinflammatory mechanism in delayed effects of early life adversity in rats: relationship to cortical NMDA receptor expression. *Brain Behav Immun* 28, 218–226. doi: 10.1016/j.bbi.2012.11.012

Wohleb, E. S., Fenn, A. M., Pacenta, A. M., Powell, N. D., Sheridan, J. F., and Godbout, J. P. (2012). Peripheral innate immune challenge exaggerated microglia activation, increased the number of inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice. *Psychoneuroendocrinology* 37, 1491–1505. doi: 10.1016/j.psyneuen.2012.02.003

Wolff, S. D., and Balaban, R. S. (1994). Magnetization transfer imaging: practical aspects and clinical applications. *Radiology* 192, 593–599. doi: 10.1148/radiology.192.3.8058919

Wright, M. O., Crawford, E., and Del Castillo, D. (2009). Childhood emotional maltreatment and later psychological distress among college students: the mediating role of maladaptive schemas. *Child Abuse Negl* 33, 59–68. doi: 10.1016/j.chiabu.2008.12.007

Wynn, T. A. (2003). IL-13 effector functions. *Annu Rev Immunol* 21, 425–456. doi: 10.1146/annurev.immunol.21.120601.141142

Xia, L., Xu, P., Yang, Z., Gu, R., and Zhang, D. (2021). Impaired probabilistic reversal learning in anxiety: Evidence from behavioral and ERP findings. *Neuroimage Clin* 31, 102751. doi: 10.1016/j.nicl.2021.102751

Yang, M.-S., Park, E. J., Sohn, S., Kwon, H. J., Shin, W.-H., Pyo, H. K., et al. (2002). Interleukin-13 and -4 induce death of activated microglia. *Glia* 38, 273–280. doi: 10.1002/glia.10057

Yang, X.-H., Huang, J., Zhu, C.-Y., Wang, Y.-F., Cheung, E. F. C., Chan, R. C. K., et al. (2014). Motivational deficits in effort-based decision making in individuals with subsyndromal depression, first-episode and remitted depression patients. *Psychiatry Research* 220, 874–882. doi: 10.1016/j.psychres.2014.08.056

Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C., et al. (2006). User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *NeuroImage* 31, 1116–1128. doi: 10.1016/j.neuroimage.2006.01.015

Zajdel, J., Zager, A., Blomqvist, A., Engblom, D., and Shionoya, K. (2019). Acute maternal separation potentiates the gene expression and corticosterone response induced by inflammation. *Brain Behav Immun* 77, 141–149. doi: 10.1016/j.bbi.2018.12.016

Zammit, S., Allebeck, P., David, A. S., Dalman, C., Hemmingsson, T., Lundberg, I., et al. (2004). A longitudinal study of premorbid IQ Score and risk of developing schizophrenia, bipolar disorder, severe depression, and other nonaffective psychoses. *Archives of General Psychiatry* 61, 354–360. doi: 10.1001/archpsyc.61.4.354

Zeng, L.-L., Shen, H., Liu, L., Wang, L., Li, B., Fang, P., et al. (2012). Identifying major depression using whole-brain functional connectivity: a multivariate pattern analysis. *Brain* 135, 1498–1507. doi: 10.1093/brain/aws059

Zhang, A., Price, J. L., Leonard, D., North, C. S., Suris, A., Javors, M. A., et al. (2020). Alcohol Use Disorder Masks the Effects of Childhood Adversity, Lifetime Trauma, and Chronic Stress on Hypothalamic-Pituitary-Adrenal Axis Reactivity. *Alcohol Clin Exp Res* 44, 1192–1203. doi: 10.1111/acer.14334

Zhang, X., Wang, B., Jin, J., An, S., Zeng, Q., Duan, Y., et al. (2014). Early deprivation reduced anxiety and enhanced memory in adult male rats. *Brain Res Bull* 108, 44–50. doi: 10.1016/j.brainresbull.2014.08.005

Zhang, X. Y., Sanchez, H., Kehoe, P., and Kosten, T. A. (2005). Neonatal isolation enhances maintenance but not reinstatement of cocaine self-administration in adult male rats. *Psychopharmacology (Berl)* 177, 391–399. doi: 10.1007/s00213-004-1963-y

Zhang, Y., Zhu, X., Bai, M., Zhang, L., Xue, L., and Yi, J. (2013). Maternal deprivation enhances behavioral vulnerability to stress associated with miR-504 expression in nucleus accumbens of rats. *PLoS ONE* 8, e69934. doi: 10.1371/journal.pone.0069934

Zhao, Y., Wang, Z., Dai, J., Chen, L., Huang, Y., and Zhan, Z. (2012). Beneficial effects of benzodiazepine diazepam on chronic stress-induced impairment of hippocampal structural plasticity and depression-like behavior in mice. *Behav Brain Res* 228, 339–350. doi: 10.1016/j.bbr.2011.12.013

Zhu, Y., Wang, Y., Yao, R., Hao, T., Cao, J., Huang, H., et al. (2017). Enhanced neuroinflammation mediated by DNA methylation of the glucocorticoid receptor triggers cognitive dysfunction after sevoflurane anesthesia in adult rats subjected to maternal separation during the neonatal period. *Journal of Neuroinflammation* 14, 6. doi: 10.1186/s12974-016-0782-5