

**Novel behavioural and
psychopharmacological strategies for
the prevention and treatment of
aversive memories in men and women**

By

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I, Vanessa Elizabeth Hennessy, confirm that the work presented in this thesis is my own. Where information has derived from other sources, I confirm that this has been indicated herein.

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Abstract

This thesis explores the interaction between physiological stress responses and aversive memory performance following trauma, with a focus on epigenetic and molecular targets for novel interventions to attenuate intrusive, sensory-perceptual memories—a core symptom of post-traumatic stress disorder (PTSD) (*Chapter 1, Section 1.1*). Given that trauma exposure alone is not always sufficient for PTSD development, sex as a biological variable is considered as a key factor influencing stress responses and effectiveness of aversive memory modification strategies (*Section 1.2*). Research paradigms and methodologies employed in subsequent experimental chapters are outlined in *Section 1.3*. *Chapter 2* examines novel psychopharmacological and behavioural strategies using a double-blind, randomised design (N=80; 40 women). The histone deacetylase inhibitor (HDACi) Sodium Valproate (400mg) or placebo was administered before retrieval + extinction or extinction alone of conditioned aversive (phobic) memory. HDACi facilitated (re)extinction but did not prevent the return of fear responses under challenge, with post-hoc analyses suggesting sex-dependent effects. *Chapter 3* investigates the impact of post-trauma hydrocortisone (30mg) administration on intrusive memories in healthy men (N=60) and women (N=59). Hydrocortisone accelerated intrusion reduction over following week, with sex x baseline hormone interactions with hydrocortisone treatment modulating frequency effects. *Chapter 4* explores mineralocorticoid receptor (MR) blockade using spironolactone (400mg) before analogue trauma in healthy men (N=78). While spironolactone had no effect on intrusion frequency, it significantly reduced the distress and vividness of intrusions. Notably, MR inhibition was testosterone-dependent with higher pre-stress levels resulting in reduced intrusion frequency, indicating a possible sex hormone interaction. *Chapter 5* summarises these

findings, integrating them within existing literature and discussing their clinical implications. Suggestions for future research are made aimed towards personalised psychiatric treatments and the development of empirically-based preventative strategies for stress-related disorders, targeting distressing, sensory-perceptual mnemonic symptoms.

Impact statement

Persistent, aversive intrusive memories are a core symptom of PTSD (DSM-IV); indeed, PTSD and phobia could be considered disorders of memory due to the intricate links between the physiological stress response and aversive memory processes. As of 2017 (Hiscox, 2020) no new pharmacological treatment specifically developed for PTSD had been developed (Bernardy and Friedman, 2017) and treatments that are available have varying effectiveness due to the heterogeneity of the symptomology of PTSD and of the populations involved (Hiscox 2023, Lewis 2020, Oosterbaan 2019). It is cautiously encouraging that *Chapter 2* showed that the use of a novel pharmacological cognitive enhancer the histone deacetylase inhibitor (Sodium Valproate, VPA) enhanced the extinction and re-extinction of conditioned fear, extending the limited prior evidence for VPA to enhance standard extinction and re-extinction of human implicit memory of conditioned fear responses in humans and rodents (Bredy and Barad, 2008; Kuriyama et al., 2011; Whittle and Singewald, 2014). VPA may therefore have the potential to enhance efficacy and tolerability of current exposure-based therapies although exploratory post hoc analysis suggests further research is needed to clarify potential sex dependent effects. *Chapters 3 and 4* add to the growing literature (Astill Wright et al., 2021) suggesting that moderating the acute glucocorticoid response either via upregulation of cortisol availability in the immediate aftermath (>6hrs) (*Chapter 3*) or via mineralocorticoid antagonism (*Chapter 4*) prior to anticipated trauma can attenuate either the frequency or affective quality of post trauma sensory perceptual aversive memories of the event. Exploratory analysis in the preventative studies here also highlights the importance of considering individual neuroendocrine status at the time of the event as a priori moderators of drug efficacy in future studies and clinical trials. Overall, the findings of

this thesis contribute to our understanding of the neurobiological and epigenetic mechanisms for resilience to threatening events and suggests targets for the further development of novel behavioural and psychopharmacological interventions for the treatment and prevention of persistent, aversive intrusive memory symptomology.

Chapters 3 and 4 were pre-specified and pre-registered prior to the commencement of data collection and analysis and all chapters followed open science practice guidelines where possible, with any exploratory analysis highlighted in the relevant chapters. Pre-registration details can be found on the Open Science Framework database. *Chapter 3* was pre-registered on 22nd May 2019 (<https://archive.org/details/osf-registrations-76yvk-v1>). *Chapter 4* (<https://osf.io/py9n5>) was pre-registered on 14 July 2021, and an addendum was posted on 13 August 2021(<https://osf.io/698q7/>). The latter described the rationale for removing one of the originally planned tasks and limiting data collection to men only due to COVID-19 restraints.

As per the UCL research paper declaration, the work in *Chapter 3* of this thesis gave rise to the following publication (Hennessy et al., 2022) and has been widely discussed in the public domain both in national newspapers (The Times, 22 October 2022, <https://www.thetimes.com/uk/healthcare/article/taking-stress-hormone-tablet-after-trauma-could-ease-impact-of-ptsd-tzzhqslxh>) and at international (ECNP, 2023) and national (BAP, 2023, awarded pre-clinical poster prize) conferences.

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Accelerated forgetting of a trauma-like event in healthy men and women after a single dose of hydrocortisone

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Epigenetic regulation and updating of memory in a model of anxiety disorder in healthy men and women.

(b) Has the manuscript been uploaded to a preprint server e.g. medRxiv?

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Chapter 2

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List of Abbreviations

<i>ACTH</i>	adrenocorticotrophic hormone
<i>BDNF</i>	brain-derived neurotrophic factor
<i>BLA</i>	basolateral amygdala
<i>BNST</i>	bed nucleus of the stria terminalis
<i>cAMP</i>	adenosine 3', 5'-cyclic monophosphate
<i>CORT</i>	cortisol and corticosterone
<i>CPU</i>	Clinical Psychopharmacology Unit
<i>CREB</i>	cAMP -response-element binding protein
<i>CRH</i>	corticotropin-releasing hormone
<i>CS1</i>	conditioned stimulus paired with aversive stimulus
<i>CS2</i>	conditioned stimulus not paired with aversive stimulus
<i>DCS</i>	D-cycloserine
<i>DNA</i>	deoxyribonucleic acid
<i>EBR</i>	extreme behavioural response
<i>EEG</i>	Electroencephalographic
<i>ERK</i>	Extracellular signal-regulated kinase
<i>EXT</i>	Extinction learning
<i>fMRI</i>	functional magnetic resonance imaging
<i>GABA</i>	y-aminobutyric acid
<i>GC</i>	glucocorticoid
<i>GR</i>	glucocorticoid receptor

<i>HAT</i>	histone acetyltransferase
<i>HDAC</i>	histone deacetylase
<i>HDACi</i>	histone deacetylase inhibitor
<i>HC</i>	hydrocortisone
<i>HPA</i>	hypothalamic-pituitary-adrenal axis
<i>HPG</i>	hypothalamic-pituitary-gonadal axis
<i>LTP</i>	long term potentiation
<i>MAPK</i>	mitogen-activated protein kinase
<i>MBR</i>	minimal behavioural response
<i>mPFC</i>	medial prefrontal cortex
<i>MRI</i>	magnetic resonance imaging
<i>NMDA</i>	N-terminal transactivation domain
<i>NC</i>	naturally/free cycling
<i>OC</i>	oral/ hormone base contraceptives
<i>PRE</i>	reactivation prior to standard extinction
<i>PTSD</i>	post-traumatic stress disorder
<i>UCS</i>	unconditional stimulus
<i>VPA</i>	sodium valproate, histone deacetylase inhibitor
<i>Zif268</i>	zinc finger protein

Glossary

Consolidation	a process through which fragile nascent engrams are strengthened into long term memories that are more resistant to alteration/decay. In the early stages memories are encoded and consolidated in the hippocampus and then through system consolidation are dispersed to long term storage in neocortical brain regions over subsequent days and during sleep.
Epigenetics	changes made to the functioning of the genome, that does not change the DNA code itself and make up the epigenetic code, e.g., DNA methylation and histone modifications alter gene expression and subsequent protein synthesis.
Extinction	induces a gradual reduction in response to a stimulus if the stimulus is repeatedly presented in the absence of reinforcement and is assumed to result in the creation of a new inhibitory memory that suppresses the original stimulus-reinforcer contingency to alter stimulus-responding.
Engram	An engram refers to the biological substrate or neural representation of a memory trace and is therefore the physical manifestation of a memory within the brain, comprising the pattern of neuronal activity, synaptic modifications, within neural networks that collectively store the sensory, emotional, and cognitive components of an experience event.
Glucocorticoids	are steroid hormones, cortisol in humans and corticosterone in animals, they have wide CNS acting on two receptor types: <i>Glucocorticoid receptors (GR)</i>; GR are widely distributed in key fear and aversive memory brain regions. In coordination with mineralocorticoid receptor activity, they moderate basal and acute stress responses. GR activity is crucially involved in successful recovery post stress onset to ensure return to homeostasis. <i>Mineralocorticoid receptors (MR)</i>; MRs peripherally regulate electrolyte balances. In the brain MR activity is involved in rapid acute, responses to stress in co-activation with catecholamines and with GR activity. MR have lower affinity for GCs than GR

and moderate slower, tonic chronic changes in basal stress over longer time frames.

Genomic action of GR and MR activity; both receptor types have nuclear receptors that upon binding cortisol (GR) or aldosterone (MR) ligands translocate to the nucleus where they regulate gene expression, acting as moderators of gene transcription with cellular specificity determined by coregulator proteins.

Non-genomic action of GR and MR activity; under acute stress conditions both GC receptors have membrane and cytosol bound receptors that work at faster timescales than their genomic counterparts. Mineralocorticoid membrane bound receptor activity not requiring gene transcription allows initial rapid response to acute stress which is constrained by glucocorticoid membrane bound receptor activity due to differential distribution of these two receptor types across brain regions.

Gonadal hormones	are. commonly referred to as ‘sex’ hormones. The major gonadal hormones are progesterone, estradiol and testosterone. These are released from the gonads and are involved in reproduction processes but are also derived in the brain and released from the adrenals during stress with interactions between the stress and sex systems moderating neural plasticity and learning under stress.
Histone acetylases	HATs (‘writers’) are enzymes that alter gene expression without altering the DNA code itself. The addition of -actyl groups to histones creates an epigenetic code that influences protein synthesis and synaptic activity by acetylases loosening of condensed chromatin to regulate access to gene transcription machinery.
Histone deacetylases	HDACs (‘erasers’) reverse the actions of Histone acetylases (HATs) to recoil the DNA into its condensed form and halt gene transcription and protein synthesis.
HDACi	Histone deacetylase inhibitor, e.g. sodium valproate, prolongs the action of Histone acetylases (HATs) to upregulate gene transcription and protein synthesis and act in brain regions primed by neural activity
PTSD	Psychological disorder that can develop following exposure to a traumatic, often life-threatening, event resulting in persistent > 4weeks re-experiencing of the event, heightened arousal, distress and avoidance symptoms. Intrusive sensory-perceptual memories triggered by cues of the traumatic event are a core symptom.

Reactivation	When a memory is retrieved in such a way that it is transformed into a labile state; reactivation is required for the initiation of reconsolidation
Reconsolidation	A protein synthesis dependent memory process, similar by distinct to original consolidation, hypothesised to reflect the destabilizing of an engram into a labile state after which it requires restabilizing back to a stable state.
Reinstatement	is a term used to describe the return of a previously extinguished fear response due to the unexpected re-presentation of the trigger cue (unconditional stimulus, UCS). Together with renewal, encountering trigger cue in new context and spontaneous return of fear responding over time these are collectively referred to as return of fear effects (ROF) and experimentally can be used to determine modification strategy success or not.
Retrieval	when a memory is activated (recalled) to be accessible to guide behaviour. A process engaged during experimental procedures to initiate reactivation and destabilization of the memory to allow memory modification. Conditions under which either the retrieval or reactivation state is initiated are still to be determin

Chapter 1

General Introduction

Section 1.1 Aversive memory under stress

1.1.1 Overview of adaptive stress response and its moderation by glucocorticoids

A healthy stress response allows prompt, appropriate responding to both environmental and physiological threats followed by a swift return to the pre-stress condition (i.e. allostasis, (McEwen, 2008)). This dynamic system allows for reaction to a wide array of stressors while maintaining homeostasis and conserving resources that requires a tightly co-ordinated orchestration of two systems. When triggered the autonomic, sympathetic-adrenal response stimulates the rapid release of adrenaline (epinephrine) and increased nor-adrenaline from the adrenal medulla and this redirects blood flow, increases respiration and diverts glucose release (Schwabe et al., 2022) to prepare for flight or fight (Canon, 1929). Additionally, the slower, hormonal response involved in stress relies on the hypothalamic-pituitary-adrenal cortex (HPA) axis particularly the release of glucocorticoids (cortisol, in humans, corticosterone in rodents) from the adrenal cortex whose role enhances the actions of the initial sympathetic-adrenal and is heavily involved in subsequently suppressing the initial activity, preventing an over extended response (Sapolsky et al., 2000) and in preparing the system for future stressors (see Figure 1.1.1). Not all stressors trigger precisely the same physiological response as this will depend on many factors (section 1.2) such as duration and type of stressor as well as previous experience, appraisal and emotional factors (Lazarus, 1995), and genetic and sex differences (Qureshi and Mehler, 2010).

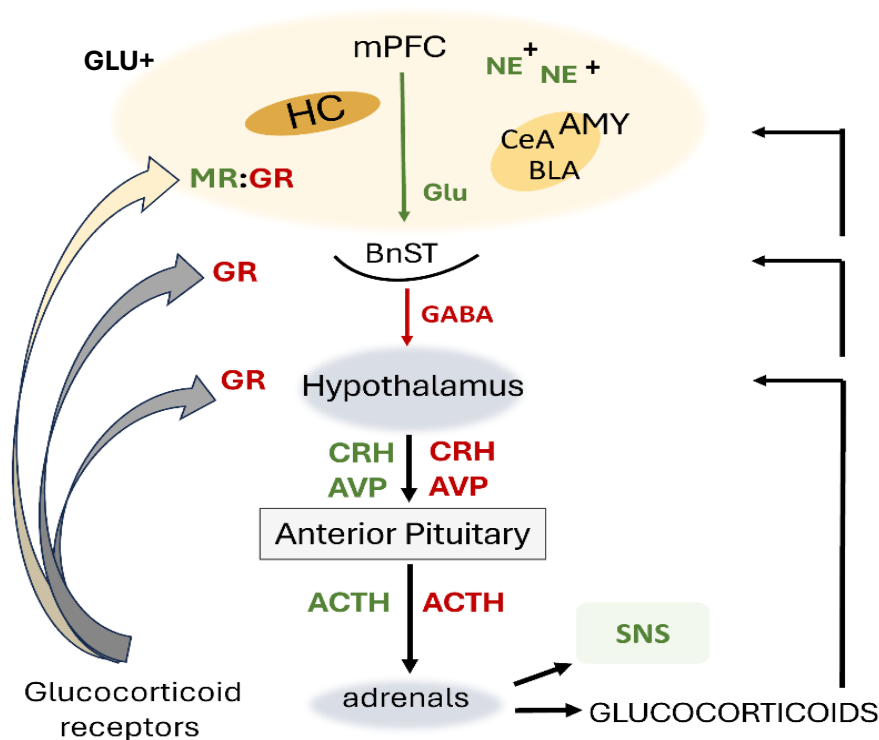
1.1.2 Mineralocorticoid and Glucocorticoid receptor involvement in the stress response

At the cellular-molecular-nuclear level, cortisol, the primary glucocorticoid in humans, plays multiple roles beyond the stress response, modulating cellular functions throughout the body and brain leading to a wide range of physiological and behavioural effects during stress and in homeostasis (de Quervain et al., 2017). The diverse effects of cortisol require high cellular specificity as well as the interaction with the outputs from other systems, such as catecholamines, endocannabinoids, oxytocin, and gonadal hormones, in determining glucocorticoid activation (Dominique et al., 2009) and its effects. Moreover, different subtypes of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) play distinct roles in glucocorticoid signalling (de Kloet and Joëls, 2020). Mineralocorticoid receptors predominantly respond to aldosterone and are involved in electrolyte balance in peripheral tissues with cortical MR's responding to cortisol due to the much higher concentrations of cortisol than aldosterone present in the brain. Glucocorticoid receptors, in addition to moderating stress responses, also regulate energy metabolism and anti-inflammatory responses.

MRs have a high affinity for cortisol (10 x greater than GRs) and nuclear MRs are thus saturated at basal stress levels. Therefore, traditionally, GRs were thought to be responsible for acute stress responding, and MRs were not thought to play a key role within this time frame and instead be responsible for slower tonic changes over time via nucleus bound receptors (Karst et al., 2022). Recent research has found GRs and MRs to be co-localized in key regions involved in fear learning and memory, such as CA1, CA3 and dentate gyrus of the hippocampus, and in the amygdala, (particularly the basolateral nuclei), and prefrontal cortex, and, importantly to work at both tonic/chronic and acute timeframes via either nucleus bound receptors functioning more slowly as transcription

factors as well as acutely via membrane and cytosol bound receptors (Karst et al., 2005; Zhou et al., 2010) (see Figure 1.1.1). Maladaptive GR/MR signalling may influence subsequent neuroendocrine responses to stress and increase an individual's vulnerability to maladaptive stress responding (Vogel et al., 2017; de Kloet and Joëls, 2020).

The integrated activity of mineralocorticoid and glucocorticoid receptors is differentiated across stress response network (Figure 1.1.1) generating both excitatory and inhibitory influences on neural and network outputs. Membrane bound mineralocorticoid receptor activity facilitates the initial ‘on-switch’ of the stress response to threat, acting as a glucocorticoid sensor, enhancing cellular excitability and promoting swift appraisal mechanisms within the mPFC (De Kloet and Joels, 2023). For the stress response to be adaptive during challenges to homeostasis e.g., a traumatic event, time-dependent cellular changes in the balance between MR/GR from the onset of initial stress induced by corticosteroid rise and after an hour post initial rise) with MR/GR activity occurring in the presence of other circulating hormones and neurotransmitters, especially (nor)adrenaline. From an hour post stress, the increase in cortisol levels activates GR-mediated mechanisms and this acts as a glutamate ‘off-switch’ especially in the basolateral amygdala (BLA) (Karst et al., 2022), see Figure 1.1.1.



Adapted from de Kloet and Joels 2020; Arnett et al., 2016

Figure 1.1.1; Mineralocorticoid and glucocorticoid receptor involvement in HPA axis stress response. The HPA axis is mostly regulated via feedback mechanisms that are modified by the action of glucocorticoids released as end products of HPA activation (green, enhancing; red, inhibitory). In response to threat, neurons within the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin-releasing hormone (CRH) and vasopressin (AVP) hormones that act upon the anterior pituitary gland to cause the release of adrenocorticotrophic hormone (ACTH). The adrenal glands respond to systemic ACTH by releasing glucocorticoids (cortisol in humans and corticosterone in rodents) (Arnett et al., 2016) and activate sympathetic nervous system (SNS). The feedback effects on HPA activity are mediated by two types of glucocorticoid receptors, mineralocorticoid (MR) and glucocorticoid receptors (GR) with GRs mediating the cessation of the stress response by inhibiting hormone release from the PVN and the anterior pituitary to prevent excessive GC release. Outside the hypothalamus (beige arrow) the balance between MR:GR density and reactivity to current stressor will determine inhibition of excessive or prolonged neuronal activity (de Kloet and Joëls, 2020). MR response of cells under stress conditions, particularly in the basolateral (BLA) amygdala (AMY) will depend on recent cellular conditions. (Joëls et al., 2011). Most of the mMR are inactivated or internalized >1 hour after the initial corticosterone rise in amygdala (BLA) which may allow GR activity to dominate and limit excessive amygdala-dependent emotional processing (Karst et al., 2022) and for a return of the stress system to homeostasis. (mPFC; medial pre-frontal cortex, BNST, bed

nucleus of the stria terminalis, CeA, central amygdala, GABA, γ -aminobutyric acid, GLU, glutamate, NE, noradrenaline).

1.1.3 Stress, neural plasticity and memory

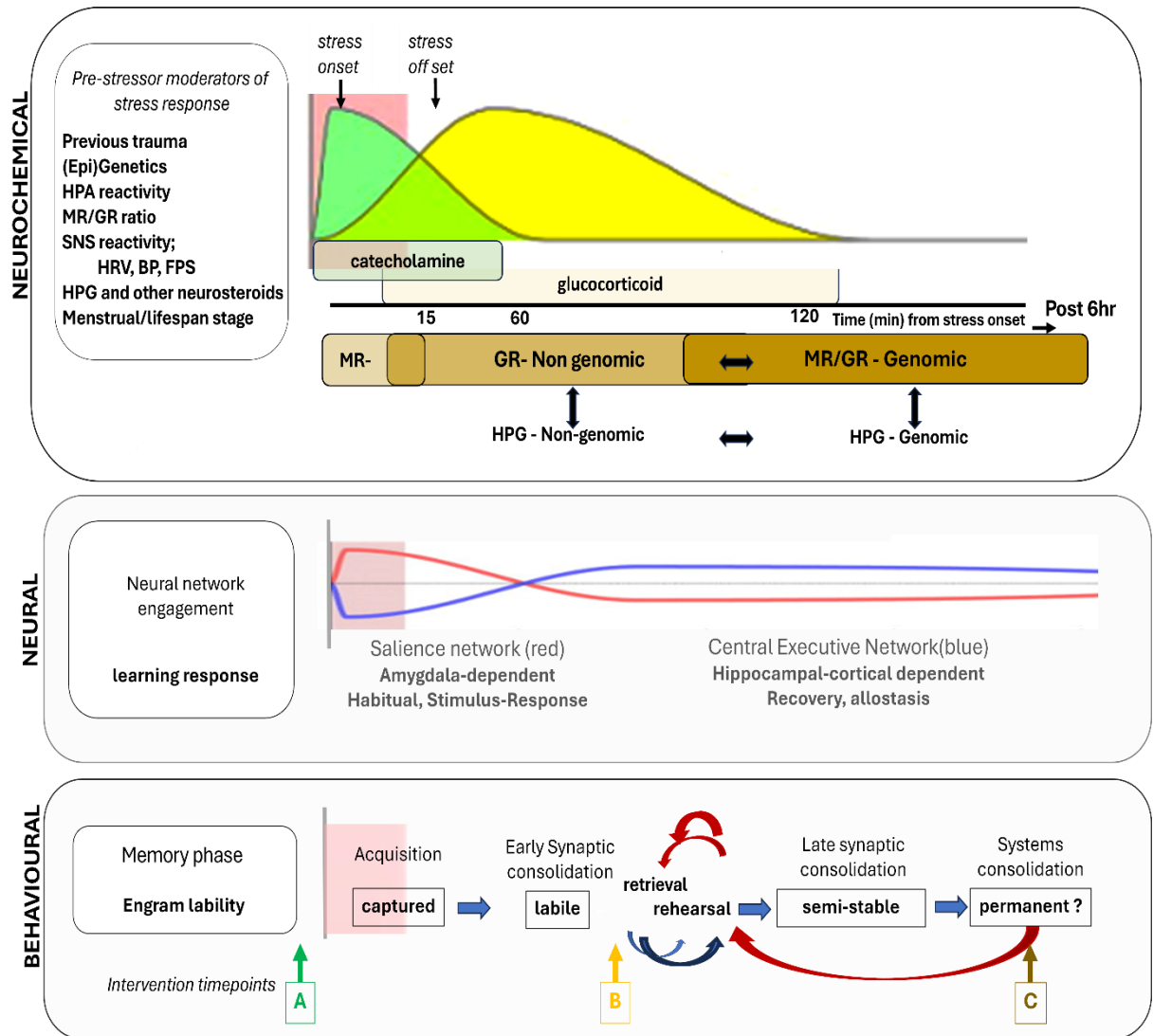
An adaptive stress response promotes structural and functional neural plasticity able to adapt to and store information about the stressful event to face future similar challenges (de Kloet et al., 2005). However, while it may be adaptive to remember as much as possible about a life-threatening event, to prevent stretching and exceeding resources (Schwabe et al., 2022) only stress relevant information needs to be extracted from the irrelevant noise. The large-scale changes in neural network connectivity and interconnectivity that enable hypervigilant, habitual responding during initial encoding, and for appraisal, need to be swiftly switched to a dynamic recovery mode if it is to be adaptive and this requires re-engagement of top-down executive control networks (see Box 1.1.1).

The amygdala nuclei, specifically the lateral amygdala (LA) is essential for the initiation (LeDoux, 1994) development of cortical plasticity underlying learning under threat (Maren, 2003). Plasticity is regulated by reciprocal connections within and between amygdaloid nuclei (particularly the central, CeA, basolateral, BLA and lateral, LA) with connections with the hippocampus, thalamus, hypothalamus, brainstem and cortical regions to store aversive memories and respond appropriately to current and future threats (Schwabe, 2017). Additionally, the hippocampus initially, but transiently, stores new aversive memories where cellular/synaptic consolidation occurs, and processes contextual information linked with the learning experience (Schwabe and Wolf, 2012). To create long term stable, memories, memories need to be transferred from the hippocampus (HPC) to the medial pre-frontal cortex (mPFC) where they are stored and maintained as independent from the hippocampus, via systems consolidation (Dudai, 2012). Memories

need to be successfully consolidated in the cortex and for them to be incorporated into an existing personal narrative and this requires prolonged memory related gene activity in both the hippocampus and the cortex (Walters and Zovkic, 2015). Results from patients with compromised amygdala processing (Hsu and Pessoa, 2007) suggests the importance of the interaction between top-down (PFC) and bottom-up (amygdala/limbic) processes and the importance of executive network inhibitory and attentional control over stimulus-response (salience network) trauma-associated emotion and memory processes (Hermans et al., 2014b) in adaptive trauma memory consolidation (Box 1.1.1 and Figure 1.1.2).

1.1.4 Stress and neural networks

The finely turned phases of the acute stress response at the cellular and synaptic level described above drives functional changes within and between defined brain regions (Kim and Kim, 2023). Following acute stress, there is large-scale change in connectivity within and between functionally different brain regions in the brain (Arnsten, 2009). The early cellular activity in the hippocampus and the amygdala enhances excitatory neural activity. Adrenal and non-genomic (predominantly MR) corticosteroid actions drive the switch dominance of the salience network to aid memory formation and reduces the dominance of the networks involved in higher order cognition (CEN) and internal monitoring (DMN) (Menon, 2011), see Figure 1.1.2. Neuroimaging studies have identified three core functionally connective networks, in a 'triple network model', (Box1.1.1), identified as moderating the acute stress response and recovery after stress (Menon, 2011).



Adapted from (Hermans et al., 2014b)

Figure 1.1.2 Neurochemical, neural and behavioural responses to a threatening event and potential timepoints for moderation of aversive memory. Top; schematic representing a swift, catecholaminergic (green) increase at start of stress response to stress and the delayed, slower rise in glucocorticoid response (yellow) with the corresponding involvement of both non-genomic and genomic glucocorticoid and mineralocorticoid receptor (GR:MR) response. Also shown is the interaction of glucocorticoid response with pre- and post- stress hypothalamic -pituitary-gonadal (HPG) neuroendocrine milieu to effect brain network shifts (middle) from dominance of salience network (red) activity and connectivity at start of stress response and later switching to dominance of executive control network (blue) activity. Stress systems returning to baseline (homeostasis) in response to sustained glucocorticoid influence

*over genomic time frames (see Box 1.1.1). Bottom; represents memory phases associated with corresponding neuroendocrine and network responses. New memory engram requires time in which protein synthesis occurs and storage of memory shifts from encoding and synaptic consolidation (hippocampal-dependent) when it is susceptible to interference/moderation followed by systems consolidation within the medial prefrontal cortex (mPFC) and transfer to long term, robust storage. However, for memory to be adaptable, under the right conditions, fully consolidated memories can be reactivated, updated and re-consolidated. Potential timepoints in which the memory engram could be moderated **A**, prior to onset of stress, **B** >6-hour post stress, **C**, post consolidation of memory (see text).*

The early switch in network connectivity at stress onset (Box 1.1.1) enhances processing of stress-relevant information that subsequently contributes to the sensory-perceptual detail often associated with memory of emotional events and will be considered further in *Chapters 4*. The delayed corticosteroid actions gradually re-engage pre-stress connectivity to restore flexibility of memory and other processes such as working memory and retrieval to contextualize emotional memories adaptively (Joels et al., 2006) and role of post trauma glucocorticoid activity will be discussed further in *Chapter 3*.

Therefore, during a stress response, neural networks do not work in isolation but in an organized, synchronous way to process and integrate information about stress stimuli and how best to respond to it now and remember how to respond at a future encounter (Hermans et al., 2014b; Schwabe, 2017) and may be amenable to moderation by novel intervention strategies discussed in the following Chapters.

Box 1.1. The Triple Network Model (Menon, 2011)

1. Salience Network (SN)

Brain Regions

- Large scale network-
- Incl. insula, amygdala, striatum and ventral tegmental area

Stress Effects

- Early amygdala driven arousal =>hypervigilance, memory formation¹
- SN activity during trauma film predicts subsequent intrusions²
- Prolonged activation increases SN - DMN connectivity correlates to subjective stress³
- Excessive activation => prioritises sensory stimuli over other input
e.g. PTSD-related hypervigilance.⁴

2. Default Mode Network (DMN)

Brain Regions

- dorsal and ventral prefrontal cortices
- medial temporal lobe (hippocampus)

Stress Effects

- Increased internal monitoring, eg. during autobiographical memory retrieval.
- Failure to suppress DMN activity can disrupt goal-directed behaviour.⁵
- Sustained activity linked to affective and addiction disorders⁶

3. Central Executive Network (CEN)

Brain Regions

- Centered in dorsolateral PFC
- Posterior parietal cortex

Stress Effects

- CEN activity at high and low stress impairs complex task performance^{7,8}.
- Impaired attention, working memory and goal-directed decision-making

¹Seeley,2019; ²Visser et al.,2022; ³Maron-Katz et al 2016; ⁴Veer et al., 2011, ⁵ Henckens, 2011; ⁶Hamilton et al 2011;

1.1.5 Glucocorticoid effects on aversive memory

Fear learning under acute stress; In the face of threat, following the initial fast sympathetic nervous system response and catecholaminergic release the slower initial rise in glucocorticoids mediated by MR activity within the hippocampus and central nucleus of the amygdala (Arnett et al., 2016) leads to swift appraisal and enhanced stimulus-response retrieval of relevant information from memory storage (de Kloet and Joëls, 2020). Additionally, the strength of encoding of salient aversive memory during stressful event is highly dependent on the influence of noradrenaline on amygdala-hippocampus activity (Merz and Wolf, 2022).

During the stress response there is a subsequent slower increase in glucocorticoid levels, such that when exogenous or endogenous GC levels are high when coinciding with high catecholamine levels, emotional memory acquisition of the event is enhanced (Hermans et al., 2014a; de Quervain et al., 2017). Brain regions involved in emotional memory learning (for example, the insular and the dorsal stratum) are similarly affected by co-ordinated interactions between glucocorticoid and noradrenergic activation with implications for controlling the switch from hippocampal ‘cognitive’ to habitual, stimulus response learning (Kim et al., 2001, Schwabe et al., 2013, Vogel et al., 2016).

Consolidation: After the stressful event it takes time for the memory to be consolidated into long term memory and both exogenous and endogenous glucocorticoids moderate memory consolidation in a dose-dependent manner with an inverted U-shaped relationship to memory performance, so that higher or lower GC levels can impair consolidation while moderate doses at learning usually enhance consolidation processes (Roozendaal et al., 1999; Andreano and Cahill, 2006). However, glucocorticoid levels alongside other co-occurring neurochemicals (Joëls and Baram, 2009; Joëls et al., 2011)

results in complex effects (section 1.1.6) on different memory processes and in different memory domains (Smeets et al., 2008; Schwabe et al., 2022). Therefore, there are inconsistencies in direction of results of glucocorticoid effects on consolidation of aversive memory.

Importantly, timing matters (Joëls et al., 2006), in the absence of catecholamine levels, at low arousal, or if GC levels rise again temporarily proximate to initial stress event memory of the event is not enhanced or can be impaired (Wiegert et al., 2006; de Quervain et al., 2017). Outside of the feared context, memory is usually impaired at elevated levels of glucocorticoids during memory retrieval, (Schwabe et al., 2022) and this is also dependent on concurrent noradrenergic activity (de Quervain et al., 2007). In humans, cortisol administered in the absence of noradrenaline reduced amygdala and hippocampal activity during neuroimaging > hour later (Henckens et al., 2010; Henckens et al., 2012).

Effects of post stress rise in glucocorticoid levels on emotional memory; Increased availability of glucocorticoids post-trauma/stress learning is thought to (up)regulate hippocampal dependent processing. The hippocampus, rich in glucocorticoid receptors, is critical to receiving and integration of information and can be protected against stress-induced neuronal atrophy with high-dose glucocorticoids if given immediately after the stressor, particularly in "extreme responders", (Zohar et al., 2011). The effects of such administration are seen in minutes and lasting more than 7 days later.

Additionally, high-dose glucocorticoids have been shown to speed up the decay of stimulus-driven elements of visual memory traces (Miller et al., 2015). Miller et al. (2015) proposed that high dose glucocorticoids (GCs) facilitate the natural decay of perceptual memories by promoting the transfer of detailed episodic memories to more

generalized, semantic memories through increased reliance on neocortical activity over hippocampal dependency. On the other hand, inhibiting glucocorticoid synthesis during fear learning (i.e. concurrent with high arousal levels) results in greater specificity of fear memory over the same timespan (Pedraza et al., 2016). The relative adaptive advantage of accuracy or generalised fear learning and role in psychopathology is likely dependent on context but evidence does suggest that glucocorticoid regulation of HPA-axis feedback mediates noradrenaline impact on detail over generalisation and contextual memory storage (reviewed (Bahtiyar et al., 2020)).

Experimental and small-scale clinical trials; contrary to some of the evidence presented above, experimental and small-scale clinical trials have shown impairing effects of hydrocortisone on emotional memory (Sijbrandij et al., 2015; Astill Wright et al., 2021) if administered soon after (analogue) trauma (<6 hr) (i.e., post a stress level rise in endogenous glucocorticoids and during the period of synaptic consolidation). Meta-analysis of Astill et al. (2021) concluded there was a small positive effect of preventative strategies targeting consolidation and that hydrocortisone was superior to control when used as a preventative measure for PTSD. These early clinical and experimental results and the pre-clinical research described above, suggests PTSD symptomology might be attenuated in the early hours after a trauma event and that mineralocorticoid and glucocorticoid receptors provide potential targets in the search for novel preventative strategies for aversive memories associated with stress related disorders. This will be the focus of *Chapter 3* and *4* of this thesis and discussed further in Section 3 of this chapter.

1.1.6 Dissociable effects of stress on different memory systems – declarative vs implicit memory.

As described above, glucocorticoids (principally cortisol in humans) have selective effects on distinct types of memory processes (Schwabe et al., 2010). Declarative memory refers to the conscious recall of facts and events and allows conscious access to a coherent narrative of autobiographical memories. Implicit memory involves non-conscious learning of perceptual sensory-emotional memories. The development of PTSD has been suggested to be due to the inadequate integration of these two systems following hyperactivation of the HPA during a stressful event (Brewin et al., 2010; Brewin and Burgess, 2014).

Under certain conditions, elevated levels of glucocorticoids can impair conscious memory retrieval in a dose and time-dependent manner (Schwabe and Wolf, 2014) so that stress-induced glucocorticoid release interferes with the ability to voluntarily recall information. On the other hand, implicit memory processes, such as procedural learning and habit formation, appear to be enhanced under high glucocorticoid conditions (Wolf, 2017) facilitating hyper consolidated sensory perceptual memories, the type associated with stress-related disorders such as PTSD (Ehlers et al., 2004). Studies have shown that individuals with PTSD demonstrate an information processing bias that interferes with hippocampal-dependent processing (Miller et al., 2017). This bias promotes robust responses to current environmental stimuli related to past trauma and indicates a disruption in the contextualization of sensory information and its integration with autobiographical memory. This lack of integration generates fragmented, disorganized, and incomplete recollections of the traumatic event (Ehlers and Clark, 2000) leading to involuntary recall when encountering trauma cues, i.e. intrusive memories. When experienced, these types of memories are characterised by their distressing and vivid

nature and sensation of ‘nowness’ (Kleim et al., 2013), Hippocampal functioning can be impaired in individuals with trauma-related disorders, including PTSD (Damis, 2022). Decreased hippocampal activity coupled with increased amygdala activity for negative emotional stimuli suggests a shift towards fear learning processes (Bisby et al., 2016; Bisby et al., 2018). This disruption in associative learning and contextual processing contributes to the encoding of stronger sensory memories and weaker contextualized episodic memories in individuals with PTSD (Brewin and Burgess, 2014).

Dual representation theory: According to dual representation theory Brewin et al. (2014), PTSD is driven by the dysregulation of two distinct imagery and memory systems: the Sensory Representational System (SRS) and the Contextualized Representational System. The sensory representational system involves inflexible perceptual memories that are automatically activated, while the contextualized system allows for flexible, consciously accessible, and context-dependent representations (Brewin et al., 2014). During traumatic events, the encoding of sensory perceptual representations is strengthened, while the encoding of context dependent information and the connections between the two systems are weakened (Brewin et al., 2014).

These distinct representation systems interact but are associated with different neural pathways. The flexible, consciously accessible declarative representations projected through the ventral visual stream to the inferior temporal cortex that are regions of the Central Executive network (see Box 1.1.1, section [1.1.5](#)) have connections with the hippocampus. In contrast, inflexible, sensory-perceptual stimuli (projected through the dorsal visual stream to superior parietal areas) are connected to the amygdala and insula that are regions of the Salience network (Brewin et al., 2014).

1.1.7 Epigenetic modification of gene expression, role of “writers” and “erasers”

DNA exists in the nucleus as a compressed structure of DNA and chromatin so that gene transcription is tightly controlled by epigenetic mechanisms. Histone protein complexes build 'nucleosomal' structures containing four histone proteins H1-H4 (Roth and Sweatt, 2009). Internal and external environmental stimuli cause transient molecular signals to modify these basic building blocks of chromatin, for example, via by the addition or removal of -acetyl (or other) groups to the ‘tails’ of the histone proteins (i.e. via acetylation, phosphorylation and methylation) creating a 'histone code' (Levenson and Sweatt, 2005; Farrelly and Maze, 2019). The combination of these epigenetic modifications affects the chromatin's structure and its function and creates a unique epigenome that influences subsequent gene expression but without changing the deoxyribonucleic acid (DNA) code itself.

Chromatin can exist in two different conformations with distinct functions (see Figure 1.1.3, bottom panel). When in heterochromatin form, it is condensed, in an inactive state, and is transcriptionally inert. When in euchromatin form it is in an open, 'uncoiled' state and makes DNA available to transcription factors which do not have access when the chromatin is condensed (Dhalluin et al., 1999). Epigenetic modification results from the recruitment of factors that either activate/uncoil the chromatin (e.g. via acetylation in response to histone acetylases (HATs), also called “writers”) or factors that inactivate/condense the chromatin (i.e. via the removal of an acetyl groups by histone deacetylases (HDACs), called “erasers” (Vecsey et al., 2007; Qiu et al., 2017).

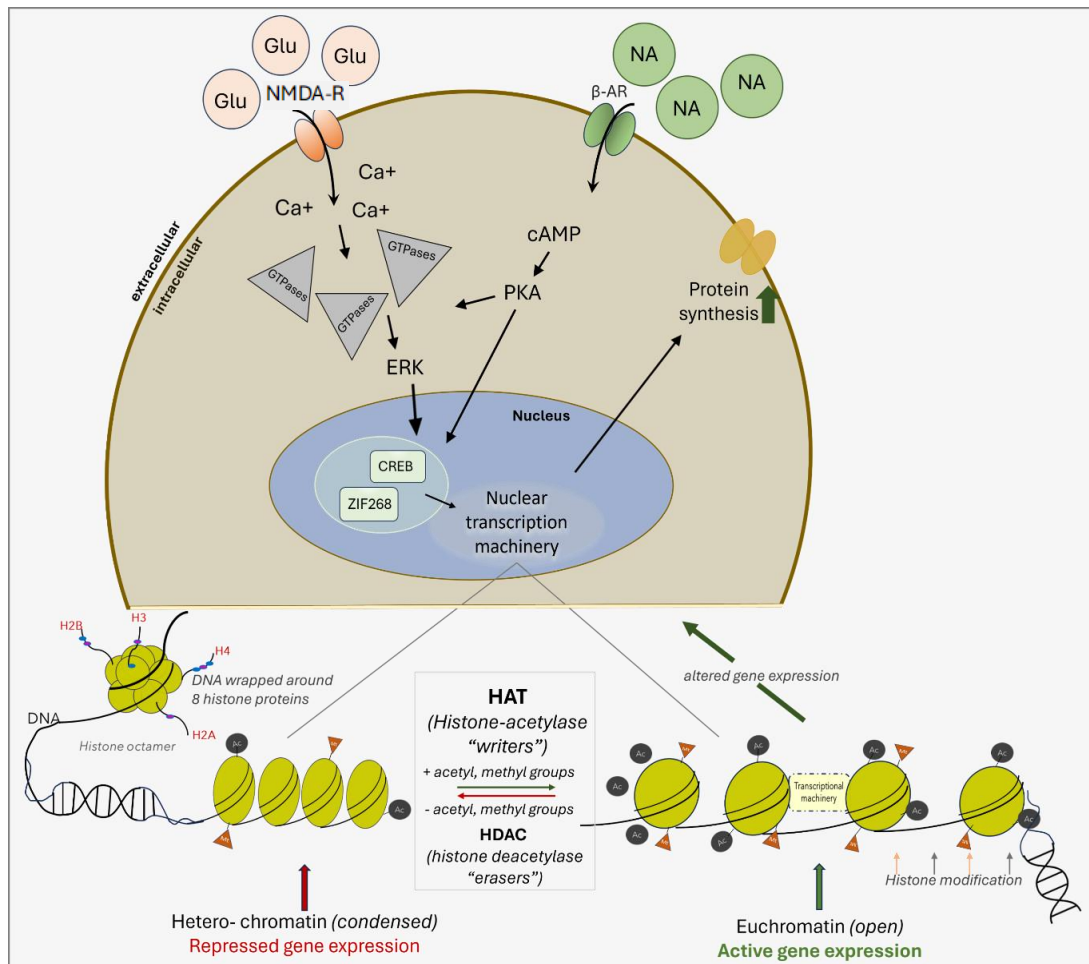


Figure 1.1.3 simplified schematic of cellular, molecular and epigenetic mechanisms underlying memory restabilisation following (aversive) memory reactivation. Adapted from (Tronson and Taylor, 2007; Whittle and Singewald, 2014; Walsh et al., 2018), **Top** Potent preventative targets for modification of consolidated aversive memories include the β -adrenergic receptors (β -AR, green) and N-methyl—D-aspartate receptors (NMDARs, orange) that initiate downstream molecular signalling cascades ending in gene transcription and protein synthesis. NMDAR initiated calcium (Ca^{2+}) influx activate GTPases such as Ras, Raf and Rap that together act with cyclic AMP activated protein kinase A (PKA) on extracellular signal regulated kinase pathway (ERK) to activate transcription factors such as CREB (cAMP response element-binding protein) and zinc finger 268 (ZIF268) which initiates gene transcription. Zif268 plus other immediate early genes e.g. c-fos are required for memory reconsolidation and extinction (Gräff et al., 2014) and their activity can be modulated via epigenetic regulation. **bottom** DNA is compressed within the nucleus wrapped around 8 histone proteins (H1 - H8) to form hetero (repressed) chromatin. Histone-acetylase enzymes ('writers') cause the chromatin structure to relax allowing access to transcription machinery and gene expression and histone modification. Histone deacetylases 'erasers' cause the chromatin to condense back to its repressed state halting protein synthesis. Inhibiting histone deacetylase prolongs gene expression and enhances protein synthesis in neural activity related brain regions see text (Burns and Gräff, 2021). Alterations to the histone

proteins, for example by the addition of methyl (black circles) or acetyl groups (red triangle), to the tails of the histone proteins in response to internal and external events creates a histone code that can modify the subsequent expression of the genetic code and upregulation of subsequent protein synthesis and subsequent learning and memory (Tronson and Taylor, 2007; Roth and Sweatt, 2009; Whittle and Singewald, 2014) (see text 1.1.6 and Chapter 2).

1.1.8 Epigenetic mechanisms involved in aversive memory maintenance and moderation

Life events can have long term consequences on the epigenome and therefore how gene expression will function at future events. The dynamic structure of chromatin (section [1.1.7](#)) enables the integration of many signals from the cell surface as well as co-ordinating appropriate transcriptional responses and this makes epigenetic changes to chromatin a potential candidate for the therapeutic manipulation of long-term memories (Kwapis and Wood, 2014; Whittle and Singewald, 2014; Cao-Lei et al., 2022).

An engram is the biological substrate and neural representation of a memory trace. It represents the physical manifestation of a memory within the brain and comprises the pattern of neuronal activity, synaptic modifications and neural circuits that collectively store the sensory, emotional and cognitive components of an experienced event. The engram is not fixed in time (Dudai, 2012) but is updated, strengthened, weakened, or left unchanged, depending on the conditions under which it is retrieved (Miller, 2021). If the engram is suitably reactivated and made labile then, similarly, but not identical to its original consolidation, the synthesis of de novo proteins is required to restabilise (i.e. reconsolidate) the memory (Nader et al., 2000; Tronson and Taylor, 2007), see Figure 1.1.3, top, and discussed further in section 1.3.9. Therefore, our ability to not only form, store and maintain long term memories but also to update them is strongly reliant on the

coordination of gene expression. Epigenetic mechanisms that regulate transcription factors drive the protein synthesis required to (re)form stable memories and subsequently update them (Maddox and Schafe, 2011; Jarome and Lubin, 2014) via underlying neural and synaptic plasticity.

Trauma induced epigenetic changes to the epigenome will impact the cellular signalling and gene expression in brain regions that play a key role in formation of contextual and episodic trauma memories, such as in the hippocampus (Stafford et al., 2012). Experimentally inhibiting HAT activity (such as with the HDAC inhibitors sodium butyrate and trichostatin A), has shown epigenetic tagging to be a highly dynamic process that supports long term memory consolidation. Increased histone acetylation enhances LTP and memory in wild type rats both in vitro and in vivo (Levenson and Sweatt, 2005) acting as a cognitive enhancer. Histone deacetylation inhibition has been shown to ameliorate memory impairments in hippocampal LTP in *Crebbp* mutant mice due to the resulting increased histone acetylation activity (Vecsey et al., 2007). Epigenetic regulation of *zif268* (an immediate early gene) as well as BDNF gene expression (Bredy et al., 2007; Gräff et al., 2014) has also been associated with the synaptic changes involved in long term fear memory formation following HDAC inhibition in the mPFC, hippocampus and amygdala (Whittle and Singewald, 2014), see Figure 1.1.3.

Epigenetic changes occur differently according to the type of memory/learning task, and this creates distinct histone codes that appear to be brain region specific (Dagnas and Mons, 2013). Histone acetylation is either enhanced or inhibited according to a balance between HAT and HDAC activity. HAT and HDAC effects occur in activity dependent genes ‘primed’ by neural activity rather than having a global effect on gene expression (Vecsey et al., 2007; Gräff et al., 2014; Burns and Gräff, 2021) with effects

subsequently evident in brain regions crucial to memory consolidation (Walters and Zovkic 2015) and reconsolidation (Bredy and Barad, 2008), see Figure 1.1.3.

Gene expression along with interaction between stress hormones, ERK signalling cascade and downstream epigenetic and gene responses in the hippocampus are central to neural plasticity (Farelly and Maze 2019) and has even been suggested as "a molecular basis of the emotion/cognition interface in stress-related learning and memory" (Johannes M. H M. Reul, 2011, p. 108). Therefore, the literature suggests that as epigenetic modifications can persist over time, are responsive to end products of the stress response, of the HPA and the hypothalamic–pituitary–gonadal axis (HPG) and have a key role in maintaining fear memories, they are excellent candidates for understanding long term behavioural adaptations to trauma such as PTSD.

However, the epigenetic mechanisms and regulators involved in moderating the gene expression and subsequent synaptic plasticity required are themselves susceptible to stress (glucocorticoid) effects (White and Wood, 2014; Jeanneteau et al., 2019; Jeanneteau, 2023). Highly stressful, i.e. high glucocorticoid level, events result in epigenetic alterations, e.g. histone acetylation and methylation that alter the way gene expression is regulated. Accumulation of dysregulating epigenetic marks, over a lifetime, but particularly following early life adversity, may drive some of the hippocampal and amygdala dysfunction seen in PTSD patients (Zovkic et al., 2013; Zovkic and Sweatt, 2013). For example, there is also a causal link found between early life stress induced epigenetic alterations in GR gene promoter, and expression of the gene regulating release of corticotropin releasing hormone from the hypothalamus and adult anxiety-related

behaviours (Roth, 2013). Such influences on gene expression may in part explain the susceptibility to forming intense fear memories that are resistant to extinction learning in those who experience repeated trauma and who are at higher risk of PTSD (Roth and Sweatt, 2011; Roth et al., 2011).

Importantly, because histone modification and its influence on chromatin is an important mechanism underlying fear memory storage and maintenance (Figure 1.1.3), It opens up the possibility of targeting specific key moderating enzymes (i.e. histone deacetylases) pharmacologically to enhance extinction memory formation and/or reconsolidation even in older extinction resistant memories such as those experienced in PTSD and long held phobias (Gräff et al., 2014; Burns and Gräff, 2021). As will be discussed in *Chapter 2*, the dynamic structure and function of chromatin makes it a potential target for therapeutic pharmacological intervention of memory related disorders (Whittle and Singewald, 2014; Cao-Lei et al., 2022; Yuan et al., 2023) and its recovery (McCarthy et al., 2009; Colciago et al., 2015) but more research in this area is needed (Kornfield et al., 2018).

1.1.9 Stress, aversive memory and PTSD

If an individual witnesses or is exposed to life threatening event, sexual violence, or severe injury that event might persistently be re-experienced as nightmares or ‘flashbacks’ leading to avoidance of the environmental cues that trigger such memories of the trauma. These mnemonic symptoms are often accompanied by trauma related hyper reactivity e.g. trouble sleeping and heightened startle response. Approximately 70% of the population will experience a trauma in their lifetime and usually such thoughts and feelings fade naturally over time (Kessler et al., 2017) but if they worsen or persist for more than one month after the event, create distress such that it interferes in daily activities

then the criteria for diagnosis of PTSD is met according to the DSMIV as set by American Psychiatric Association (2013).

The WHO World Mental Health Survey (Kessler et al., 2017) reports that of those who experience trauma a sub-group will go on to develop PTSD with approximately 3.9% of the global population experiencing PTSD in their lifetime. However, of those who do develop PTSD 60% will still be experiencing symptoms a year on (Kessler et al., 2017; Kessler et al., 2022). Estimating risk of vulnerability to developing PTSD is complex. Prevalence rates vary, depending on the type of trauma experienced and depending on the individual/sub-group who has experienced that type of trauma. Experiencing interpersonal violence carries the highest risk for PTSD with prevalence for rape and sexual assault between 13 and 15% and the category, ‘domestic’ sexual violence, accounting for 42% of person years with PTSD (Kessler et al., 2017). Experiencing trauma is itself a risk factor for developing PTSD following a subsequent trauma. In the general population, women experiencing trauma are at generally higher risk than men of developing PTSD with a reported PTSD rate of 13% in women and 6.2% in men (Ramikie and Ressler, 2018). Other populations are also at higher risk, particularly displaced populations, with estimates of prevalence 7 to 8 time higher in this population than globally (Aysazci-Cakar et al., 2022), probably due to the higher likelihood of repeated trauma in conflict hit regions (Fossion et al., 2015).

Experiencing a trauma has long lasting effects not just on psychological outcomes but at bio-, neuro- and physiological levels. However, reflected in the fact that not everyone who experiences trauma goes on to develop stress-related disorders, PTSD patients are reported to have dysregulated HPA responses, with hypo-glucocorticoid levels although with increased cortisol reactivity to stress (Yehuda and LeDoux, 2007). These symptoms may be due to decreased cortisol levels with inflammatory markers such as C-

reactive protein and interleukin 6 levels have been reported as elevated in PTSD but not non-PTSD trauma victims (Hori and Kim, 2019). Elevated catecholamine levels contributes to hyperarousal in PTSD compared to healthy controls and trauma exposed non PTSD (Young and Breslau, 2004; Bremner, 2007) as well as a deficit in inhibitory control of amygdala due to hypoactivity in medial prefrontal cortex (mPFC) that contributes to hypervigilance and anxiety (albeit with inconsistencies in the literature). Brain imaging studies also show reduced hippocampal volume in PTSD compared to non-PTSD individuals (Bremner, 2007). Some of these differences possibly pre-date trauma suggesting that some cortisol-related dysregulation in PTSD may reflect preexisting vulnerability to PTSD memory symptoms rather than consequence of experiencing trauma (Yehuda and LeDoux, 2007). A failure of the HPA to contain SNS response during trauma may lead to over consolidation of memory at the time and subsequently maintained by lower cortisol levels relative to noradrenaline levels when intrusions occur subsequently (Nicholson et al., 2014).

There are difficulties and controversy in diagnosing PTSD due the range and heterogeneity of presentation of symptoms (Walton et al., 2017). This is coupled with difficulty in accessing health care in some situations/populations (Schein 2021) that can lead to significant delays in accessing appropriate treatment (Hiscox et al., 2023). Systematic reviews find that psychological therapies are effective treatments for PTSD with the strongest evidence for cognitive behavioural therapy with a trauma focus although in military and veterans' populations results with behavioural therapies are less encouraging (Lewis et al., 2020). Eye movement desensitisation and reprocessing (EMDR) were found effective but similarly with less effectiveness in military personnel and showed no benefit of early intervention with EDMR for rape victims compared to watchful waiting (Covers et al., 2021). A recent meta-analysis of treatments for PTSD

after sexual assault showed that one third of rape victims are not symptom free at follow up (Oosterbaan et al., 2019). Additionally, there are high drop-out rates from current psychological therapies for PTSD (14-18%) particularly for those with a trauma focus (Lewis et al., 2020) and, of those who are helped, rates of relapse have been reported as high as 50% (Kessler et al., 2022). Clearly, for many PTSD patients there is pressing need for novel well tolerated, effective, robust treatments (Holmes et al., 2014; Krystal et al., 2017) and reliable, deliverable preventative strategies.

Section 1.2 - The role of sex and gonadal hormones in the development and treatment of persistent aversive memories

1.2.1 Sex differences in vulnerability and resilience to stress related disorders

Women are at higher risk of developing PTSD than men (Haskell et al., 2010; Haering et al., 2024) and of experiencing greater degree of symptoms in anxiety disorders (Maeng and Milad, 2015). Women diagnosed with PTSD report more intrusive memories (Hsu et al., 2018) and avoidance type symptoms, more severe debilitating and persistent symptoms and resulting in a poorer quality of life for women than men with stress disorders (Seedat et al., 2005; Perrin et al., 2014) although see (Hiscox et al., 2023). Response to stress can also trigger other mental health and physical health conditions that are known to have differentiated progression and severity between the sexes (Becker et al., 2007). The Centre for Disease Control Prevention (CDC) estimate that approximately 1 in 4 women in the USA will experience a sexual assault in their lives. Women experience symptoms for longer and women who experience sexual violence are at most

risk with 40% of rape victims experiencing PTSD symptoms at 3 months (Kessler et al., 2017). Women are at higher risk for experiencing sexual violence (Farahi and McEachern, 2021) although this does not wholly account for the gender difference in PTSD prevalence (Ramikie and Ressler, 2018).

Sex differences in PTSD presentation are sensitive to trauma-type and this could be missed in samples limited to one population, e.g. veterans (Hiscox et al., 2023) with, for example, the highest rates of PTSD are as a response to rape, particularly in women (Ferree et al., 2012). Additionally, differences on a range of biological factors differ by sex in PTSD, for example immunological status, sleep, and comorbidities with other illnesses. However, for both sexes there is a poor outlook for treatment options with few currently developed specifically for treatment of PTSD in both sexes (Dabrowska, 2023) with some patients inappropriately prescribed drugs known to exacerbate PTSD symptoms (Hiscox et al., 2023).

Research exploring the interactions between biological and non-biological mechanisms is increasing rapidly and revealing the importance of recognising their contribution to both development but also treatment and recovery following trauma (Seligowski et al., 2020; Hiscox et al., 2023). Biological sex influences on PTSD and other stress related disorders are varied and complex and will interact with socio-cultural differences. Experimentally identifying biological sex differences in response to novel strategies to ameliorate stress disorder symptoms may improve treatment outcomes but could also help resolve the sometimes-contradictory results that exist in the existing research literature more generally.

Aversive memory research has traditionally focused on male rodents to avoid confounding results caused by hormonal fluctuations in females, but, despite NIH mandates to address sex bias in research, including the 1993 directive to include women in clinical studies and a 2014 initiative to further promote equity (Shansky, 2019; Shansky and Murphy, 2021), the underrepresentation of females in preclinical models of aversive memory processing may still be hindering our understanding of how sex and sex hormones influence the aetiology and treatment of anxiety and fear disorders (Dabrowska, 2023).

The rest of this section explores the current understanding of how sex and stress hormones interact to influence resilience or vulnerability to stress and identify potential targets for novel interventions for stress-related disorders in both sexes, a topic expanded upon in subsequent chapters.

1.2.2 HPA-HPG interactions; the implications for sex differences in response to threat

Crosstalk between the hypothalamic-pituitary adrenal (HPA) and hypothalamic-pituitary gonadal (HPG) axes may underlie some of the sex differences in behavioural responses to stress as well as response to both novel and current treatments (Bangasser et al., 2010; Sze and Brunton, 2020), see section 1.2.6. The reciprocal modulation between the HPA and HPG systems act indirectly via connections to the hippocampus, mPFC, and amygdala to influence responses in critical fear learning circuitry regions (Figure 1.2.1a) which are affected by both sex and stress steroid hormones (Ney et al., 2019). Biochemistry of steroid hormones are also interconnected (Figure 1.2.1b). For example, testosterone is converted via aromatization to estradiol (Maeng and Milad, 2015).

Crucial to this crosstalk between the two networks (see Figure 1.2.1a) is the relationship between levels of cortisol (the principal glucocorticoid in humans) and aldosterone as end products of the HPA axis and peripheral estradiol (principally E2), progesterone (P4) and testosterone, as products of the HPG axis (Toufexis et al., 2014; Brann et al., 2021). Testosterone and estrogens can modulate the HPA axis due to the widespread expression of gonadal hormone receptors in HPA neural circuitry (including in hippocampus, hypothalamus and the hypothalamic paraventricular nucleus, PVN). In rodents estradiol is reported to enhance HPA activity and disrupts glucocorticoid negative feedback on HPA axis activity although opposing effects dependent on varying experimental conditions are also reported (Heck and Handa, 2019). In humans, women are reported as having higher HPA reactivity and greater release of stress hormones (Goel et al., 2014) reviewed by Kudielka and Kirschbaum (2005). Testosterone administration in women is reported to inhibit HPA response (Hermans et al., 2007) and subsequent behavioural fear responding (Hermans et al., 2006). Reciprocally, stress induced HPA activity, in conjunction with available progesterone, can inhibit estradiol and testosterone secretion (Toufexis et al., 2014) with implications for aversive memory persistence following trauma (Ney et al., 2019).

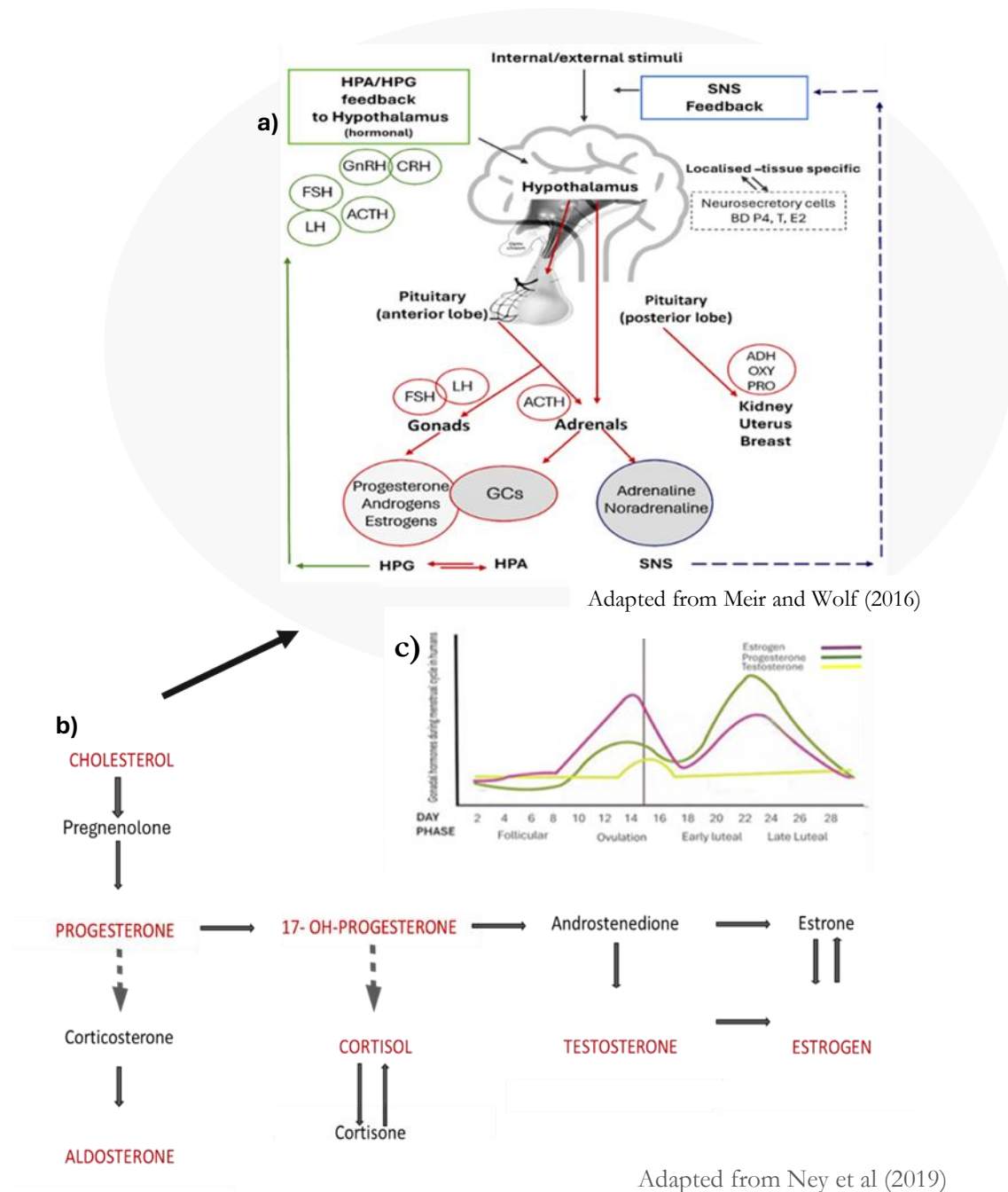


Figure 1.2.1 Hypothalamic pituitary adrenal (HPA) and Hypothalamic pituitary gonadal (HPG) interactions, biosynthesis pathway and human menstrual cycle. a) The pituitary gland controls homeostasis within the body via hormone release (red and green circles) the release of which are moderated via feedforward (red) pathways and feedback (green) mechanisms in the control of organism wide functions. The anterior pituitary is central to moderating the physiological response to aversive events (see Figure 1.1.1) with the final products of the HPA axis being glucocorticoids (GC)

released from the adrenal glands. The pituitary gland is also part of the HPG axis regulating luteinizing hormone (LH) and follicle-stimulating hormone (FSH) resulting in the release of 'sex' hormones from the gonads in both males and females. Interactions between the end products of both axes in concert with sympathetic nervous system activity moderated levels of adrenalin and noradrenalin (blue)) determines behavioural and mnemonic outcomes during and post an aversive event (adapted from Meir Drexler et al. (2016). Brain-derived (BD) levels of gonadal hormones directly influence long term potentiation (LTP), neuroplasticity and protection from neuro toxicity in fear learning brain regions (e.g. amygdala and hippocampus). GC levels alters biosynthesis of brain derived gonadal hormone a potential further mechanism for stress/gonadal hormone interaction effects (Brann et al,2022). b) simplified biosynthesis pathways of stress and gonadal steroid hormones formed from cholesterol; dotted arrows indicate missing steps (adapted from Ney et al (2019). c) depiction of relative gonadal hormones in women across the menstrual cycle. In humans, the menstrual cycle is typically divided into a pre-ovulatory phase, the follicular, and a post ovulatory, the luteal, phase. The early follicular phase, starting at day 1 of menstruation, is characterized by lower concentrations of estradiol (red) and progesterone (green) compared to the higher concentrations of these hormones during the luteal phase between days 13 and 24 and during ovulation. Testosterone shown in yellow. Ratio of gonadal hormone levels and their interaction with HPA products across the cycle is likely to impact experimental manipulation of aversive memory phases (Ney et al.,2019; Soni et al, 2013).

1.2.3 Gonadal hormone fluctuations across the menstrual and life cycle

Hypothalamic–pituitary–adrenal (HPA) and the hypothalamic–pituitary–gonadal (HPG) axis interact to promote behavioural change following trauma (Ney et al., 2019; Sze and Brunton, 2020; Nillni et al., 2021) which results in differences in stress reactivity at different phases of the menstrual cycle (Tolin and Foa, 2008; Maeng and Milad, 2015; Andreano et al., 2018; Cook et al., 2021). The increased prevalence of stress-related disorders after puberty are suggestive of a role for gonadal hormones in fear memory consolidation and extinction (Li and Graham, 2017). From puberty until menopause, the menstrual cycle in naturally cycling women is characterized by fluctuations in gonadal hormone levels, principally estradiol and progesterone as well as testosterone (Cook et

al., 2021) across approx. 28 days in humans. Levels of estradiol, progesterone, and testosterone are controlled via HPG and HPA interactions, with, on average, higher levels of testosterone in men than women, and large fluctuations in estradiol and progesterone across a natural menstrual cycle compared to men and women taking hormone contraceptives.

Following the mid-cycle luteinizing hormone (LH) surge, estradiol levels rise swiftly and remain relatively stable across the luteal phase compared to progesterone levels that rise significantly. The ratio and rate of change, rather than absolute levels between these hormones may have important consequences for their interaction with stress steroids (Soni et al., 2013; Herrera et al., 2016; Pineles et al., 2016; Ney et al., 2019) to influence an individual's vulnerability or resilience to developing aversive intrusive memories and to potential treatment and preventative strategies.

Sex hormone levels in men, which also include estradiol and progesterone as well as testosterone, are relatively constant within the same individual although there is considerable between-person variability in basal and stress-induced hormone levels as well as marked changes, particularly in testosterone levels, over the lifespan (Society, 2007; Travison et al., 2007; Wu et al., 2008; Escobar-Morreale, 2018). In recent years there has been an effort in both human and animal research to explore how these gonadal hormone fluctuations impact on stress reactivity and subsequent formation, maintenance and moderation of emotional memory and vulnerability to PTSD (Shansky, 2015; Ney et al., 2019; Raeder et al., 2019; Shansky, 2019; Nillni et al., 2021).

1.2.4 Role of gonadal hormones in fear (un)learning in women and men

Estradiol

Estradiol plays a critical role in fear memory and stress interactions due to its widespread effects on brain regions like the amygdala, mPFC, and hippocampus, which have a high density of estradiol receptors (Jasnow et al., 2006; Lynch et al., 2016; Taxier et al., 2020). Estradiol influences all phases of memory processing (Lonsdorf and Merz, 2017), with, in general, higher estradiol levels being associated with enhanced memory formation and consolidation (Lebron-Milad et al., 2012; Garcia et al., 2018). And for higher estradiol levels enhancing fear extinction and extinction recall (Milad et al., 2009a; Milad et al., 2009b; Garcia et al., 2018).

However, the literature suggests that there are inconsistencies in estradiol effects across all phases dependent on interaction with concurrent stress levels (Antov and Stockhorst, 2014). Under high stress, women with high estradiol levels show enhanced memory formation (Antov and Stockhorst, 2014). This may contribute to stronger trauma memories in women who experience a traumatic event during high-estradiol phases of their cycle and may, in part, explain higher clinical PTSD rates in women than men. Neural activity in brain regions relating to emotion regulation during viewing a traumatic film has been linked to estradiol status (Miedl et al., 2018) with underlying neural activity between and within the mPFC related to hormonal differences, particularly estradiol levels, rather than sex per se (Bierwirth et al., 2021; Carpenter et al., 2022), to mediate stress effects on learning and memory.

The effects of estradiol on extinction and extinction recall have implications for current clinical practice as existing gold standard treatments for PTSD and phobia are based on extinction principles. However, there is inconsistency in the literature in direction of estradiol memory effects (Garcia et al., 2018) dependent on estradiol levels

at time of both extinction learning and extinction recall. Additionally, the role of estradiol is further complicated by its conflicting effects on extinction retention dependent on current PTSD status (Pineles et al., 2016; Pineles et al., 2020). Additionally, studies of experimental/analogue-trauma paradigms have found contradictory results to effects seen in clinical settings, with high estradiol phases being associated with both increased flashback-type memories (Bryant et al., 2011) while others report lower estradiol phases linked to stronger trauma-film intrusive memories (Wegerer et al., 2014) although the different methodologies and populations make direct comparisons difficult (Garcia et al., 2018). Importantly, it will be important to explain these discrepancies between results from clinical and non-clinical populations to accurately assess estradiol influence on both vulnerabilities to stress related disorders and therapeutic outcomes for stress-related disorders in women (Garcia et al., 2018).

The role of estradiol in men has been less thoroughly studied except as they compare to women. Some research suggests men exhibit extinction recall comparable to high-estradiol women (Milad et al., 2010) while other studies find men's performance more like low-estradiol women (Milad et al., 2006). Men generally show lower neural activation than high-estradiol women in brain regions including the insula, cingulate cortex, amygdala, and hippocampus during fear memory processes (Hwang et al., 2015) with evidence for high vs low estradiol effects during extinction learning. Additionally, the medial prefrontal cortex is a crucial brain region involved in fear extinction and is sexually dimorphic (Goldstein et al., 2010; Brann et al., 2022) sensitive to estradiol levels (Zeidan et al., 2011; Merz et al., 2013) and also responsive to stress (Arnsten, 2009; Maeng and Shors, 2013). The mPFC may act to neurobiologically differentiate by sex the effects of estradiol, stress on fear learning and aversive memory.

Progesterone

Progesterone is the mediator in the pathway that ends with the production of both testosterone and estradiol as well as cortisol. Progesterone strongly influences HPA/HPG interactions (see Figure 1.2.1) to moderate subsequent cortisol release via GABAergic signalling to the HPG (Ney et al., 2019). As an intermediate in the synthesis of cortisol, progesterone may alter cortisol bioavailability during stress as reported in women (Herrera et al., 2016).

Progesterone enhances memory performance under stress, for example, women administered a high dose of progesterone showed an improvement in memory of threatening images that correlated with increase in cortisol levels (Felmingham et al., 2012a). Progesterone also inhibits mineralocorticoid function, with the potential to moderate MR dependent initial stress responses (discussed further in section 1.1.2 and *Chapter 4*). In general, in women, memory performance is enhanced in studies when stress is high or during mid luteal phase of menstrual cycle i.e. progesterone will enhance cortisol bioavailability and memory consolidation processes to result in increased intrusion frequency more robustly under stress (Andreano et al., 2008; Ferree and Cahill, 2009; Soni et al., 2013; Bayer et al., 2018; Krinke et al., 2022). However, there are inconsistencies in results in studies conducted in the follicular phase and/or with no stress induction (Wegerer et al., 2014). Additionally, opposite effects of progesterone on emotional memory may be dependent on PTSD diagnosis. As a diagnosis of PTSD is often associated with cortisol dysregulation, an interaction between progesterone and cortisol reactivity during stress may be mediating these results on emotional memory in women.

In men; progesterone has a role in stress response in men (Breier and Buchanan, 1992; Herrera et al., 2016) and has anti-androgenic effects (Barros et al., 2015). Most

human studies limit their analysis of progesterone to confirmation of menstrual status and few sample gonadal hormones in men in memory studies (Garcia et al., 2018) although see (Cheung et al., 2013; Antov and Stockhorst, 2014). Therefore, more research is needed to clarify their specific role in fear memory processes and their moderation and will be discussed further in Chapter 3.

Testosterone

Testosterone generally provides protective effects against anxiety and depression, particularly in men with low testosterone levels (Aydogan et al., 2012; McHenry et al., 2014; Zitzmann, 2020). It modulates fear memory through genomic and non-genomic mechanisms, reducing HPA axis activity and glucocorticoid levels while enhancing plasticity in regions like the hippocampus and amygdala (Sapolsky, 2005; McHenry et al., 2014). Elevated testosterone-to-cortisol ratios improve fear extinction learning (Pace-Schott et al., 2013), though its conversion to estradiol complicates direct effects (Maeng and Milad, 2015). Blocking this conversion impairs extinction recall, indicating testosterone may act indirectly through estradiol (Graham and Milad, 2014). Testosterone's effects on fear memory remain inconsistent, with some studies showing neuroprotection against trauma and others reporting enhanced fear conditioning (Chen et al., 2014) or not (Anagnostaras et al., 1998).

There is insufficient literature to draw conclusions about testosterone's role in development or maintenance of PTSD (Seligowski et al., 2020) and its influence is likely through interaction with other neuroendocrine products. However, its protective effects have been reported in men having higher serum testosterone levels pre-combat deployment (women were not included in this study) as being at less risk of developing

PTSD (Reijnen et al., 2015). Its role in potential novel strategies for prevention and treatment merits further study and will be discussed further in *Chapter 4*.

1.2.5 Hormone contraception use and aversive memory

Most hormone contraception formulae combine synthetic estradiol (ethinylestradiol) and progesterone (e.g., levonogestrel). This prevents ovulation by chronically maintaining low levels of endogenous gonadal hormones (Golobof and Kiley, 2016). Persistently low levels of estradiol and progesterone have functional consequences for connections from the medial PFC to the amygdala and HPA especially in females (Walf and Frye, 2005; Maeng and Milad, 2015) and consequences for subsequent gonadal hormone response under stress conditions. Hormone contraceptives have been linked to a generally blunted mood (Jentsch et al., 2019), blunted response to stress, and lower levels of fear reduction in phobic fear at follow up post exposure therapy (Raeder et al., 2019) and qualitatively different memory recall (e.g. between gist vs detail, see section 1.1.5) compared to naturally cycling women (Nielsen et al., 2013; Graham, 2022). Like cortisol, estradiol follows an inverted U-shaped dose effect with levels that are too high or low having differing effects on hippocampal neural activity (Bayer et al., 2018). Experiencing trauma following extended hormone contraceptives use that keeps endogenous estradiol levels artificially low may contribute to resistance to extinction and greater fear recovery during extinction recall due to hormone contraceptives induced changes in neural activation, BDNF expression and synaptic plasticity in fear related brain regions (Pluchino et al., 2009; Merz et al., 2012).

Hormone contraceptive use therefore may have influence on aversive memories and PTSD development as well as treatment. Women with a diagnosis of PTSD using hormone contraceptives had enhanced extinction of fear compared to women not taking hormone contraceptives and men (Bartholomew et al., 2022). Potentially, this may be due to the higher affinity of synthetic to endogenous estradiol and may provide a protective influence against intrusive memories following experimental analogue trauma (Maslahati et al., 2023) and in emergency settings albeit at differing levels depending on formulation (Ferree et al., 2012).

Experimentally, it cannot be assumed that treatment effects will transfer between women taking hormone contraceptives and naturally cycling women or to men. Additionally, worldwide use of hormone based contraceptives is high (Lacasse et al., 2024) any novel intervention would need to account for these effects on the outcomes while remembering that, as with naturally cycling women, there will be individual variability in hormone levels between women that is best accounted for with hormone level assays.

1.2.6 Gonadal hormones and epigenetic regulation of (aversive) memory

Epigenetic control at the chromatin level (section 1.1.7) is highly influential on gene transcription and highlights the important role histone modifications play in learning and memory (Colciago et al., 2015; Burns and Gräff, 2021) and in aversive memory in particular (Gräff et al., 2014). Interactions between estradiol receptors and the epigenome results in estrogen-mediated gene transcription to enhance memory and learning in a variety of species including humans (Tuscher et al., 2018). It is suggested that there are interactions between estradiol and enzymes that co-activate (HATs) or co-repress (HDACs) chromatin (section 1.1.7) to promote gene transcription (Frick et al., 2011) in

genes (e.g. BDNF) that play a role in neuroplasticity (Luine and Frankfurt, 2013). Variations in BDNF levels are also influenced by gonadal hormone x glucocorticoid interactions (Spencer et al., 2008; Luine and Frankfurt, 2013) suggesting that the enhancing effects of estradiol on emotional memory may be via epigenetic moderation of BDNF (Fortress et al., 2014). However, while the relative epigenetic influence of sex and gonadal hormone differences on aversive memory has yet to be fully determined sex should be considered as a variable in future research exploring cognitive enhancers targeting epigenetic mechanisms as potential pharmacological interventions for stress related disorders and will be discussed further in *Chapter 2*.

1.2.7 Stress and gonadal hormones and the moderation of aversive sensory perceptual, intrusive, memories in women and men

As described (section [1.1.6](#)) stress level glucocorticoids interact with sex and gonadal hormones to differentially affect different types of memory domains. Modifying this interaction and its effect on sensory perceptual memories of the type associated with stress related disorders is less well researched (Garcia et al., 2018; Hsu et al., 2021) than in the fear conditioning literature more generally although the research will clearly inform research of intrusive memory moderation (section [1.2.6](#)). From current clinical data there suggests there is a (weak) association between menstrual or hormonal status and subsequent aversive intrusions (e.g., Ferree et al., 2012; Pineles et al., 2016) but see Bryant et al. (2011). There is also mixed evidence for higher estradiol and the luteal phase (higher estradiol and progesterone) being associated with better fear inhibition in clinical samples although this too is challenged by Pineles et al. (2016) result. Gonadal hormone reactions during stress have been shown to interact with individual stress reactivity; i.e. peri-estradiol levels were shown to have a time-dependent effect in high anxiety compared to low anxiety women, with estradiol being positively correlated with intrusions early

(day 1 and 2) after experimental trauma but switching to protective against intrusions in the longer-term (Franke et al., 2022). This suggests that moderating stress x estradiol response around the time of trauma might have consequences for subsequent intrusive memory formation and may be a target for pharmacological intervention as further discussed in *Chapter 3*. A systematic review and meta-analysis report (Hsu et al., 2021) suggests that there is some support for estradiol and progesterone as moderators of both emotional negative recall and spontaneous, intrusive memories but direct comparisons between the sexes is rare. Hsu et al. (2021), in a recent review and meta-analysis (Hsu et al., 2021), details nine studies that specifically look at gonadal hormone differences in moderation of intrusive memories formation in either clinical settings or using the trauma film paradigm (Ferree and Cahill, 2009; Bryant et al., 2011; Ferree et al., 2011; Ferree et al., 2012; Cheung et al., 2013; Soni et al., 2013; Wegerer et al., 2014; Rombold et al., 2016b) with three of these using self-report of menstrual phase rather than direct measurement of gonadal hormone levels (Ferree and Cahill, 2009; Bryant et al., 2011; Ferree et al., 2012). The relatively small number of studies reflects the limited research on interaction of gonadal hormones levels rather than sex alone as a moderator of pharmacological prevention of intrusive memory consolidation and will be explored in both sexes in *Chapters 3* and in men in *Chapter 4*.

There is also a small amount of research investigating the interaction with sex and gonadal hormone levels in cued fear discriminative responses and reconsolidation paradigms (Day and Stevenson, 2020). However, there is a potential role for estradiol in discriminating between fear and safety cues and also enhancing inhibition of fear response to safety cues (Day and Stevenson, 2020) as well as the generalization of fear to safety signal following test of reinstatement. In terms of sex differences in moderation of reconsolidation of conditioned aversive memory, something explored in *Chapter 2*, Meir

and Wolf, in two identical but separate studies (Meir Drexler et al., 2015; Meir Drexler et al., 2016), found sex dependent effects in response to hydrocortisone administration prior to reactivation-extinction of cued fear provided protective effects in men only. Sterminger et al., found no sex effect when controlling for sex in a similar protocol using propranolol, although only female data was parsed out in the latter post hoc analysis (Sterminger et al., 2022). In contrast, another study found retrieval prior to extinction without pharmacological adjunct reduced return of fear in women but not in men (Chen et al., 2021). No gonadal hormone data was available from these reconsolidation studies.

The presence or absence of stress is an important factor as sex moderate learning strategies (ter Horst et al., 2013) and emotional memory depending on stress levels (Meir Drexler and Wolf, 2017). This will have implications for treatment. This may be the case not only for preventative treatments that moderate pre and post the trauma stress levels but also for treatments that pharmacologically or behaviourally moderate exposure-based therapy which triggers and targets stress responses to emotional cues and its effects on sensory-perceptual sequelae. It is unrealistic to study all effects simultaneously, but important to keep in mind the complexity of the system in which the small part under investigation sits and its challenges/limitations but also the opportunities this poses; a challenge taken up with exploratory analysis in the following chapters of this thesis.

Section 1.3 Aversive memory modification; paradigms and research methods identifying targets for potential behavioural and/or psychopharmacological intervention strategies

1.3.1 Fear conditioning as a model of PTSD and anxiety disorder

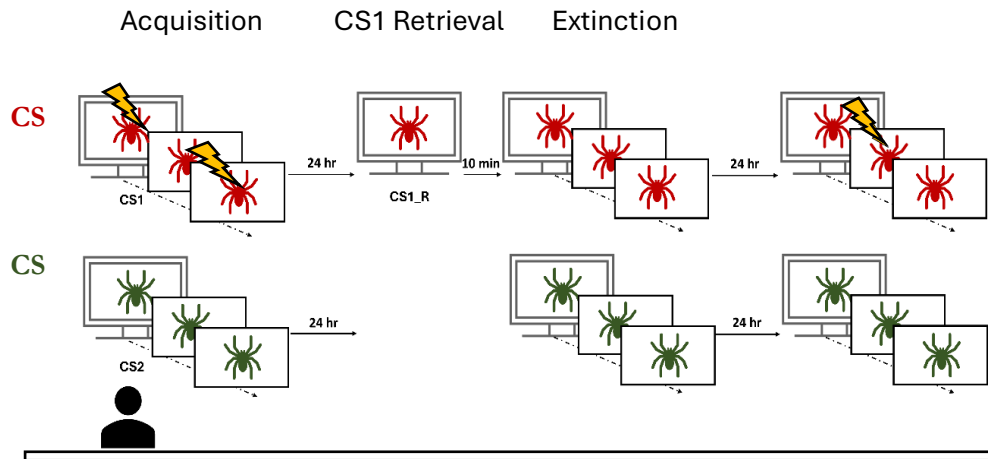
PTSD is an unusual mental health condition as it can usually be linked to a single traumatic, life-threatening event, indeed this is a diagnostic criterion for the disorder (DSM-V) (American Psychiatric Association, 2013). Pavlovian and associative fear conditioning aims to model the acute stress effects that most individuals experience at the time and recovery following a trauma event, e.g. intense fear, anxiety but that over time gradually fade and homeostasis/allostasis is re-established (McEwen, 2002; McEwen, 2008; McEwen et al., 2012). However, for some individuals the intense feelings of fear and anxiety do not subside but are sustained often triggered by repeated cued reminders of the event. The repeated reminders, instead of adaptively limiting the response to specific trauma cues and reducing the fear response to such cues over time, lead to increased and generalized (dis)stress and hypersensitivity in those suffering from PTSD.

Animal and human fear conditioning (Pavlov, 1949) aims to reflect the conditions under which PTSD is triggered (Rau et al., 2005; Yehuda and LeDoux, 2007) with

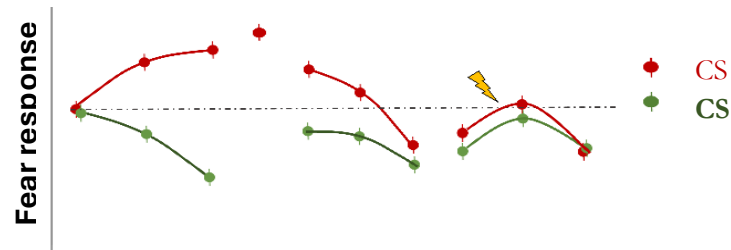
responses to it monitored. The animal model is useful in answering questions that are difficult or unethical to explore with human volunteers or in the clinic. However, care must be taken inferring answers from animal models to humans. The rodent model is used extensively in fear conditioning studies (Schroyens et al., 2021) and while fear circuitry is similar between rodent and human, brains structure and function are not identical (Delgado et al., 2006; Kesner and Hopkins, 2006). Importantly, unlike in humans, it is not possible for rodents to report introspections or tell researchers how they ‘feel’. Animal models are instead capturing a fear motor response (LeDoux, 2014). Therefore, care is needed in translating methodology from animal to human studies (LeDoux, 2017; Haaker et al., 2019) and how results are extrapolated to model the impact of stress or intervention effects on humans. This is particularly difficult for concepts such as distress and vividness of intrusive memories described in *Chapters 3 and 4*.

Predicting and adjusting associations between what is expected from a particular event and what actually occurs allows adaptive learning about potential future events and in predicting future threats that can improve an individual's chances of survival (Sevenster et al., 2014). The failure to correctly predict an outcome drives most updating/ learning and this feature of associative learning is the basis for exposure therapies, i.e. the expected adverse outcome does not occur at the presentation of situations or cues that have previously been associated with a traumatic event and repeated ‘no-fear’ outcomes update expectations until the stimulus-fear association is weakened and the fear/threat response is reduced (Maren, 2011; Sevenster et al., 2012) and see Figure 1.3.1 and Figure 2.1.

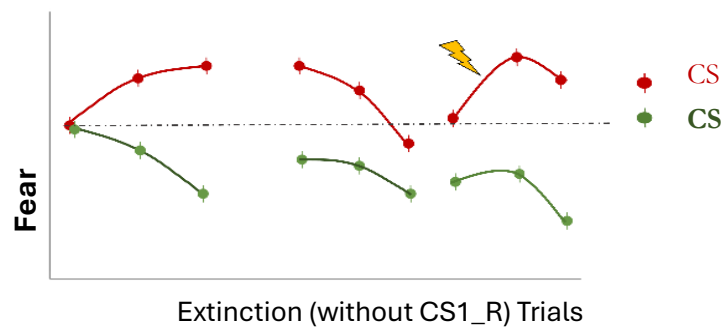
a Retrieval + Extinction Protocol



b Acquisition CS1-R Extinction



c Retrieval + Extinction



d

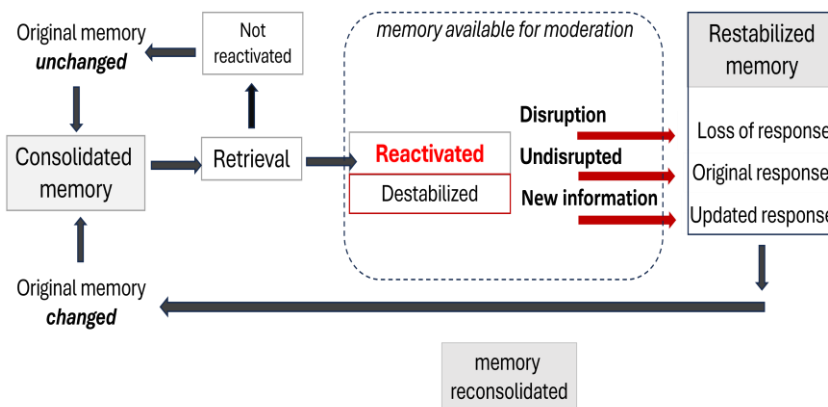


Figure 1.3.1.a Human associative fear conditioning – an example protocol and employing prior retrieval of CS1 followed by standard extinction (PRE) training trials. At acquisition an unconditional stimulus, US, (e.g. aversive shock) is presented concurrent with a relevant conditioned stimulus (CS1) (e.g., spider picture, red line) on a full or partial reinforcement schedule. Another similar unconditioned stimulus (CS2) (e.g. different spider picture, green line) is never paired with the unconditional stimulus. The outcome measure (e.g. explicit, e.g. shock expectancy ratings or implicit, e.g. SCR) is recorded during acquisition, at retrieval of conditioned stimuli (CS-1-R) where CS1 is presented without US followed by extinction trials and during recovery. After extinction training, during which all trials to both CSs are never paired with a shock, participants undergo test of reinstatement (a single representation of the US) to assess the return of the conditioned fear responding to CS1 relative to CS2. Reinstatement measures either implicit (e.g. SCR) or explicit (e.g. Shock expectancy) awareness of the likely learnt contingency over subsequent trials and indicates if extinction memory was disrupted, or if the original aversive memory has been reinstated. **Figure 1.3.1 b, c, d:** graphical example of corresponding outcome measure from shock expectancy ratings (dotted line represents at chance responding) to PRE (a) compared to standard extinction, i.e. without CS1 retrieval prior to extinction (b). It is anticipated that PRE protocol if memory is successfully reactivated at retrieval would protect against return of fear response to CS1 compared to CS2 at test of reinstatement by d) updating original memory rather than creating a new inhibitory memory as occurs with extinction learning without prior retrieval.

Intrusive aversive memories, of the type associated with PTSD and phobia, are similarly linked to re-experiencing of an involuntary conditioned fear response triggered by learned association between an environmental cue associated with the traumatic event (Rothbaum and Davis, 2003). Any cue, particularly if it occurs in time close to the onset of the traumatic event can become a conditioned stimulus/trigger for intrusive memories even though often patients may not be consciously aware of which cues trigger the intrusions and cues may not be obviously related to the original context so that cues are generalised to other stimuli (Debiec et al., 2013). Human associative learning experiments generally focus on what is learnt, consolidated and retrieved (Ney et al., 2022) rather than

the perceptual elements or memory elements more associated with naturalistic, highly subjective, spontaneous aversive memories. Outcomes are typically conditioned responses measured by physiological responses such as skin conductance, potentiated startle eye blink, heart rate, heart rate variability although these outcomes reflect different processes that may dissociate (Lonsdorf and Merz, 2017).

In human studies it is possible to successfully capture expectancy and anxiety ratings through self-reports (Lonsdorf and Merz, 2017). These measures are typically captured over the various memory phases i.e. acquisition, extinction and subsequent retention test (see Figure 1.3.1) to assess the strength or relapse of the inhibitory extinction memory trace under challenge or passage of time (Bouton, 2002; Myers and Davis, 2007). Extinction retention strength can be accessed via return of fear (ROF) manipulations to experimentally induce renewal, reinstatement or spontaneous recovery (Figure 1.3.1). Human conditioning studies use differential stimuli, i.e., the pairing of one neutral or high salience, species fear-relevant, conditioned stimulus (CS1, conditioned stimulus) (Luck et al., 2020), with a an electric shock or loud/aversive noise (unconditional stimulus, US) while another similar stimulus (CS2) is not. The CS2 acts as the safety signal and the differential between the two signals captures fear learning or extinction, while controlling for habituation. In animal models presenting the CS1 in a novel context is more usual than presenting differential cues (Lonsdorf and Merz, 2017). The reinforcement schedule in both models can be total/continuous (the CS1 is always followed by a shock) or partial (some presentations are not accompanied by the US) at varying levels of reinforcement scheduling (Grady et al., 2016). Partial reinforcement schedule at acquisition is thought to prevent extensive habituation to the US and over rapid extinction learning seen with continuous reinforcement during acquisition. Instead, a partial reinforcement acquisition schedule can induce conditioned responding that is more resistant to extinction and less

likely that the retrieval trial, if given, is deemed a first trial of extinction. Reinforcement schedules at acquisition can alter subsequent conditioned fear responding depending on other protocol factors (Kitamura et al., 2020) and, as used in *Chapter 2*, a priori awareness of the CS-US contingency before acquisition learning, i.e. participants are told that CS1 will be but that the CS2 will never be accompanied by a US results in less variance in differential fear conditioning levels. Therefore, associative conditioning is purported to model the acquisition of the learnt link between cues at time of trauma (CSs), the trauma being the US and aversive intrusive memories modelled by the experimental behavioural and physiological outcome measures, the conditioned response (CR) (Ney et al., 2022).

Using variations of the fear conditioning protocol has made it possible to examine a wide range of fear learning related behaviours, measure response outcomes across the learning and memory phases, between individuals as well as to assess different interventions (Fullana et al., 2018; Ney et al., 2019; Schenker et al., 2021; Ressler et al., 2022) to predict fear extinction learning and relapse (Lonsdorf and Merz, 2017). The paradigm's parallels with memory processes activated during exposure based therapies means the fear conditioning paradigm provides an excellent model to explore potential new behavioural and pharmacological manipulators of amelioration of aversive learning in humans (Schiller et al., 2010; Beckers and Kindt, 2017; Ecker, 2021) including targeting epigenetic mechanisms (Kuriyama et al., 2011) and glucocorticoids manipulation (Meir Drexler et al., 2015).

1.3.2 Limitations of Associative fear conditioning

The simplicity of the cues described above allows for controlled exploration of fear learning mechanisms but is also a major limitation of experimental associative designs (Scheveneels et al., 2016; Beckers and Kindt, 2017) alongside the fact that usually any tests of return of fear are within days, weeks at most of their acquisition. Intrusive memories are conceptually similar to experimentally conditioned responses but they are much richer and contained in complex contexts that are not represented in the simple stimuli used experimentally. Additionally, robust trauma memories treated in the clinic are often not days but decades old and research suggests (Gräff et al., 2014) that manipulation of remote memories does not result in the activation of the same neuroplasticity-related genes that occurs in new (i.e. day old) memories. The updating mechanisms being targeted in fear conditioning studies may not be the same mechanisms as those being targeted in exposure therapies, meaning that interventions shown to attenuate day old, simple memories in the lab may not successfully translate into treating decades old, complex real life trauma memories in the clinic. Experimental designs may need to more closely operationalise memories as experienced by clinical populations they hope to help (Walsh et al., 2018; Scheveneels et al., 2021).

1.3.3 Trauma film and complex imagery paradigms

Although the role of emotional memories in psychiatric disorders is widely recognised and there is an extensive literature studying stress effects on voluntary memory recall while distressing involuntary memories of the type elicited in PTSD has until recently often been less studied in experimental studies (Schwabe et al., 2022). This is partly because assessment of involuntary memory relies on verbal report, meaning it

cannot be studied in non-human animals. Human experiments looking at involuntary emotional memory in healthy volunteers use distressing pictures or short video clips, followed by diary-based daily recording of intrusive memories (Wegerer et al., 2014). Recently, more studies have adopted an approach that involves viewing of a ‘stressful’ film depicting a (fictional) narrative of interpersonal violence which better reveals the cognitive, perceptual and physiological responses of participants (James et al., 2016; Iyadurai et al., 2019).

The use of the trauma film paradigm (Horowitz, 1969; Soni et al., 2013; Das et al., 2016; Kamboj et al., 2020) shows realistic visualizations of the type of events likely to be considered traumatic, (i.e. perceived as likely to cause serious injury or to be life-threatening, (DSM-5; American Psychiatric Association, 2013)). The use of moving, realistic or immersive images allows investigation of pre, peri and post responses to a known analogue of trauma, at a defined time relative both to the delivery of an intervention and to collection of outcome measures consistently across participants (Holmes and Bourne, 2008). It has been widely used and has proved to reliably generate similar spontaneous 'intrusive' memories of the type experienced in PTSD (Rattel et al., 2019a) within an experimental setting from which to model memory formation following trauma and how either behavioural (Holmes et al., 2009) or pharmacological interventions (Das et al., 2016; Kamboj et al., 2020) can moderate voluntary and intrusive memories. Other sequelae of trauma can also be captured such as self-reported levels of distress and vividness associated with intrusive memories via diary entries and impact on a range of physiological, behavioural and declarative responses captured in lab before, during and after trauma. It is proving a useful experimental psychopathology model of symptoms following trauma from which to investigate underlying mechanisms that moderate PTSD symptoms (discussed further in *Chapters 3 and 4*) and results from such studies can help

integrate knowledge from associative learning studies and guide future clinical interventions to reduce post trauma symptomology (Lau-Zhu et al., 2021; Ney et al., 2022).

1.3.4 Limitations of the Trauma Film Paradigm

A criticism of standard fear conditioning is that it elicits adaptive aversive memories rather than the overly intense, inflexible, maladaptive post-trauma memories (Beckers et al., 2013) and it is these maladaptive memories that are often resistant to extinction/prolonged exposure and reconsolidation-type manipulation (Gazarini et al., 2023). This criticism could also be levelled at the trauma film paradigm. Although delivering a richer, more complex analogue of fear than fear conditioning models, these studies do not usually report increases in stress hormone levels at post real-life trauma levels, indeed arousal following analogue trauma film, as measured by skin conductance responses, can be lower than for fear conditioning studies (Ney et al., 2022). Intrusive memory levels over the following days are typically low and adaptively, fade rapidly over the subsequent week. However, intrusion frequency is fairly consistent between studies and reporting methods (Rattel et al., 2019a) and frequency is not substantially lower than following real life trauma (Porcheret et al., 2020). The method has also been shown to be predictive of clinical outcomes (Horsch et al., 2017; Iyadurai et al., 2019; Kanstrup et al., 2021); using a spatial-cognitive 'vaccine' to reduce intrusive memories after trauma was developed based on research using an analogue trauma film paradigm (Stuart et al., 2006; Holmes et al., 2009; James et al., 2016) suggesting it's validity as a model for development of other interventions.

1.3.5 Aversive memories and extinction learning

For those with PTSD, trauma memories do not naturally ‘fade away’, suggesting a maladaptive, consolidation of the sensory-perceptual link between trauma cues in the environment and trauma memories these elicit. Invasive, intrusive memories can also be spontaneously recalled in response to *non*-threatening environmental cues due to the formation of a particularly potent stimulus-response to cues relating to events that occurred at the time of the trauma. Most existing exposure-based therapies aim to inhibit these potent fearful, distressing and often highly robust memories by weakening the cued response to the feared stimuli associated with the trauma event using conditioned fear extinction learning principles as described above (Figure 1.3.1). Currently, exposure based therapies are the 'gold standard' in treating anxiety and stress-related disorders. However, through relapse, resistance to treatment or extreme distress during therapy approximately half of patients are unsuccessfully treated long term (Fu, 2023). Therefore, there is a considerable proportion of individuals who require alternative treatment options to ensure long-term fear reduction (Milton et al, 2023).

Why can extinction learning fail to protect against relapse?

Extinction learning creates a new memory rather than altering or replacing the original fear memory, the extinction memory and the original fear memory exist alongside each other and this can prevent stable, long-lasting fear extinction (see Figure 2.1). Even when successfully inhibited following exposure therapy, the original memory of the CS/US memory association can prevail over the extinction memory if, i) the threat or US is experienced again, i.e. reinstatement (Bouton, 2002), ii) spontaneous return over time, the CS or environmental cue can evoke a stress response even if previously inhibited (Quirk, 2002) or, iii) when cues similar to the original fear stimuli are encountered but in a different context to the extinction context, this creates enough ambiguity between the

'safety' extinction memory and the original fear memory to elicit a fear response (Maren and Holmes, 2016).

Extinction of a fear response is controlled by different circuits within the same structures as for threat encoding. Extinction learning aims to enhance top down inhibitory connections from the medial prefrontal cortex to both the basal nuclei and intercalated cells of the amygdala and this reduces output from the central amygdala nuclei to the hypothalamus and to the periaqueductal gray (Myers and Davis, 2007; Maren, 2011; Orsini et al., 2011). Increased inhibitory control of central medial amygdala output allows enhanced contextualization of amygdala-dependent sensory perceptions of the threat memory and reduces fear expression (Sussman et al., 2016) reviewed in Senn et al. (2014). Both animal and human research suggest that in extinction impaired individuals, such as in PTSD patients, there is a failure in these pro-extinction circuits to prevent sustained amygdala activity due to decreased activity in medial prefrontal gyrus (Milad et al., 2014). Adaptive fear learning is impaired if components of or functional connectivity between networks (see section [1.1](#) and Box 1.1.2) is impaired and this results in maladaptive fear responses and resistance to extinction.

Remote memories; Identical extinction protocols that are seen to work in recent memories are seen to fail in remote memories (Costanzi et al., 2011). This may be due to fear memory generalization that occurs over time as the brain areas involved in the storage of a memory change with the age of the memory and different molecular and cellular mechanisms underlie their maintenance. This generalization of memories over time, creates memories that are more gist than detail (see section [1.1.6](#)), allowing the memory to be adaptive as the environment changes (Gräff et al., 2014). However, this makes the original conditioned stimuli of older fear memories harder to recall accurately and may make identifying the correct trigger to target in therapy difficult resulting in unsuccessful

fear memory updating (Silva and Gräff, 2023). Remote memories have been shown to be resistant to modification by reconsolidation methods and recalling remote memories does not engage histone acetylation mediated plasticity (Silva and Graff, 2023; see also section 1.1.7). The criteria for a diagnosis of PTSD is the presence of intrusive memories for longer than 4 weeks meaning that any treatment for existing PTSD needs to meet the challenge of moderating older more robust memories. However, although remote memories are more resistant to established fear reduction methods than recently acquired memories, under the right circumstances, it has been shown to be possible to attenuate return of fear responses to these more robust memories (Silva and Graff, 2023).

1.3.6 Conditions for memory destabilization at retrieval of (aversive) memory

A previously consolidated fear memory can be destabilized by a brief presentation of the conditioned stimulus (CS) generating the reactivation of a memory, rather than purely retrieval. This reactivation can make that memory available to interference or updating before it is then reconsolidated (restabilized). If extinction learning occurs within this post reactivation time window, that is, before the memory is restabilized (thought to be <6hrs), it is possible to alter the original memory trace rather than create a new inhibitory memory as is the case in standard extinction learning protocols (section 1.3.6, Figure 1.3.1). Reconsolidation shares similar but not identical cellular, molecular and neural mechanisms with the original consolidation of memory (Tronson and Taylor, 2007). The distinction between consolidation and reconsolidation may be due to different brain areas or time scales involved, as well as some distinct molecular processes. For example, following reactivation of a memory, the recruitment of dopaminergic, cannabinoid, beta adrenergic and NMDA receptors, and histaminergic signalling occurs

until a transitional 'limbo' state is reached (Merlo et al., 2014) where either reconsolidation or extinction learning could occur. In an animal model, memory lability has been associated with specific biomarkers, such as the reduced expression of specific proteins (e.g. post-synaptic density scaffolding protein Shank (Jarome et al., 2013)). In humans, only retrospective self-reported violations of expectancy have been identified as a proxy for such a biomarker of destabilization (Das et al., 2018) and establishing a reliable behavioural or biomarker for successful memory reactivation has so far proved a challenge for researchers of fear memory updating (Bui and Milton, 2023).

Following successful destabilization of a memory, the memory needs to be re-stored i.e., reconsolidated. Reconsolidation engages L-type voltage-gated calcium channels that result in the plasticity and remodelling required for memory reconsolidation. This includes activation of pathways (see Figure 1.1.3) that regulate translation and transcription (Tronson and Taylor, 2007) such as the transcription factors C/EBP and Zif268 and the kinase ERK, distinct to reconsolidation over consolidation. Other regulators of gene expression such as epigenetic modifications (see section [1.1.7](#)) and hormonal/neurotransmitter regulation associated with memory storage and learning more generally are recruited so that, similar to consolidation, neural synaptic modifications occur following reconsolidation (Burns and Gräff, 2021).

However, the effect of extinction on destabilized memories is complex and influenced by various factors related to the CS/US association, individual characteristics, and the memory manipulation strategies employed. These factors are not fully understood or well-described in the literature (Monfils et al., 2009b; Merlo et al., 2014; Bui and Milton, 2023) but include boundary conditions (see Box 1.3.1) requiring to be met for reactivation (rather than retrieval), destabilisation and potential for memory updating at reconsolidation (Craske et al., 2014).

Box 1.2 *Boundary conditions that influence reconsolidation interference.* (Bui and Milton, 2023)

i. **Timing:** The timing and nature of the associations between stimuli or cues during the original encoding can impact the accuracy of reconsolidation-induced destabilization and can be influenced by the duration of re-exposure to a cue, triggering retrieval, extinction, or neither process (Sevenster et al., 2012; Cassini et al., 2017; Rodriguez-Ortiz and Bermúdez-Rattoni, 2017).

ii. **Associations:** sensory memories and associations made within the time and place they occur can determine whether there is generalization to other elements of a complex memory. Real-life trauma memories involve more numerous associations encoded at the same time during the traumatic event than pairings in classical conditioning studies. These associations can hinder successful reactivation/reconsolidation but also present additional targets for intervention if the appropriate conditions can be identified (Debiec et al., 2013; Maddox et al., 2019).

iii. **Features:** The age and strength of the memory will influence its sensitivity to reconsolidation interference. Intrusive memories, acquired under severe stress as observed in PTSD, are particularly resistant to interference, especially those stored over long periods (Alberini et al., 2006; Gräff et al., 2014; Burns and Gräff, 2021; Chen et al., 2021; Silva and Gräff, 2023).

v. **Context:** The context in which memory reactivation occurs, whether the context is the same or different to when the memory was originally formed, can also play a significant role in destabilization processes. As during the original learning, the contextual cues present during reactivation can influence the subsequent memory updating or reconsolidation processes (Jarome et al., 2015)

iv. **Prediction error:** For memory destabilization to occur it is proposed that a discrepancy between the expected and actual outcomes associated with the presentation of a cue is required. This is often referred to as a prediction error and its violation can lead to processes such as retrieval, reconsolidation, or new learning (Pedreira et al., 2004; Sevenster et al., 2013; Sevenster et al., 2014; Bui and Milton, 2023).

1.3.7 Aversive memory updating during reconsolidation window

Once an (aversive) memory is reactivated and during reconsolidation window (< 6hr) there is a period of heightened, learning-dependent, neuronal plasticity (Tronson and Taylor, 2007) where the original memory's synaptic strength can either be maintained or

altered (Bonin and De Koninck, 2015); a memory can be either strengthened if recall is accompanied by higher valence, unchanged, if no new information is encountered, or updated/weakened if valence is lower at recall (Silva and Gräff, 2023).

At the cellular level, a new cellular tagging technique to 'visualize' aversive memories corroborates evidence from other disciplines that for a traumatic memory to be changed it must be successfully recalled (Josselyn and Tonegawa, 2020). Fear memory recall induces learning dependent synaptic plasticity (section [1.1.2](#)) and subsequent metaplasticity of the fear memory or 'engram' (Finnie and Nader, 2012) i.e. the neural representation of the memory in the brain. By boosting the intrinsic excitability of original fear 'memory' cells via various treatments, it is possible to promote further learning-dependent neural plasticity (Pignatelli et al., 2019) and lasting behavioural change. Animals with impaired fear attenuation have down regulated immediate early genes (IEGs) (Rudenko et al., 2013). IEGs are key in directing transcriptional cascades important for synaptic plasticity underlying learning and memory including associative fear learning (Davis et al., 2003). Additionally, *zif268*, itself an IEG that promotes synaptic plasticity (Figure 1.1.3), has been found to be upregulated following reconsolidation-updating in recent fear memories (Lee et al., 2004). Importantly, using plasticity-promoting treatments, upregulation IEGs parallels an increase in brain-derived neurotrophic factor (BDNF) and long term potentiation (LTP), a *synaptic* correlate of learning and engram modification. The resulting increase in number of dendritic spines and synapses (a *structural* correlate of learning and engram modification) can lead to enhanced fear reduction (McEwen et al., 2012; Gräff et al., 2014). There is also evidence that substantially different neural circuits are involved in reconsolidation-updating from extinction learning (Silva and Gräff, 2023). Reconsolidation targets the original amygdala-cortical fear memory engram while extinction relies on amygdala-

hippocampal-ventromedial prefrontal cortex interactions (Milad et al., 2014). Additionally, differences in aversive engram neural patterns are seen in humans following reconsolidation vs extinction suggesting that alterations in PFC-amygdala connectivity is crucial for reconsolidation-updating particularly of older aversive memories (Björkstrand et al., 2016; Su et al., 2022).

1.3.8 Novel behavioural manipulation of aversive memory as potential treatment for PTSD and anxiety disorders

The goal for a successful therapy would be the complete, permanent eradication of the fear response to the previously feared stimuli (Ecker and Bridges, 2020) whilst leaving the declarative memory of the event unaffected. Since the early studies in animals (Monfils et al., 2009a) and then in humans (Schiller et al., 2010), a number of similar studies in humans (Schiller et al., 2013; Liu et al., 2014; Steinfurth et al., 2014; Agren et al., 2017), and reviewed in Ecker (2021), have reported successful behavioural manipulation to ‘rewrite’ the original aversive memory. Under the right conditions (Figure 1.3.1, Box 1.2), this is reported to result in permanent attenuation of fear responding. A brief retrieval of the target memory prior to extinction learning (PRE) aims to reactivate and make it amenable (labile) to updating/attenuating (Figures 1.3.1), explored in *Chapter 2*. However, there have been some failures to replicate the retrieval plus extinction behavioural strategy to robustly prevent renewal of fear responses both in humans (Golkar et al., 2012; Kindt and Soeter, 2013; Meir Drexler et al., 2014; Chalkia et al., 2020) discussed in reviews (Lee et al., 2017; Elsey et al., 2018) and in animal studies (Schroyens et al., 2021). The inconsistencies in the literature have led to suggestions that the reconsolidation hypothesis itself needs to be reconsidered (Clem and Schiller, 2016; Gisquet-Verrier and Riccio, 2019) although a meta-analysis (Kredlow et al., 2016) found a significant small to moderate effect for post retrieval extinction strategy to reduce return

of fear in humans suggesting its potential for use in clinical practice (Beckers and Kindt, 2017).

1.3.8.1 Behavioural manipulation with histone deacetylase inhibition as cognitive enhancer during retrieval and extinction of conditioned fear.

Attempts to rescue extinction deficits have regularly failed in older memories even with almost identical or modified behavioural reconsolidation protocols to those that were found successful in treating recent memories (Gräff et al., 2014). However, there has been considerable success at using pharmacological administration to *block* post retrieval reconsolidation to attenuate fear memories in humans, notably with propranolol (Kindt et al., 2009; Sevenster et al., 2013; Soeter and Kindt, 2015) as with behavioural manipulation, there are also reported failures in both human and animal studies (Bos et al., 2012; Bos et al., 2014; Thome et al., 2016; Schroyens et al., 2017a) especially in older fear memories (Milekic and Alberini, 2002) although not all (Debiec et al., 2013) cases. The challenge is engaging and targeting the original engram cells because memories that are stored long-term are widely distributed across cortical and thalamic circuits (Frankland and Bontempi, 2005; Wheeler et al., 2013). Targeting the cellular and molecular mechanisms that induce or *upregulate* learning-dependent neural plasticity during reconsolidation could promote the updating of fear and aversive memories, in even extinction-resistant memories of the type associated with PTSD by using histone deacetylase inhibitors as cognitive enhancers to attenuate establish aversive memories.

Gene expression is required to generate the protein synthesis that enables long term memories to be formed or maintained and regulators of such gene expression, such as histone deacetylases, (section [1.1.8](#)) therefore, have influence over memory processes including implications for aversive memories.

Early animal studies showed that treatment with NMDA receptor antagonists (Przybylski and Sara 1997), or protein synthesis inhibitors (Nader et al., 2000) after memory retrieval interfered with memory reconsolidation and impaired subsequent fear memory recall. Alternatively, it may be possible to *enhance* protein synthesis, facilitating long-term potentiation (LTP) and, increase synaptic plasticity that underpins the neuronal plasticity required during extinction post reactivation using a cognitive enhancing drug as an adjunct to the behaviour memory modification strategy, retrieval-extinction (Whittle and Singewald, 2014). By enhancing the updating of the existing aversive memory with new, safety, information into the existing memory during the reconsolidation-updating window, there is the potential to moderate even remote, extinction-resistant memories (Silva and Graff, 2023). Such effects have been reported from studies using histone deacetylase inhibitors (HDACis) such as sodium valproate (Bredy and Barad, 2008), reviewed in Whittle and Singewald (2014) and with NMDA receptor agonists, e.g., D-cycloserine (DCS), for a review see Norberg et al. (2008).

Sodium Valproate (VPA) has been shown to potentiate the inhibitory effects of γ -aminobutyric acid (GABA) and is prescribed as an anti-epileptic, mood stabilizer and prescribed in the treatment of PTSD (Adamou et al., 2007). The drug has a good safety profile, being well-tolerated in most patients with adverse effects being usually mild to moderate and hypersensitivity reactions are rare (Peterson and Naunton, 2005). It has been suggested that enhancement of sodium valproate's GABAergic activity may assist in the initiation of extinction training possibly via its impact on GABAergic signalling through a variety of mechanisms, including blocking voltage gated sodium and calcium channels (Zanatta et al., 2019). It antagonises androgen and progesterone receptors, causing an upregulation of androgen synthesis in the gonads and functions as an aromatase inhibitor of the conversion of testosterone to estradiol (Verrotti et al., 2011). Sodium valproate

(VPA) also functions as a ‘cognitive enhancer’ via histone deacetylase inhibition (HDACi) and upregulation of gene expression and subsequent learning dependent synaptic and neural activity in fear learning brain regions (e.g. hippocampus) during extinction learning (Whittle and Singewald, 2014).

PTSD-like extinction impairments were rescued in rodent studies (Bredy et al., 2007; Bredy and Barad, 2008; Roth, 2013) that explored the HDAC inhibiting properties of VPA, as a psychopharmacological adjunct in the updating of fear memories in a series of studies using fear memory reactivation and extinction protocols. HDAC inhibitors such as VPA (Figure 1.1.3) regulate chromatin condensation (Shahbazian and Grunstein, 2007) and increase transcription of brain derived neurotrophic factor (BDNF) via acetylation in promoter region of BDNF ExonIV gene promoter, as well as upregulation of acetylation of NMDA receptor genes and subsequent long term potentiation (LTP) increase in extinction relevant neural pathways (i.e., the amygdala, ventromedial pre-frontal cortex and hippocampus) following systemic delivery of VPA during extinction learning (Gräff et al., 2014; Silva and Gräff, 2023). In animal models it was shown to enhance extinction and prevent return of behavioural fear responding (Bredy et al., 2007; Bredy and Barad, 2008) that resulted in long lasting attenuation of conditioned fear although its effects were highly dependent on protocols used. Animal studies suggest that, under the right conditions (Bredy et al., 2007; Bredy and Barad, 2008), HDAC inhibition can promote the structural and epigenetic plasticity occurring during memory updating (Vecsey et al., 2007) to enhance attenuation of conditioned fear responses at test of reinstatement, potentially via upregulate histone acetylation-mediated neuroplasticity in the hippocampus (Huang et al., 2020) and BDNF in the prefrontal cortex (Bredy et al., 2007). There are limited results from the human literature although two studies that used VPA (400mg) in conjunction with standard extinction protocols (Kuriyama et al., 2011;

Kuriyama et al., 2013), rather than post retrieval extinction (PRE), found similar results to animal models (Bredy et al., 2007; Bredy and Barad, 2008) with a prevention of physiological (skin conductance) fear responding 24 hours after extinction learning, although explicit fear responses were not collected. Building on previous findings, *Chapter 2* investigates whether sodium valproate, a psychopharmacological cognitive enhancer and HDAC inhibitor, can synergistically enhance the attenuation of conditioned fear responses when used in combination with a behavioural reconsolidation protocol (i.e. a strategy aimed at timepoint C in Figure 1.1.2).

1.3.9 Novel psychopharmacological interventions to prevent development of persistent aversive memories

Spontaneous, intrusive memories of the type experienced in those with long-term PTSD, are potent and once established makes their treatment particularly challenging. It would therefore be highly beneficial if it were possible for those types of long-lasting sensory-perceptual memories and re-experiencing symptoms to be prevented before they could be fully consolidated and dispersed in long term memory. As described in section 1, the effect of the stress response to trauma on memory formation is a complex “neuro-symphony of stress” (Joëls and Baram, 2009; Joëls et al., 2012) which makes it a challenge to moderate successfully. However, the complex relationship between the timed orchestration of the elements of the stress response and subsequent emotional memory outcomes presents potential targets that may be moderated to prevent the development of robust, difficult to treat intrusive memories.

1.3.9.1 Moderating cortisol levels using hydrocortisone after trauma event as preventative strategy for persistent aversive memories (Chapter 3)

Our understanding of how the stress response acts on glucocorticoid receptor-rich brain regions (i.e. hippocampus, especially the dentate gyrus, basolateral and central nuclei of the amygdala and prefrontal cortex) to influence neural network activity in the initial stages of memory consolidation (McEwen et al., 2016; Meir Drexler and Wolf, 2017; Schwabe et al., 2022) and on subsequent retrieval (Wolf, 2017), suggest an opportunity to pharmacologically intervene at the early stage of the consolidation period to prevent formation of maladaptive fear memories but after a trauma has occurred. There is also a considerable benefit in cross-pollination of ideas studying the moderation of both long term and newly acquired aversive memories.

As with reactivation-extinction protocols (section [1.3.7](#)), there is a brief (< 6 hour) opportunity for intervention immediately after a stressful event when protein synthesis and synaptic changes are occurring before a memory becomes fully developed (Dudai, 2012). During a stress response the co-ordinated response of noradrenaline and high levels of glucocorticoids act synergistically to enhance the memory of salient elements of the event (Joëls et al., 2011). However, if high levels of glucocorticoids are present outside of the noradrenaline response and temporally close to initial glucocorticoid rise then sensory-perceptual memory of the event can be attenuated (de Quervain et al., 2017). This time window immediately following a trauma event therefore presents an opportunity to behaviourally (James et al., 2016) and potentially pharmacologically moderate the consolidation of trauma memory particularly intrusive memories of the type associated with PTSD (Astill Wright et al., 2019; Iyadurai et al., 2019).

Results from small scale clinical studies from emergency settings following traumatic events (e.g. motor accidents) showed that a single high dose of cortisol within

6hrs of the event did reduce the risk of developing PTSD (Zohar et al., 2011). Other studies reported reduced risk of PTSD following repeated high dose cortisol treatment within 12 hours of high risk medical treatments (Schelling et al., 1999; Schelling et al., 2001; Schelling et al., 2004; Weis et al., 2006) or after traumatic injury (Delahanty et al., 2013) with two meta-analyses finding small to large effects of hydrocortisone to reduce PTSD symptoms (Sijbrandij et al., 2015; Astill Wright et al., 2021). Experimentally, altering the glucocorticoid response to stress by using a single, stress level dose of the drug hydrocortisone (30mg), the tablet form of endogenous cortisol, following viewing a distressing film (Kamboj et al., 2020), but not before (Rombold et al., 2016b) has been shown to attenuate subsequent intrusive memories in human and animal experimental studies (Zohar et al., 2011).

Chapter 3 reports the results of hydrocortisone administration immediately after a trauma-like event to examine the effects of supraphysiological levels of exogenous cortisol (glucocorticoids) on the frequency of spontaneous sensory-perceptual memories and re-experiencing symptoms over the following week (i.e. a strategy aimed at timepoint B in Figure 1.1.2). Previous work on which this extends was conducted in women taking hormone contraceptives to control for fluctuations in female hormones across the cycle (Kamboj et al., 2020). As discussed in section 1.2, stress and gonadal hormones may interact to influence fear learning and intrusive memory (Kok et al., 2016; Hsu et al., 2018; Hsu et al., 2021) therefore, the experimental study described in *Chapter 3* extended this previous research to include men and baseline salivary gonadal hormone levels were sampled in both sexes.

Hydrocortisone – is the exogenous form of the human hormone cortisol (steroid glucocorticoid) and is a widely prescribed drug for both its anti-inflammatory and immunosuppressive activity in a range of physical disorders. It has the same mechanism

of action as endogenous cortisol and at an acute, one off dose raises the bioavailable levels of cortisol and following the administration of the tablet form of hydrocortisone, peak plasma levels will be reached in approximately 60 minutes augmenting current endogenous levels (Jung et al., 2014). Endogenous levels will fluctuate through the day (Lightman and Conway-Campbell, 2010) following a circadian rhythm (at a nadir between 11pm and 1am (within the range 0.5-9.7nmol), peaking early morning between 6 and 9am (range 4.5-25.3nmol) (Dahlgren et al., 2009) as well as oscillating considerably within that rhythm due to ultradian pulsatile fluctuations (Lightman and Conway-Campbell, 2010) which have greater frequency and amplitude in the morning hours (in humans).

Endogenous cortisol is secreted as a final product of the HPA axis (Figure 1.1.1) and blood cortisol levels can increase 6-12 fold in response to maximal stress (Dorin et al., 2012). Cortisol exerts negative feedback which subsequently has inhibitory effects at pituitary and hypothalamic levels (section [1.1.1](#)). Hydrocortisone is a relatively cheap drug, already in use in emergency clinical practice as an anti-inflammatory and for use as a single-use potential preventative measure post-trauma could be used safely in a wide demographic and potential sex differences in response to treatment will be discussed further in *Chapter 3*.

1.3.10.2 MR antagonism using spironolactone as a moderator of aversive memory development when administered prior to a threatening event (Chapter 4)

As described (section [1.1.2](#)) stress induced levels of cortisol interact with two receptor types – glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) to influence subsequent behaviour and memory for the event (de Kloet and Joëls, 2020; Karst et al., 2022). The crucial role of MRs in emotional memory processes is starting to

be better elucidated although the effects of stress on encoding/early consolidation of involuntary memory (rather than voluntary memory) are not fully determined nor the differential role of MR versus GR in the aetiology of PTSD (Vogel et al., 2016; Kanatsou et al., 2019; de Kloet and Joëls, 2023).

When bound to cortisol, intracellular mineralocorticoid (MR) and glucocorticoid (GR) receptors act as transcription factors, mediating slow genomic responses during stress adaptation and moderating tonic changes in basal cortisol levels. Alternatively, membrane-bound versions of MR/GR mediate rapid, non-genomic effects on neuronal excitability (Groeneweg et al., 2012), see also section 1.1.2. Under acute stress conditions MR response moderates activity in basolateral amygdala and hippocampal (CA1 and dentate gyrus) processing where density of MRs is prevalent (Karst et al., 2005) in concert with subsequent glucocorticoid receptor activity (de Kloet and Joëls, 2020). The rapid action of MRs during acute stress conditions aligns with the need for quick adaptations to stressful situations, potentially shaping the immediate emotional and cognitive responses to acute stress and subsequent memory of the event (Wingenfeld and Otte, 2019). However, maladaptive aversive memory formation is characterised by fragmented voluntary memory and exaggerated perceptual priming that has been linked to an over reliance on stimulus response learning and reduced ‘cognitive’ learning (Wingard and Packard, 2008; Brewin et al., 2010). Therefore, while a time limited MR mediated shift to fast stimulus-responding in the face of threat is adaptive (Schwabe et al., 2013; Schwabe and Wolf, 2013) dominance of or prolonged MR activity at stress onset can result in an increase in re-experiencing a trauma episode and increase in frequency of distressing and vivid intrusive memories.

Therefore, it is important to determine the co-ordinated yet differential effects of GR- and MR-mediated stress effects on aversive sensory-perceptual and voluntary

declarative memory formation for both a better theoretical understanding but also for potential use as targets for psychopharmacological moderation.

Spironolactone a mineralocorticoid receptor (MR) antagonist, competitively blocks aldosterone, a key component of the renin-angiotensin-aldosterone system, regulating electrolyte balance via peripheral MRs (Patibandla et al., 2020). Within the brain MR's, respond to the much higher concentration of cortisol to aldosterone, and act at both non-genomic and genomic timeframes (de Kloet and Joëls, 2020) as describe above. Clinically, spironolactone is used as a potassium-sparing diuretic principally for management of cardiac failure (Cannavo et al., 2018) and is well tolerated. Experimentally, MR antagonism, prior to a stressor in men, increased cortisol availability and impaired working memory (Cornelisse et al., 2011a) under stress suggesting involvement of MR involvement in regulating HPA activity during acute stress to influence domain specific memory performance (Cornelisse et al., 2011a).

MRs play a role in early amygdala (basolateral)-driven stress appraisal (Karst et al., 2022) which may modulate the vividness and reliving of traumatic intrusions (Brewin et al., 2010). While MR hippocampal dependent effects on emotional memory (Groeneweg et al., 2012) may differ from those on voluntary declarative recall (Cornelisse et al., 2011a). Further research is needed to understand its role in post-trauma responses and interactions with glucocorticoid receptor activity and gonadal hormones (de Kloet and Joëls, 2020) Given its low cost, safety, and availability (Accord-UK.Ltd, 2022). spironolactone could be explored as a pre-trauma intervention for individuals at high risk of stress exposure, such as first responders or complex surgical patients, (a strategy targeted at timepoint A in Figure 1.1.2). This will be examined further in *Chapter 4*.

Chapter 2

Epigenetic regulation and updating of
memory in a model of anxiety disorder in
healthy men and women

2.1 Introduction

Synaptic plasticity and learning enable rapid behavioural change in the face of threatening or noxious stimuli. These abilities provide humans with a highly adaptive advantage in facing similar future threats, to minimise future harms. However, such adaptive capacity may come at a cost. Extreme emotional or traumatic events can hijack these learning mechanisms and rapidly generate highly potent, distressing memories which become consolidated into long lasting, maladaptive threat memories that are resistant to current treatments aimed at their extinction. Such memories are a core feature of stress disorders such as PTSD which is partly characterized by chronic, intrusive or ‘flashback’ memories of the emotional event (Brown and Kulik, 1977). The intrusive sensory-perceptual memories are thought to perpetuate PTSD by maintaining hyperarousal, stress and re-experiencing of fear responses. Existing behavioural exposure based therapies, aim to suppress fear responses to stimuli associated with traumatic events via extinction learning, the repeated presentation of feared stimuli in the absence of harmful outcome. Extinction learning operates by creating a new inhibitory ‘safety memory’, which leaves the original threat memory intact but suppresses its retrieval (Bouton, 2002). This is effective in some cases, but results in high relapse rates (Olatunji et al., 2010) via spontaneous recovery, renewal and reinstatement of threat associations. Long term efficacy for treatment currently sits at best around 50%, highlighting a considerable need for more effective and long-lasting methods to silence these highly distressing decontextualized memories.

2.1.1 Reconsolidation-update approaches to silencing maladaptive memories

Prior research has examined the potential of combined pharmacological-behavioural approaches to enhance the permanence of suppressing sensory-perceptual elements of aversive memories (Whittle and Singewald, 2014; Walsh et al., 2018; Singewald et al., 2023). One promising mechanism is via the manipulation of memory reconsolidation mechanisms; whereby consolidated memories may be directly altered (Figure 2.1a). Specifically, using a ‘reminder’ to reactivate a memory in the presence of new information may make that memory unstable and liable to direct updating with new ‘safety’ learning (i.e. extinction), prior to the memory being *reconsolidated* (Nader et al., 2000). This ‘retrieval-extinction’ procedure (PRE) (Schiller et al., 2009; Schiller et al., 2012) requires only a modification of existing extinction therapies (Figure 2.1b), but there is evidence from animal (Monfils and Holmes, 2018) and human experimental studies as well as in clinical settings (Kredlow et al., 2016; Astill Wright et al., 2021; Ecker, 2021) this can produce a long-lasting reduction in conditioned fear responses that are resistant to reinstatement and renewal effects (Beckers and Kindt, 2017). However, not all studies have been able to demonstrate retrieval-extinction benefit over extinction alone with or without pharmacological adjunct (Schroyens et al., 2017b; Rotondo et al., 2022) and with some direct replication failures (Chalkia et al., 2020). This has led to the identification of certain key parameters that determine which of several mutually exclusive memory processes are activated following reminders: Pure retrieval, destabilisation/reconsolidation, ‘limbo’ or extinction (Merlo et al., 2014).

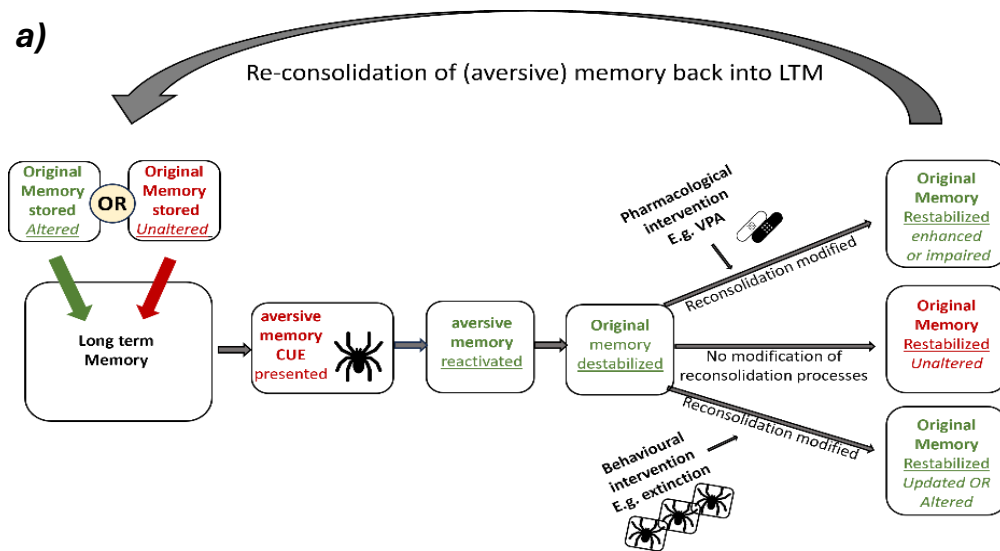


Figure 2.1a) Reactivation of a fear memory prior to either pharmacological or behavioural interference or updating (or a combination) can lead to altered reconsolidation of the fear memory engram and reduce fear response and return of fear effects

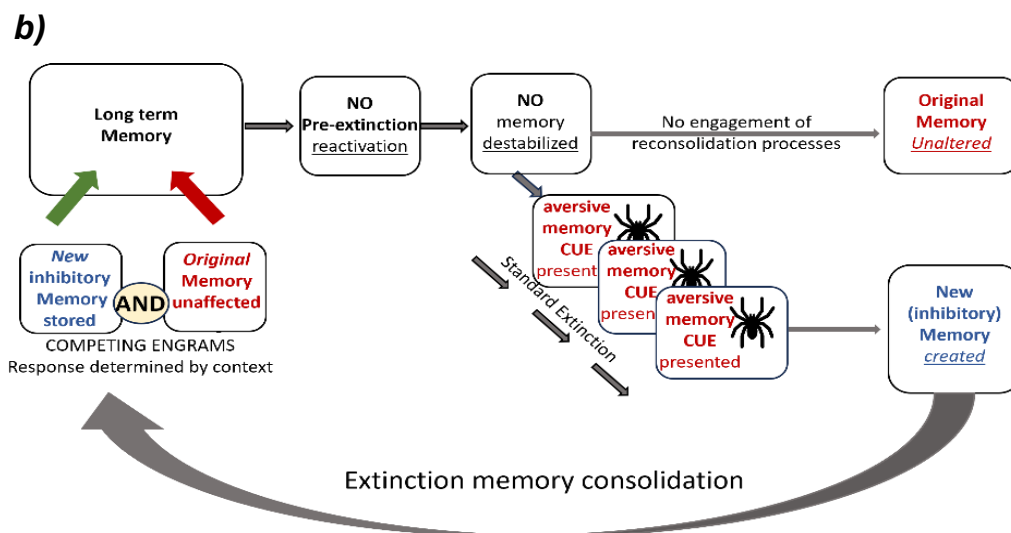


Figure 2.1 b) Standard extinction creates a new ‘safety’ memory in response to the fear cue that competes and inhibits fear responses but does not alter the original fear memory engram. Modified from (Paulus et al., 2019)

2.1.2 Boundary conditions constraining memory destabilisation at retrieval

A key parameter determining the switch between these processes is ‘prediction error’; a mismatch between learned and actual outcomes upon exposure to feared stimuli (Chen et al., 2021). When a previously consolidated memory is retrieved with the ‘right’ degree of prediction error (Sevenster et al., 2012; Sevenster et al., 2014) it may destabilise and undergo update and reconsolidation. During the ‘reconsolidation window’ – thought to last up to 6 hours (Monfils et al., 2009b), during which the memory is labile, it may be updated with new information prior to restabilising into long-term storage. In contrast, extinction occurs following repeated presentation of a CS in the absence of the US, leading to multiple prediction errors and a gradual acute reduction of the fear response through inhibition of the original fear memory (Bouton, 2002). However, if extinction occurs *following* memory destabilisation, i.e. within the ‘reconsolidation window’ - the result may be an updated, CS-US association which, in some cases, is resistant to return of fear (Monfils and Holmes, 2018).

A current obstacle to clinical implementation of these approaches is the fact that memory destabilisation is not reliably triggered by reminders. Rather, several ‘boundary conditions’ constrain memory destabilisation (Bui and Milton, 2023). One of these is memory strength and age, with more remote and entrenched memories being more resistant to destabilisation. This may mean that long-held fear memories that may have been retrieved and reconsolidated many times over decades, as can occur in untreated PTSD, may not be successfully targeted with retrieval -extinction procedures alone (Silva and Gräff, 2023). An intriguing possibility to surmount this issue is by ‘priming’ the cellular plasticity mechanisms that subserve memory destabilisation. For example, by

introducing pharmacological adjuncts that affect histone acetylation and methylation, alongside the novel behavioural approach, it may be possible to target the epigenetic transcriptional mechanisms that long term memory plasticity ultimately relies upon (Bredy and Barad, 2008).

2.1.3 Epigenetic priming and memory reconsolidation

In the absence of nuclear signalling cascades that initiate transcription and protein synthesis; DNA is tightly wound into a complex with histone proteins called chromatin (Figure 2.2). When protein synthesis is required (such as during reconsolidation) histone acetyltransferases (HATs) cause the euchromatin complex to relax into an open chromatid state allowing transcriptional machinery access to the genetic code required for RNA synthesis. Following translation, histone deacetylases promote the recoiling of the DNA chromatin, preventing ongoing transcription/translation and further learning (Vecsey et al., 2007). Histone deacetylase inhibitors (HDACis) prevent this recoiling from active to ‘silent’ chromatin and maintain DNA in an accessible state. This provides an opportunity to enhance memory-related synaptic plasticity in engram networks by ‘epigenetically priming’ (Figure 2.2c) the translation of required plasticity proteins (Gräff et al., 2014; Silva and Gräff, 2023), potentially leading to long-lasting, more efficacious ‘corrective learning’ interventions, even for older more remote memories that are more resistant to plasticity (Stafford and Lattal, 2011; Stafford et al., 2012). Sodium Valproate (VPA), a commonly used anti-epileptic and mood stabilizer, acts as histone deacetylase inhibitor (HDACi) along with its other central pharmacological mechanisms (largely GABAergic) and could potentially be used to boost the efficacy of interventions that rely upon memory formation, destabilisation or reconsolidation (Bredy et al., 2007; Bredy and Barad, 2008).

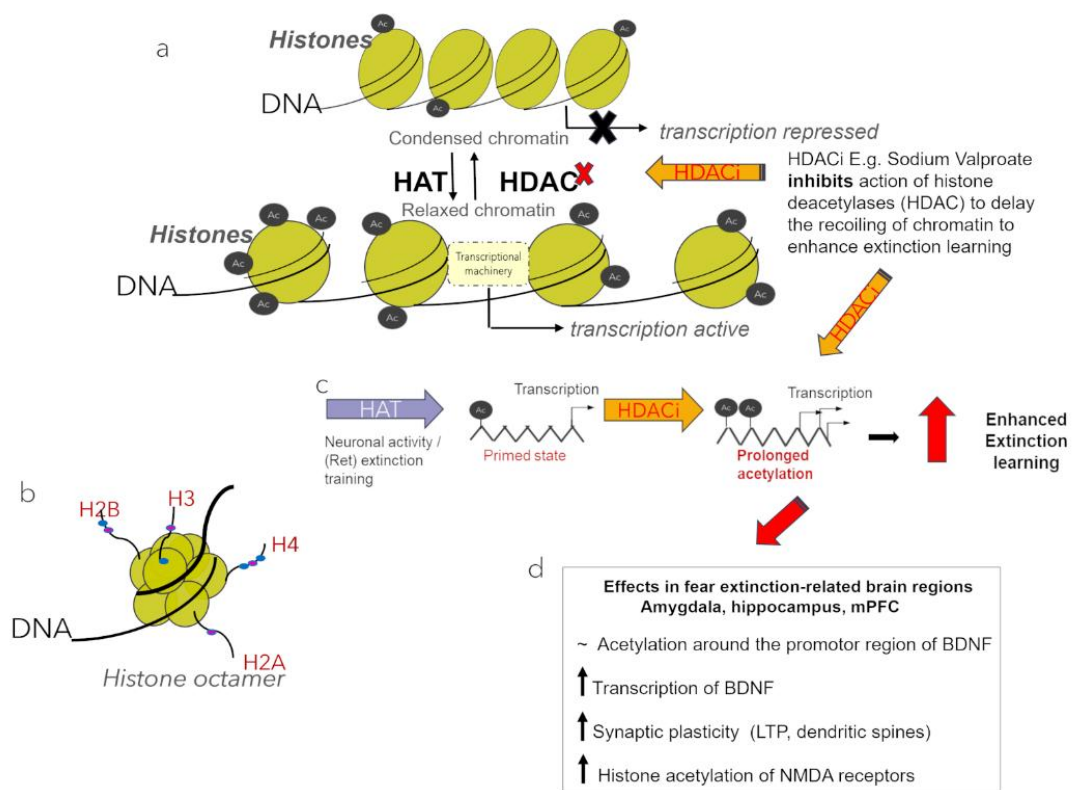


Figure 2.2 Epigenetic regulation of extinction-related brain regions in response to extinction learning. *a*) Depiction of the condensed chromatin. The histone octamer is a tightly wound DNA complex of histone proteins. *b*) Moderation by gene expression through epigenetic changes (e.g. addition methyl (blue circle) or acetyl groups (pink, black circles) to lysine residues on histone tails). The condensed state persists when transcription machinery is silent and until protein synthesis is required (such as during extinction) when histone acetyltransferases (HATs, also called ‘writer’) cause the condensed chromatin complex to relax, allowing transcriptional machinery access to the genetic code and learning to occur. Following translation, histone deacetylases (HDACs, ‘erasers’) promote the recoiling of the DNA chromatin, repressing transcription and further learning (Vercsey et al., 2007). Histone deacetylase inhibitors (HDACis, e.g. Sodium Valproate) prevents this recoiling from active to ‘silent’ chromatin and maintains DNA in an accessible state *c*) The ‘priming’ by neural activity and prolonged HAR response (Burns and Graff, 2022) following HDACi action increases acetylation and transcription of BDNF and enhances synaptic plasticity in fear extinction related brain regions. *d*) leading to enhanced, robust extinction learning (Bredy et al., 2007; Wang et al., 2022) schematic adapted from (Whittle and Singewald, 2014)

This possibility has been empirically demonstrated in several preclinical paradigms. Lattal and colleagues (2008) found that systemic or intra-hippocampal administration of the HDACis sodium butyrate or Trichostatin A immediately prior to a brief (3 min) extinction session produced a reduction in contextual fear responding of the magnitude normally only seen following a long (24 min) extinction session, consistent with potentiation of extinction through HDAC inhibition.

Reactivation and reconsolidation of a memory similarly relies on transcriptional processes (Tronson and Taylor, 2007). The administration of an HDACi such as VPA prior to memory reactivation would be expected to act synergistically with the retrieval-extinction procedure; either by enhancing the reconsolidation of the updated memory trace or lowering the ‘threshold’ for the memory to destabilise at retrieval. A lack of memory destabilization at retrieval has been suggested to underlie previous failures with the retrieval-extinction protocol (Gräff et al., 2014). Priming the epigenetic mechanisms necessary for memory reconsolidation rendered remote fear memories susceptible to update via a retrieval-extinction procedures; something which was not achieved with retrieval-extinction alone. Immunohistochemical analysis in this study revealed that retrieval alone failed to induce histone deacetylation-dependent synaptic plasticity in remote fear memories. However, the exact same retrieval procedure performed after intraperitoneal administration of a class I specific HDACi produced the same levels of fear reduction in remote memories as for recent memories following the retrieval-extinction procedure (Graff et al., 2014). Given that impaired extinction learning and fear inhibition (Singewald et al., 2015; Ell et al., 2024) are predictors of symptom persistence in PTSD and anxiety disorders (Jovanovic and Ressler, 2010; Sijbrandij et al., 2013), these effects with HDACis suggest a promising means to enhance the efficacy of memory

therapeutics in PTSD, by making robust trauma memories amenable to reconsolidation-based intervention.

Although specific HDACis are not licensed for human use, sodium valproate (a common mood stabiliser and anti-epileptic) exhibits class I and IIa HDACi inhibitor activity that acts on *BDNF* promotor I and IV and has been shown capable of enhancing extinction and reconsolidation of fear learning in rodent model (Bredy and Barad, 2008). A recent review highlights epigenetic changes in BDNF, e.g. of *Bdnf* exon-IV, in a rodent model, as being associated with memory, stress, and neuropsychiatric disorders in humans (Cao-Lei et al., 2022). In line with animal studies (Bredy et al., 2007; Bredy and Barad, 2008; Sah et al., 2019), the first human trials of memory modulation by VPA have shown encouraging results (Kuriyama et al., 2011; Kuriyama et al., 2013). A single dose of VPA in conjunction with extinction attenuated the reinstatement of fear conditioning, suggesting VPA may enhance the decoupling of the US-CS association during extinction learning in humans (Kuriyama et al., 2011) and prevent reinstatement of fear. This study capitalised on these properties of sodium valproate to assess potential epigenetic modulation of reconsolidation-update mechanisms in humans.

2.1.4 Sex differences in histone modification

Pre- and neonatal research has shown important developmental sex differences in histone modification. These are predominantly driven by steroid hormone activity through their enzymatic involvement in acetyl and methyl epigenetic changes, (e.g. in estrogen and androgen receptor gene interactions with histone modifying enzymes in many brain regions (Tsai et al., 2009). These interactions result in and maintain sex differences in the brain. Patterns of histone acetylation associated with specific genes are thought to be

dictated by gonadal steroid hormones and receptors together with histone acetyltransferase (HAT) or HDAC activity of coactivators and corepressors (McCarthy et al., 2009). In this way, testosterone-regulated histone acetylation results in epigenetic changes that drive ‘masculinization’ of a bed nucleus of the stria terminalis (BNST), involved in adaptive modulation of stress responses via its role in regulation of HPA (Choi et al., 2007; Choi et al., 2008). Additionally, HDAC inhibition, via VPA administration, has been shown to disrupt this epigenetic control of this sexual differentiation of the BNST (Murray et al., 2009). Alterations in histone proteins have also been reported in patients in a range of psychiatric disorders with age (Tang et al., 2011) and sex-specific effects (Qureshi and Mehler, 2010).

Furthermore, at the behavioural level there is previous research showing sex differences in fear conditioning in PTSD (Inslicht et al., 2013) with brain regions involved in both fear conditioning and PTSD status (e.g. amygdala, ventral medial PFC and hippocampus) being sexually dimorphic (Goldstein et al., 2001). Sex or sex hormone effects on responses to fear stimuli have been reported across all fear memory phases (Garcia et al., 2018) although there are only a small number of studies that compare the sexes directly, especially in exploring fear discrimination specifically (Day and Stevenson, 2020). Men have shown enhanced skin conductance response during fear acquisition compared to women (Milad et al., 2010) with sex differences in stress-arousal neurocircuitry during fear conditioning and extinction (Lebron-Milad et al., 2012). Women subjectively rate fear higher than men (Lonsdorf and Merz, 2017) and some studies have shown stronger conditioned stimulus differentiation between conditioned fear stimuli (CS⁺) and safety stimuli (CS⁻) due to reduced response to safety signals in women. As such, it is important to examine sex differences when investigating potential HDAC-modulatory effects of VPA on fear memory.

2.1.5 The current study

The current randomised, placebo-controlled study uses a model of threat learning (Pavlovian fear conditioning) to test the epigenetic regulation of memory extinction and reconsolidation in men and women and its potential as an addition to current therapies to manipulate maladaptive fear memories. A factorial design was employed, examining two memory processes (classical extinction vs. retrieval-extinction) in combination with drug or placebo, to tease apart the effects of VPA on each process.

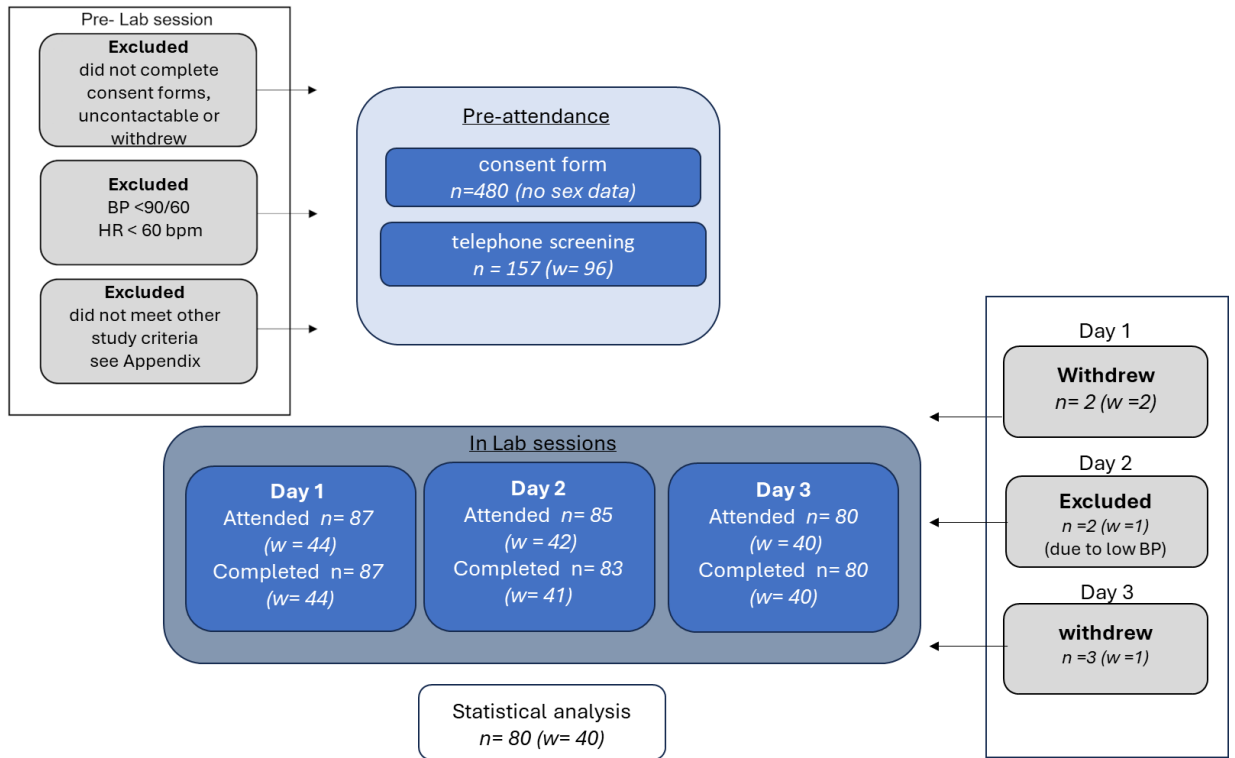
In line with Kuriyama and colleagues, VPA was hypothesised to enhance extinction, resulting in greater protection against return of fear at test following a ‘standard’ extinction paradigm and in line with Graff and colleagues (Gräff et al., 2014). VPA was hypothesised to enhance the labialisation potential of consolidated fear memories upon retrieval, resulting in greatest protection against fear memory recovery when retrieval-extinction is combined with VPA. There is not enough prior research to hypothesize a direction of effect between the sexes instead exploratory analysis of the data was conducted to inform future research.

2.2 Materials and Methods

2.2.1 Participants and Design

Eighty participants (men = 40; women = 40) were recruited via on-campus in UCL and via online advertising. All participants provided fully informed and written consent prior to participation. Participant ages ranged from 18 to 55 (mean $\pm$ SD age, 27.5 ± 9.5 years). Inclusion and exclusion criteria (full list in appendix 2.1) were assessed via telephone pre-screening interview and inclusion criteria included but not limited to (i) ages 18-65 (ii) normal body mass index ($>18<30$), no current or pre-existing medical conditions, (iii) contraindications to use of a single 400 mg dose of Sodium Valproate, iv) not pregnant or breast feeding (v) blood pressure $<90/60$ and heart rate < 60 bpm at drug administration, two participants (1 man, 1 woman) were excluded for this reason (Figure 2.3). Three women and two men withdrew their participation from the study and were replaced. Participants were paid compensation for their time. Medical oversight was available throughout and the study was approved by the UCL Research Ethics Committee (Project ID Number: 3901/002) and conducted in accordance with the Declaration of Helsinki (see appendix 2.2).

Participants taking part in the study were randomly assigned to one of four groups in a 2 (drug condition) x 2 (memory intervention) factorial design: The cells of the design matrix were placebo+ extinction (PBO+EXT), placebo+post-retrieval-extinction (PBO+PRE): sodium valproate+extinction (VPA+EXT) or sodium valproate+post-retrieval-extinction (VPA+PRE). Equal numbers of men and women were assigned to each group.



BP = blood pressure, **HR** = Heart rate, **W** = Women

Figure 2.3 Participant flow diagram depicting participant recruitment from response to advertisement to inclusion in final data analysis. Two participants, one man and one woman (W), were excluded before drug administration due to BP <90/60 on Day 2.

2.2.2 Stimuli

Conditioned stimuli (CS) were species-relevant fear stimuli (i.e. pictures of spiders; IAPS numbers 1200 -1201), strengthening conditioned fear acquisition (Mineka and Öhman, 2002). A partial reinforcement schedule (62.5%) during acquisition was used to generate more CS-US associations that were more robust to prediction error and prevent the reactivation trial on Day 2 acting as an extinction trial (LaBar et al., 1998). One of the pictures (CS1) was accompanied by the US on 62.5% of its presentations while the other 37.5 % were not reinforced. The other picture (CS2) was never accompanied by the US. Picture assignment to each CS was counterbalanced across participants.

The UCS was a 10ms square pulse electric shock delivered to the left wrist via Ag/AgCl electrodes. The intensity of the US was calibrated to each participant by gradually increasing the level from 20V in 5V increments delivered to the left wrist for 10ms. The level of shock was set at a moderately aversive level (i.e., seven on a scale of 1, not at all painful, to 10, maximally painful) and at which each participant determined to be ‘uncomfortable but not painful’. The intensity was calibrated prior to acquisition (Day 1) and remained the same over the following days. Startle probes were 40ms bursts of white noise at 104 dB with a near-instantaneous rise time, presented via on-ear headphones.

All CSs were presented centrally on the screen for 8s. On trials with startle probes, these sounded 7 seconds after CS onset. On CS1+ /US trials, the US was delivered 500ms after CS1⁺ had left the screen (Figure 2.4). Startle probes were also occasionally administered during intertrial intervals (Noise Alone; NA) following (Kindt and Soeter, 2013). ITI length was quasi-randomised across trials (5,10 or 15ms).

2.2.3 Measures of conditioned fear response:

Skin conductance response: Skin conductance responses (SCR) were recorded using the same BiopacTM MP150 and EDA100C unit with wet gel foam electrodes attached to the middle phalanges of the index and middle fingers of participants’ left hands. Offline analysis was conducted using the Acknowledge 4.0 software package with automated peak detection routines cross-checked with visual inspection of phasic SCRs. Event-related SCRs were taken as phasic rises >0.02 mSiemens between 1 and 7 seconds post-CS onset.

US-Expectancy measures: Declarative fear assessments (shock expectancy) were measured during presentation of each conditioned stimulus. During presentation of each CS participants were asked to rate the expectancy of receiving a shock by pressing

computer keys numbered on a scale from -5 (certainly not receiving a shock) through 0 (uncertain) to + 5 (certain to receive a shock). Participants needed to make their assessment within 5s of CS appearing on the screen, that is, 2s prior to the delivery of the startle probe (Figure 2.4).

2.2.4 Subjective assessments

Trait measures of anxiety were assessed at the start of each session using trait version of the State and Trait Anxiety inventory, STAI; (Spielberger, 1983), Anxiety Sensitivity Index, ASI, (Reiss et al., 1986), state affect with the Positive and Negative Effect Schedule, PANAS, (Watson et al., 1988) and Brief Mood Introspection scale, BMIS, (Watson and Tellegen, 1985). Beck Depression Inventory, BDI, (Beck et al., 1996) and Davidson Trauma scale, DTS, (Davidson, 1996) were completed on Day1 only. In addition, levels of spider fear were assessed on each day using the Spider Phobia Questionnaire ,SPQ, (Klorman et al., 1974). On Day 2, participants reported their subjective state using a Bodily Symptom Scale, BSS, completed immediately prior to and 60 mins following administration of VPA/PBO.

2.2.5 Drug preparation

Drug administration was oral and double-blind. Sodium valproate (400mg) and placebo (lactose powder) were in identical opaque capsules. Drug condition was pre-randomised and drugs sealed in a numbered envelope by an experimenter not involved in the study. The appropriate envelope was given to the experimenter conducting the data collection on the day. Participants were instructed to take the capsules directly from the envelope so as to minimize unblinding of either participant or experimenter.

2.3 Experimental procedure

2.3.1 Acquisition (Day 1)

Following informed consent, blood pressure and heart rate was checked. Participants then completed the STAI, SPQ, ASI, PANAS, BMIS, BDI and DTS and were instructed on how to provide the US expectancy measures that were to occur over the learning task (Figure 2.4).

Participants were informed that two pictures would be presented on the computer screen and that one picture would sometimes be followed by a shock and one picture would never be followed by a shock. They were asked to try to learn over the course of the trials when a picture would be followed by a shock and to indicate how certain they were of receiving a shock using the US expectancy rating scale. Acquisition consisted of 8 CS1 trials (5 CS1⁺ paired with shock), 8 CS2⁻ trials (never paired with shock) and 8 NA (noise alone) trials in a fixed, pseudorandomised order. At the end of acquisition, participants were explicitly asked to remember what they had learnt to promote a stronger memory of the CS/US association (Norrholm et al., 2006).

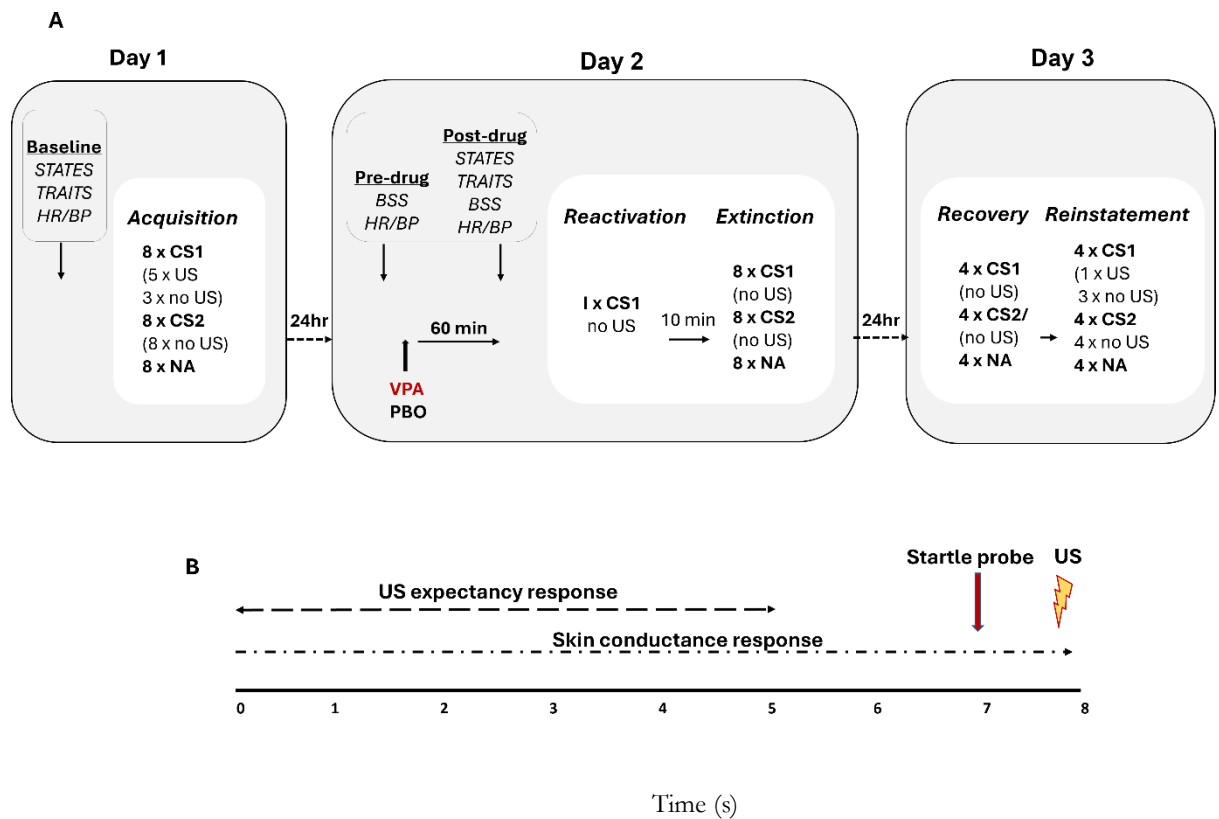


Figure 2.4 Schematic representing three day protocol: Day 1, differential conditioning acquisition. CS1 represents US reinforced conditioning trials at 62.5% reinforcement while CS2 were the control trials, i.e., a matched CS that was never accompanied by US. **Day 2**, extinction (EXT) learning +/- prior retrieval (PRE). PRE, CS1 was presented on the screen in the absence of the US; EXT a blank screen was presented instead of the CS1. During extinction all CS trials were unreinforced. **Day 3**, recall/recovery and reinstatement of shock with assessment of return of fear responses. During Noise alone (NA) trials a blank screen was presented accompanied by a startle probe.

B represents the time intervals for presentation of the startle probe and US and response measurements during trials

2.3.2 Drug administration, (non-) reactivation and extinction, (Day 2)

Vital signs and behavioural questionnaires completed prior to administration (double blind) of 400mg sodium valproate or matched placebo. Sixty minutes later (allowing peak plasma levels of valproate to be achieved (Anthony et al., 1976). Participants completed post-drug behavioural questionnaires and before completing the (retrieval) extinction task. Prior to the task they were told that the pictures and procedure would be the same as on day 1. This was to minimise occasion effects and allow for prediction error when contingency expectations were violated. Shock and electrodermal electrodes were attached as on Day 1.

Retrieval (in PRE+VPA, PRE+PBO) consisted of a single unreinforced CS1⁻ trial followed by a single NA trial. EXT+VPA and EXT+PBO groups saw a blank screen followed by a NA trial. All groups then waited 10 minutes before continuing to extinction. The two interventions were thus matched for total length. Extinction consisted of 8 CS1⁻ CS2⁻, and NA trials (Figure 2.4) with no reinforcement at any point to either stimulus. After this task, vital signs were then taken and participants asked to guess which treatment they received. They were asked to refrain from taking alcohol or any drugs for 24 hours.

2.3.3 Fear memory recall, recovery/reinstatement (Day 3)

During the recall phase 4 CS1⁻, CS2⁻ and NA trials were presented in a pseudorandomised order followed immediately by the reinstatement challenge, where a single CS1⁺ shock pairing was delivered. Reinstatement of responding was assessed over a subsequent presentation of 3CS1⁻, 3CS2⁻ and 8 NA trials (all unreinforced).

At the completion of the session, participant's questions were answered, they were paid and thanked for their time.

2.4 Data handling and Statistical Approach

2.4.1. Data handling

We followed the skin conductance acquisition and analysis procedures laid out by Schiller et al (Schiller et al., 2009; Schiller et al., 2012). Skin conductance responses (SCRs) were recorded as amplitude of phasic skin conductance waveforms occurring between 0.5- and 7-seconds following CS onset. Waveforms with a peak amplitude below 0.02 μ Siemens were regarded as sub-threshold and scored as 0. All SCRs were +1 log-transformed to normalise positive skew in the distribution. All electrophysiological signal processing was performed using standardised routines in *AcqKnowledge* 4.0 for BIOPAC. Expectancy ratings (-5 to +5) were converted to a scale 1 to 10 making 5 guess-level.

2.4.2 Data Assumptions

Data distributions were inspected visually using histograms and QQ plots. Where sphericity was violated in repeated measures ANOVA, significance levels and degrees of freedom were adjusted according to the level of Mauchly's epsilon (Stevens, 2012). Where non-integer degrees of freedom are reported in the results (section 2.5 below), these represent adjusted values.

2.4.3 Confirmatory analysis

Acquisition and extinction trials were reduced to 4 'Epochs', each consisting of mean response to two consecutive trials of the same CS type. Linear mixed effects models (using ML) were used to estimate fixed effects of CS type (CS), drug, intervention and epoch and their interactions, with participant intercept as a random effect (formula: $\sim 1 | id$). Model selection was based on the Akaike Information Criteria (AIC), a function of

model fit and complexity. Where AICs of two models were similar (< 2 different), the more parsimonious model was selected. Models were fit in a forward stepwise procedure (starting with an intercept-only empty model) to determine final minimal adequate models beyond which significant improvement in model fit was not achieved by addition of terms. Terms in the final best fit model were then analysed using Wald chi square tests. Significant two-way and higher interactions were assessed with simple effects analyses, with follow-up comparisons as appropriate. All post-hoc tests at Epoch or trial level analysis were Bonferroni corrected for number of Epochs/trials and denoted by *p.adj*. For cases where data were missing, Little's test for randomness of missing values was used, including Drug and Intervention as categorical indicators; no variable had more than 12.5% data missing [χ^2 (408, N = 80) = 434.83, $p = .172$].

At start of Day 3 recall of conditioned responding, 'occasion setting' was assessed at Trial 1. Three unreinforced trials followed which acted to assess recovery/re/extinction of any return of conditioned fear responding to this initial trial. Neither these three trials nor the trials following the re-presentation of the UCS as test of reinstatement of conditioned fear were reduced to Epochs. As such there were four trials assessing recall/recovery followed by four trials assessing reinstatement/re-extinction.

For *between*-session spontaneous recovery of conditioned responding prior to re-extinction, we followed Schiller et al (2012), comparing expectancies during the final trial of extinction for each CS to the first trial of recall, with 2 (CS) x 2 (Trial) x 2 (Drug) x 2 (Intervention) linear model.

2.4.4 Exploratory sex analysis

There is rapidly accumulating data from ours and other labs that biological sex (and gonadal hormones) play a crucial moderating role in fear learning and

extinction and retrieval processes in both experimental (Milad et al., 2006; Hwang et al., 2015; Lonsdorf et al., 2015; Keiser et al., 2017) and clinical populations (Inslicht et al., 2013; Raeder et al., 2019) although in the limited literature there are mixed results from studies looking at sex differences in response to manipulation of memory reconsolidation mechanisms specifically. Sex differences were found between two studies following retrieval+extinction protocols with glucocorticoid administration. A significant effect on fear memory was reported in men (Meir Drexler et al., 2015) but not women, although in two trials assessing reconsolidation of traumatic memories for PTSD in male veterans (Gray et al., 2019) and female veterans (Gray et al., 2021) without pharmacological adjunct, no sex differences were reported. However, there is increasing evidence for sex differences in aversive memory processes (Garcia et al., 2018) and in their modification (Dabrowska, 2023) leading to calls to account for sex as a biological variable when assessing mechanisms underlying novel strategies and how these modify aversive memories in both sexes in pre-clinical and clinical populations (Cahill, 2012; Lonsdorf and Merz, 2017; Beltz et al., 2019; Arnegard et al., 2020; Shansky and Murphy, 2021). The analysis was therefore extended to evaluate the effect of including sex in the above models, modelling its main effect and interaction with drug, intervention, CS and trial.

2.5 Results

2.5.1 Descriptive characteristics

Table 2.1 Mean (SD) descriptive statistics for self-report measures

	Sodium Valproate (N=40, W= 20)		Placebo (N = 40, W = 20)		Men (N = 40)	Women (W) (N = 40)
	Extinction N=20, W=10	Retrieval+ Extinction N=20, W=10	Extinction N=20, W=10	Retrieval+ Extinction N=20, W=10		
Baseline						
Shock intensity (US)	49.65(12.13)	46.92(9.82)	46.01(10.23)	47.62(13.83)	47.17(10.30)	47.40(13.50)
Age (years)	28.72(9.00)	25.63(9.01)	26.72(8.48)	29.63(9.01)	29.40(12.60)	25.50(7.50)
Blood pressure (Systolic)	113.70(15.90)	115.85(16.60)	115.25(11.50)	113.15(14.40)	121.85(14.00)	107.20(13.50)
Blood pressure (Diastolic)	75.80(11.60)	76.00(10.30)	70.80(8.87)	72.20(9.46)	74.70(10.10)	73.60(10.10)
Heart rate (bpm)	75.20(13.80)	75.90(8.67)	73.20(13.20)	77.80(13.60)	75.10(10.10)	78.70(12.40)
STAI	31.90(7.46)	30.20(7.80)	29.80(9.62)	29.00(6.44)	46.30(3.80)	46.90(3.90)
ASI	15.90(6.55)	17.65(6.52)	15.65(5.74)	19.05(8.71)	19.80(8.40)	16.90(6.60)
SPQ	20.80(3.41)	20.90(4.20)	21.30(3.29)	20.90(4.30)	51.85(6.10)	51.10(4.30)
PANAS (positive)	34.80(8.12)	31.50(8.04)	33.20(6.93)	35.40(7.77)	49.10(7.30)	43.80(7.30)
PANAS (negative)	14.45(4.36)	13.50(8.04)	13.45(2.26)	12.75(2.24)	12.78(4.39)	14.05(0.80)
BDI	5.30(4.65)	6.15(4.48)	6.00(6.14)	5.45(4.13)	5.40(4.60)	6.20(4.000)
DTS	2.23(0.86)	2.81(1.12)	2.50(1.06)	2.18(0.83)	25.50(11.40)	22.80(8.60)
BMIS	41.75(5.91)	38.90(6.71)	41.75(5.36)	41.70(4.64)	39.70(6.10)	40.90(5.70)

Self-reported measures of state anxiety, STAI, anxiety sensitivity, ASI, spider fear, SPQ, Positive and Negative affect, PANAS, depression BDI, and distress tolerance, DTS, and demographic and physiological measures on Day 1 (see text). At baseline no drug or intervention differences were observed for baseline descriptive measures ($p > .05$).

Men and women (W) scored equally on all measures except

**Systolic blood pressure (men > women; $F(1, 79) = 8.435, p < .001, \eta^2 = .29$)*

PANAS positive scores (men > women; $F(1, 78) = 7.306, p = .008, \eta^2 = .086$)

At baseline, confirming the effectiveness of randomisation procedure, no group differences were found on behavioural or cardiovascular measures (Table 2.1). There was no difference between groups after manipulation on day 2 or between baseline and day 3. Changes in the responses to the Behavioural Symptom Scale (BSS) questions from pre to post VPA/ PBO administration did not reveal any significant interaction between time point (pre and post drug) and drug condition [$F(1,78) = 0.538, p = .668, \eta_p^2 = .138$]. A $2 \times 2 \chi^2$ analysis showed no significant relationship between drug received and participants' drug guess ($\chi^2 (1, N = 80) = 0.101, p = .750$), indicating successful blinding.

There was a main effect of Day on state anxiety [$F(2, 152) = 32.19, p < .001, \eta_p^2 = .1$] with higher anxiety on day 1 than days 2 and 3 [$F(1, 76) = 12.763, p = 0.001, \eta_p^2 = .144$] and no change between the latter two [$F(1, 76) = .044, p = .834, \eta_p^2 = .001$]. For PANAS-rated affect, a Day x subscale interaction was found [$F(2, 152) = 8.017, p < .001, \eta_p^2 = .095$]. This reflected a Day effect for positive affect overall [$F(1, 76) = 13.843, p < .001, \eta_p^2 = .27$], with reductions in positive affect from day 1 to day 2 [$t(78) = 5.14, p < .001, r = .5$], but a rebound from day 2 to day 3 [$t(78) = 4.02, p < .001, r = .41$], in line with acquisition and extinction of fear.

2.5.2 US Expectancy (expectancy of shock)

2.5.2.1 Acquisition

The final model included fixed effects of Epoch and CS and their interaction (formula: Expectancy $\sim$ Epoch + CS + Epoch * CS, + 1|ID) , total model $R^2 = 0.55$, marginal R^2 of fixed effects = 0.48.) Within this model there was a main effect of Epoch ($\chi^2 (3) = 22.16, p < .001, \eta_p^2 = .157$), a main effect of CS, ($\chi^2 (1) = 561.26, p < .001, \eta_p^2 = .684$) and CS x Epoch interaction ($\chi^2 (3) = 100.64, p < .001, \eta_p^2 = .401$). This interaction indicated that there was a significant increase in shock expectancy from first Epoch to end

for CS1 [$t(74) = 4.63$, $p < 0.001$, $d = .76$] and a decrease to CS2 [all t s > 5 , p s < 0.001] consistent with the CS/shock contingencies having been rapidly acquired (Figure 2.5) as there was no significant difference CS1 vs CS2 in response at first presentation.

Model fit was not improved by adding Sex as a predictor of expectancy during conditioning, indicating that men and women acquired conditioned explicit fearful responding equivalently.

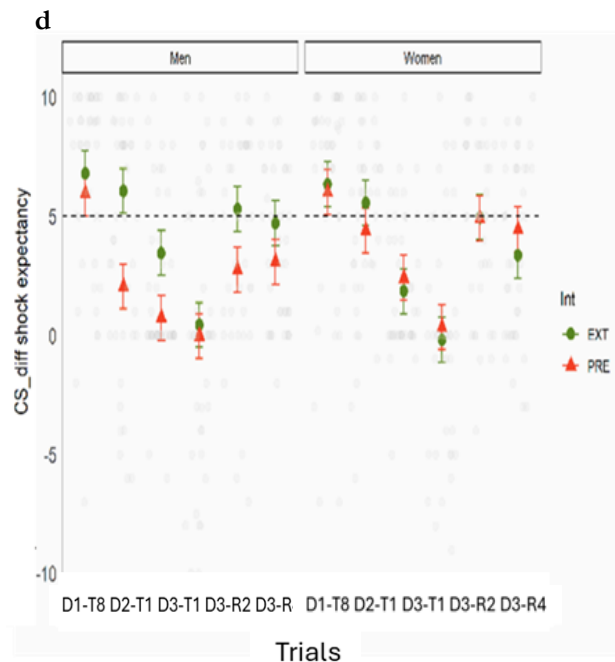
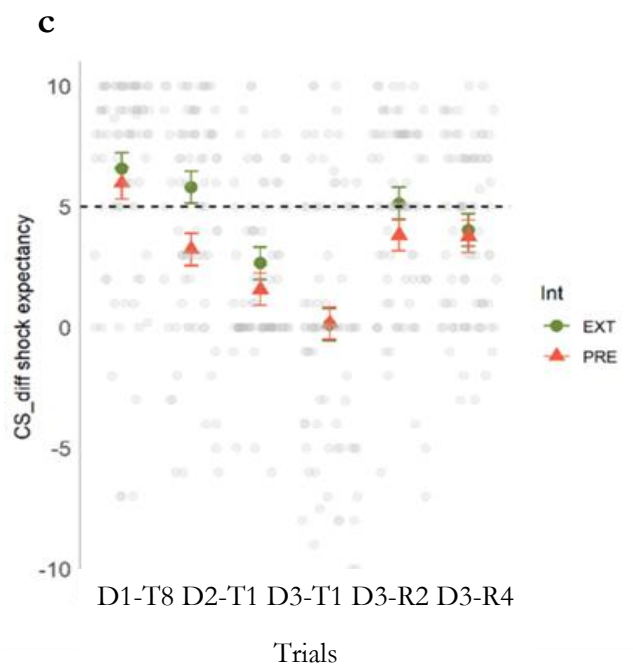
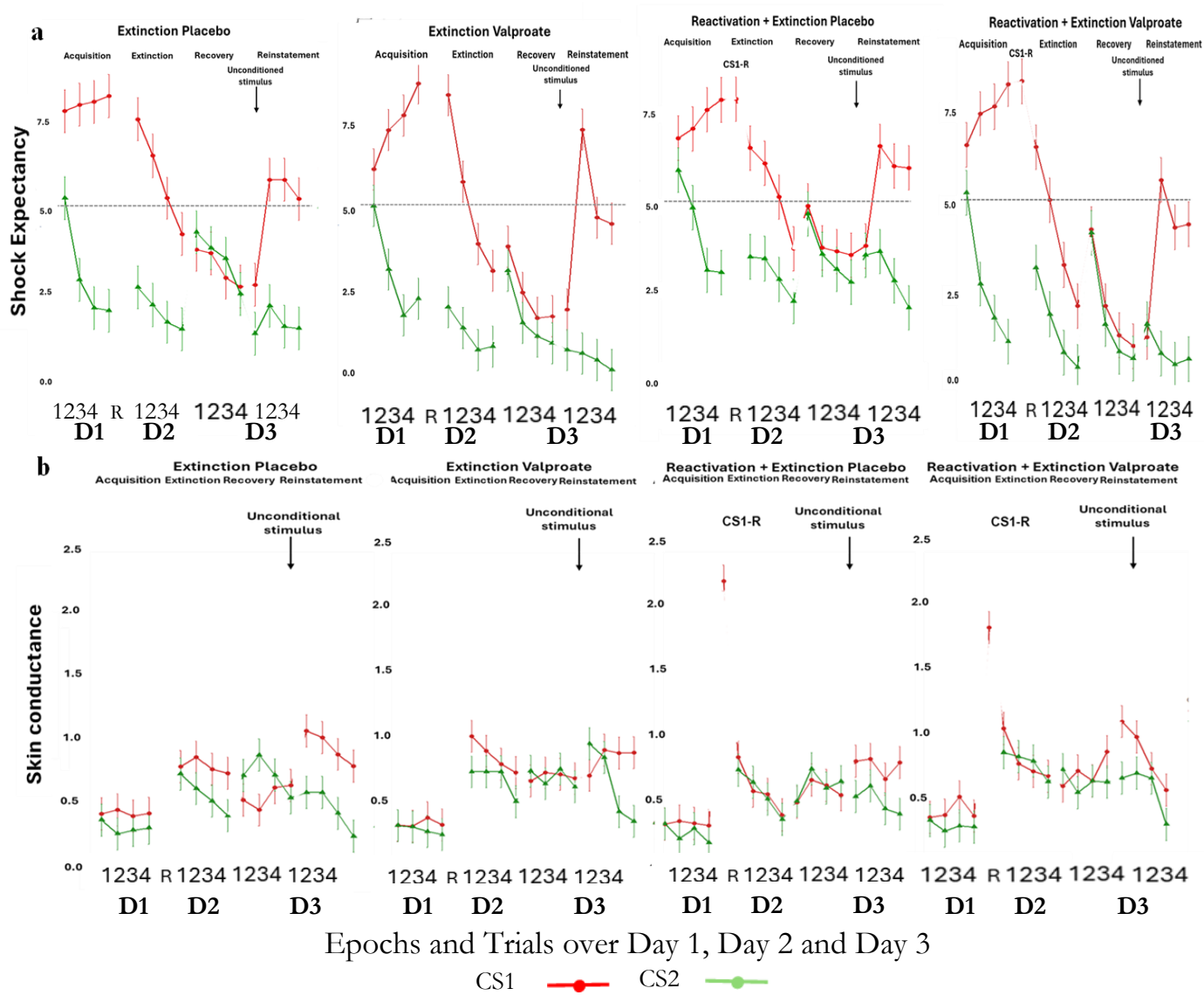


Figure 2.5 Response measures (US expectancy, SCR across the three day protocol: CS1 (conditioned stimuli, red line, red circle) and CS2 (unconditioned stimuli, green line, green triangle) (Day1, 8 Trials collapsed to 4 Epochs), Day2, Reactivation trial shown to the PRE groups (response to CS1-R – retrieval/reactivation of CS1) followed by 4 standard extinction Epochs with VPA and without (B). Extinction session (all participants (Day 2, 8 Trials collapsed to 4 Epochs as shown), recovery (Day3, 4 Trials) followed by reinstatement (Day3, four Trials) with representation of unconditional stimulus prior to second Trial within the reinstatement session. **a)** Mean US expectancy of shock rating by Group, **b)** Mean skin conductance response by Group, see text. **c)** Shows CS differential (CS1-CS2) expectancy ratings at last Trial acquisition (D1-T.8), first Trial Extinction (D2-T1), last Trial extinction (D2-T8), first Trial Recall (D3-T1), test of reinstatement of fear (D3-R2) and last Trial of reinstatement (D3-R4) moderated by i) Intervention, **d)** as for c) split by sex. PRE, red triangle. EXT green circle. Dotted line shows score at chance level of shock expectancy. Error bars represent ± 1 S.E.M

2.5.2.2 Extinction (Day 2)

The final model for extinction data included Epoch, CS, Intervention and Drug (Total model $R^2 = 0.53$, marginal R^2 of fixed effect = 0.40). All main effects and two-way interactions were highly significant, although the 3-way interaction was not (see Table 2.2).

Table 2.2 Extinction, day2 - Intervention and Drug effects

<i>Model Term</i>	<i>Statistics</i>	<i>Effect description</i>
Epoch	$\chi^2_4 = 158.90, p < .001, \eta^2_p = .524$	Reduction in expectancy over epochs
CS	$\chi^2_1 = 448.09, p < .001, \eta^2_p = .472$	CS1 expectancy > CS2 : partial extinction
Drug	$\chi^2_1 = 5.52, p = 0.02, \eta^2_p = .071$	PBO expectancy > VPA expectancy
Epoch x Drug	$\chi^2_4 = 16.03, p = .002, \eta^2_p = .074$	Extinction over time VPA > PBO
Epoch x CS	$\chi^2_4 = 23.87, p < .001, \eta^2_p = .227$	Greater reduction to CS1 than CS2
CS x Intervention	$\chi^2_1 = 14.25, p < .001, \eta^2_p = .003$	CS1 shock expectancy EXT > PRE CS2 shock expectancy PRE > EXT
CS x Intervention x Epoch	$p = .45$	Non-significant
Model $R^2 = 0.53$, marginal R^2 of fixed effect = 0.40, -2LL = -1501.0 AIC = 3034		

Confirmation of extinction learning: Shock expectancy for CS1 (Figure 2.5a) decreased from significantly higher than ‘chance’ (dotted line) at Epoch 1 [$t(79) = 8.16, p < 0.001, d = .69$] to significantly lower than ‘chance’ by Epoch 4 [$t(79) = 4.93, p = 0.001, d = 0.55$]. Conversely, there was no significant decrease between Epochs (all $ps_{adj} > .05$) to CS2. While this indicates successful extinction of shock expectancy to CS1, significant differential shock expectancy (CS1 > CS2) remained at the final trial [$t(39) = 3.10, p = .004, d = 1.0$] suggestive of incomplete/partial extinction.

Retrieval trial: In the PRE groups, there was a significant reduction in shock expectancy between the retrieval trial and first trial of extinction [$F(1,38)=12.592$, $p = .001$, $\eta^2_p = .249$], potentially indicating that extinction was initiated by the retrieval trial (Figure 2.6a,b), or interactive effects between the retrieval trial and subsequent extinction.

Importantly, the intervention condition (PRE vs EXT) (Figure 2.6a) appeared to *acutely* impact shock expectancy over extinction in a dissociable manner for CS1 and CS2. Mean expectancy to CS1 was higher for standard extinction than PRE [$t(160)=2.22$, $p=.027$, $d = .24$]. Conversely, shock expectancy for CS2 was higher for PRE than standard extinction ($t(160) = 2.11$, $p = .04$, $d = .21$) (Figure 2.6c). In terms of conditioned response, (CS1-CS2), this resulted in an interaction between Intervention and first/last trial of extinction [$F(1,72)=6.20$, $p < .02$] (see Figure 2.6b,c). This indicated significantly lower conditioned expectancy in PRE on Trial 1 of extinction (3.23 ± 4.54 vs 5.8 ± 4.21 , $t(76) = 3.07$, $p_{adj} = .012$, $d = .70$]. However, this effect was fleeting, with non-significant differences thereafter ($p_{adjs} = .39$). Comparison of responses to each CS suggested that the difference was largely driven by increased expectancy to CS2 in PRE after Trial 1 (rather than attenuation of CS1 responding). Despite this, the three-way interaction (CS x Intervention x Epoch) was non-significant. Qualitatively, the lingering CR observed in both groups as the end of extinction was at quite different levels of significance (PRE; $p_{adj} = .04$; EXT; $p_{adj} = .0007$).

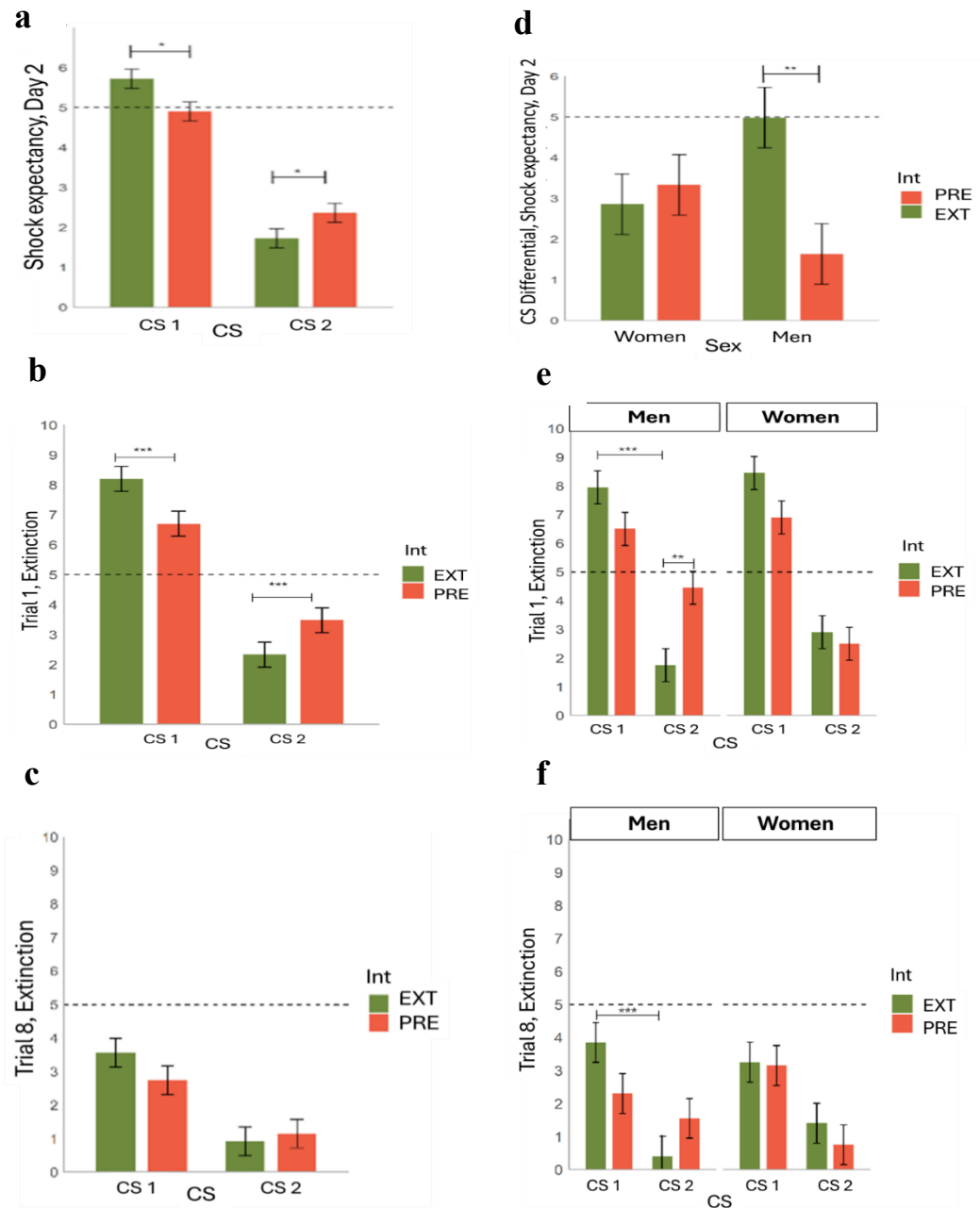


Figure 2.6. a, b, c) Mean expectancy scores during extinction session, day 2, following PRE (reactivation prior to extinction) vs extinction alone (EXT). d, e, f). sex dependent effects between intervention groups a) During extinction there was differential responding between intervention groups in their responses to CS1(left) and CS2(right) with those previously exposed to the retrieval trial (i.e., PRE, in red) having significantly lower expectancy of shock to CS1 ($p = .027$, $d = .24$) than those having standard extinction (EXT, in green) this pattern is reversed for expectancy of shock to CS2 ($p = .04$, $d = .21$) starting from Trial 1. At Trail 1 (b), greater expectancy of shock for CS1 in the Extinction group (green) compared to PRE(red) ($p.\text{adj} = 0.01^{**}$, $d = .41$) and the reverse intervention effect to CS2 ($p.\text{adj} < 0.001$, $d = .87^{***}$) resulting in a significant difference in CS differential between Intervention groups $\text{PRE} < \text{EXT}$ ($p.\text{adj} = .01$) However, by Trial 8 (end of extinction) (c) there was no statistical difference between groups $p.\text{ads} > .05$)(d) Mean expectancy scores during extinction were sex dependent with men showing a significant

*difference in expectancy PRE<EXT resulting in a significant mean CS differential (CS1-CS2) between intervention groups during the session ($p = 0.004$, $\eta^2 = .99$ **). At first trial of extinction (e) in men, there was an Intervention x CS interaction ($p_{adj} < .01$) with no significant difference between CS at Trial 1 in the PRE group ($p_{adj} = .12$) but a significant difference in the EXT group ($p_{adj} < .001$ ***) driven by PRE>EXT response to CS2 ($p_{adj} = .01$) but no significant difference between groups in response to CS1 ($p_{adj} = .144$). At last trial of extinction (f) In men, significant difference (PRE<EXT) in CS differential ($p = 0.02$) with significant difference between CS1 and CS2 EXT ($p_{adj} = .001$) but not in PRE ($p_{adj} = .78$) whereas in women we found no intervention or drug effects or interactions during extinction. Dotted line represents chance rating score. Error bars = S.E.M.*

Drug effects: Drug condition did not impact expectancy on the retrieval trial [$F(1,38)=1.065$, $p=.309$)] and there was no difference in reduction in shock expectancy between drug groups from the retrieval trial to the first trial of extinction [$F(1,38)=.750$, $p=.392$)] and no Drug difference at Epoch 1 ($p > .05$). However, VPA appeared to acutely enhance the speed of extinction learning. Expectancy of shock to CS1 showed a significant Drug x Epoch interaction [$F(3) = 5.50$, $p = .002$, $\eta^2 = .06$)] with VPA induced overall mean reduced expectancy by Epoch 3 [$t(78) = -3.81$, $p = .04$, $d = 0.47$]. Highly significant reductions in shock expectancy were observed from Epoch 1 to 3 in the VPA group ($t(62) = 3.84$, $p < .0001$, $p_{adj} < .001$, $d = .93$), but not in PBO, resulting in significantly lower expectancy in VPA compared to PBO at Epoch 3 ($t(146) = 2.97$, $p = .003$, $p_{adj} = .02$, $d = .35$). A trial-by-trial analysis, showed VPA (but not PBO) induced a steeper reduction in shock expectancy to *both* CSs early (from Trial 1, [$F(3,273) = 7.22$, $p < .0001$, $\eta^2 = .06$)]), indicating that VPA enhanced the speed of global safety learning (Figure 2.7a).

2.5.2.2.i Exploratory analysis of Sex x US expectancy, Extinction

Exploratory analysis found that inclusion of sex as a factor in the best fitting above improved model prediction of expectancy data during the extinction task (total model $R^2 = 0.59$, marginal fixed effects $R^2 = 0.41$). Within this model there was a Sex x CS x Intervention interaction ($\chi^2(1) = 28.45, p < .014, \eta p^2 = .08$) subsumed under a Sex x Epoch x CS x Drug x Intervention interaction ($\chi^2(1) = 5.02, p = .02, \eta p^2 = .06$) see Figure 2.6d,e,f and Figure 2.7b.

At Reactivation: there was a trend towards a main effect of sex during the retrieval trial [$F(1,38) = 3.77, p = .06, d = 0.62$], (Women > Men = $M = 8.55(SD=1.24)$, $M = 7.45(SD=2.19)$). These interactions with Sex were investigated by analysis of effects within each sex separately.

2.5.2.2.i.a Extinction – Analysis in Men only

For males, the final best fitting model terms are reported in Table 2.3 below. Multivariate simple effects analysis showed an Epoch x Drug interaction [$F(3,58) = 4.541, p = .017, \eta p^2 = .10$] see Figure 4f, but no Drug interactions with CS ($p > .05$) in males. The impact of VPA on expectancy occurred after Epoch 1 [$F(1,38) = 13.82, p < .001, \eta p^2 = .27$] with no significant differences in expectancy between drug groups at start of extinction, but a trend difference (VPA < PBO) by Epoch 2 ($p_{adj} = 0.07$), which reached significance by Epoch 3 ($p_{adj} = .04, d = .60$) This pattern reflected a similar pattern of accelerated extinction learning seen in pre-planned analysis (see Figure 2.7b). Intervention x Drug interaction did not survive simple analysis it is of interest to note that exploratory post hoc analysis showed that an effect of drug was seen only in the PRE group ($p_{adj} = .006, d = .86$) during extinction.

The CS x Intervention interaction [$F(1,278) = 30.91, p < .001, \eta^2 = .20$] reflected a significant CR in both intervention groups (PRE $p < .001, d = .13$; EXT $p < .001, d = .35$) but greater expectancy in standard extinction than PRE for CS1 ($p_{adj} = 0.012, d = .41$), but greater expectancy for CS2 in PRE ($p < 0.001, p_{adj} < .001, d = .87$). This pattern of responding was in line with the overall results reported above (not stratified by sex, Figure 2.6e,f), yielding a reduced CR in PRE [$F(1,38) = 9.58, p = 0.004, \eta^2 = .99$] that remained until the final Trial ($p = 0.02$). As above, this was largely driven by intervention effects on CS2 as when split by CS the main effect of intervention during extinction remained significant only for CS2 [$F(1,36) = 33.08, p < .002, \eta^2 = .18$] and resulted in a significant difference in men in expectancy scores PRE < EXT with mean CS differential (CS1-CS2) between intervention groups during the extinction session ($p = 0.004, \eta^2 = .99$) (Figure 2.6 d).

Table 2.3 Extinction, day2 - Intervention and Drug effects on expectancy ratings in men

<i>Model Term</i>	<i>Statistics</i>	<i>Effect description</i>
Epoch	$(\chi^2(3) = 32.50, p < .001, \eta^2 = .47)$	Reduction in expectancy over epochs
CS	$(\chi^2(1) = 35.88, p < .001, \eta^2 = .50)$	CS1 expectancy > CS2 : partial extinction
Drug	$(\chi^2(1) = 7.60, p < .001, \eta^2 = .17)$	VPA < PBO over extinction session
Intervention	$p = .43$	Non-significant
Epoch x Drug	$(\chi^2(3) = 4.363, p < .017, \eta^2 = .11)$	Larger effect of VPA > PBO to enhance extinction over Epochs than analysis of whole sample
CS x Intervention	$(\chi^2(1) = 9.21, p = .004, \eta^2 = .20)$	CS1 expectancy EXT > PRE CS2 expectancy PRE > EXT
total model conditional $R^2 = 0.52$; marginal fixed effects $R^2 = 0.42$ -2LL = -771.6 AIC = 1567		

2.5.2.2.i.b Extinction – Analysis in women only

In women, the model that best predicted expectancy of shock during extinction included only Epoch and CS (total model conditional $R^2 = 0.45$; marginal fixed effects $R^2 = 0.34$). Within this model we found substantial main effects of Epoch ($\chi^2(3) = 81.50, p < .001, \eta^2 = .60$) and CS (CS1 > CS2) ($\chi^2(1) = 180.60, p < .001, \eta^2 = .49$) as well as

an interaction between CS-type and Epoch ($\chi^2(3) = 17.42, p < .001, \eta^2p = .28$), in line with the main non-stratified effects above. However, in contrast to the men, we found no intervention (Figure 2.6d,e,f) or drug (Figure 2.7b) effects or interactions with these terms indicating a sexual dimorphism in responses to retrieval-extinction and VPA and that these effects may only be observed in men.

2.5.2.3 US Expectancy scores during Recall and Recovery (Day 3)

Between-session Spontaneous Recovery: Assessment of spontaneous recovery following the analysis approach of Schiller et al (2012) showed a CS x Trial interaction from last trial of extinction to first trial of recovery [$F(1, 76) = 8.02, p = .006, \eta^2p = .095$]. This reflected a residual effect of CS (CS1>CS2) at the end of extinction, day 2, [$F(1,76) = 31.34, p < .001, \eta^2p = .292$]. There was significant offline extinction (Plendl and Wotjak, 2010) evidenced by a *lack* of differential responding to CSs during the first trial of recovery, day 3, [$F(1,76) = 0.052, p = .82$]. A main effect of Trial [$F(1,76) = 91.608, p < .001, \eta^2p = .547$] indicated an ‘occasion setting’ effect of the new testing session, with non-discriminative increases in shock expectancy from the end of extinction (day 2) to first trial of recall (day 3). A main effect of Drug [$F(1,76) = 5.165, p = .026, \eta^2p = .071$] indicated that the enhanced extinction and safety generalisation following VPA was maintained to recall, with lower overall shock expectancy in the VPA group than PBO. An Intervention x Trial interaction [$F(1, 76) = 5.831, p = .018, \eta^2p = .071$] was also found, driven by a potentiated increase in non-discriminative (i.e. to both CSs) shock expectancy ratings from end of extinction to first trial of recovery in PRE group [$F(1, 76) = 71.832, p < .001, \eta^2p = .486$] compared to EXT group (Figure 2.5a,c).

Recall/re-extinction trials; The best-fitting model of expectancy ratings during the four recall/ recovery trials included fixed effects of Drug and Trial (formula:~1|id + Drug +Trial: total model $R^2=0.39$, marginal R^2 of fixed effect =0.12). See Table 2.4.

Table 2.4 Recall/Recovery Trials, Day3 – Drug effects on expectancy ratings

<i>Model Term</i>	<i>Statistics</i>	<i>Effect description</i>
Trial	$(\chi^2(3) = 64.87, p < .001, \eta_p^2 = .76)$	Reduction in expectancy/re-extinction from Trial 2
Drug	$(\chi^2(1) = 12.30, p < .001, \eta_p^2 = .14)$	VPA < PBO over recall trials
Trial x Drug	$(\chi^2(3) = 7.96, p = .05, \eta_p^2 = .05)$	VPA > PBO enhanced re-extinction of expectancy after ‘occasion setting’ at Trial 1
total model conditional $R^2 = 0.54$; marginal fixed effects $R^2 = 0.36$ -2LL = -1588.6 AIC = 3183		

Analysis of the interactions showed equivalent shock expectancy between Drug groups on Trial 1 of recall ($p_{adj}=.37$), but subsequently lower expectancy of shock in the VPA group at Trial 2 ($p_{adj} = .001, d = .32$) and onwards ($p_{adj}s < .001$), consistent with enhanced *re-extinction* over the recall trials in VPA group (Figure 2.5 a). No other significant effects or interactions were found.

2.5.2.4 Reinstatement, Day 3

Over the four trials that included the test of reinstatement of fear at Trial 1, the best fitting model included fixed main effects of Trial, CS and Drug (formula:~1|id: total model $R^2=0.54$, marginal R^2 of fixed effect =0.36). see Table 2.5.

Table 2.5 Reinstatement, Day 3, Drug effects on expectancy

<i>Model Term</i>	<i>Statistics</i>	<i>Effect description</i>
Trial	$(\chi^2(3) = 56.82, p < .001, \eta_p^2 = .12)$	An increase in expectancy post-UCS followed by a reduction over remaining trials
CS	$(\chi^2(1) = 306.98, p < .001, \eta_p^2 = .67)$	CS1 > CS2
Drug	$(\chi^2(1) = 11.68, p < .001, \eta_p^2 = .03)$	Expectancy VPA < PBO over reinstatement trials
Trial x CS	$(\chi^2(3) = 68.72, p < .001, \eta_p^2 = .03)$	CS1 > CS2 increase from pre (T1) to post (T2) UCS
CS x Trial x Drug	$(\chi^2(3) = 8.90, p < .001, \eta_p^2 = .02)$	Expectancy to CS1 VPA > PBO enhanced re-extinction over post UCS trials. VPA enhanced habituation to CS2 at test (T2) so that CS diff VPA > PBO
total model conditional $R^2 = 0.54$; marginal fixed effects $R^2 = 0.36$ -2LL = -1481.8 AIC = 3017.5		

A Trial x Drug interaction to CS1 [$F(3, 232) = 4.59, p = .004, \eta_p^2 = .06$] reflected similar reinstatement of shock expectancy in both drug groups ($p.adj = >0.999$) at above chance level post-UCS, see Figure 2.7c). Post reinstating shock (Trial 2), expectancy to the safety stimulus (CS2), remained lower in VPA than PBO [$F(1,78) = 10.10, p = .002, \eta_p^2 = .29$], leading to a larger difference between CSs (Figure 2.7d) than in VPA than PBO [$F(1,78) = 7.87, p = .006, \eta_p^2 = .09$]. This is consistent with enhancement of safety signal learning to CS2 in VPA group compared to PBO. This reflected VPA expectancy scores significantly lower than PBO over the post-reinstatement trials ($p.adj < .001, d = .74$). Furthermore, after the reinstating shock, the PBO group maintained high shock expectancy for the rest of the session ($p.adj > .99$). In contrast, there were significant and sustained reductions in expectancy to CS1 immediately post- reinstatement in VPA (trial 2 vs. 3 $p.adj = .01, d = .73$). Although scores did not return to pre-test levels, descriptively CS1 ratings *did* drop below chance level only in the VPA group (Figure 2.5a).

Additional analysis of CS differential at Trial 2 alone revealed a main effect of group [$F(1,72)= 3.65, p =.01$] with significant drug effects only on standard extinction EXT (EXT+VPA > EXT+PBO; $p_{adj} = .04, d=.35$).

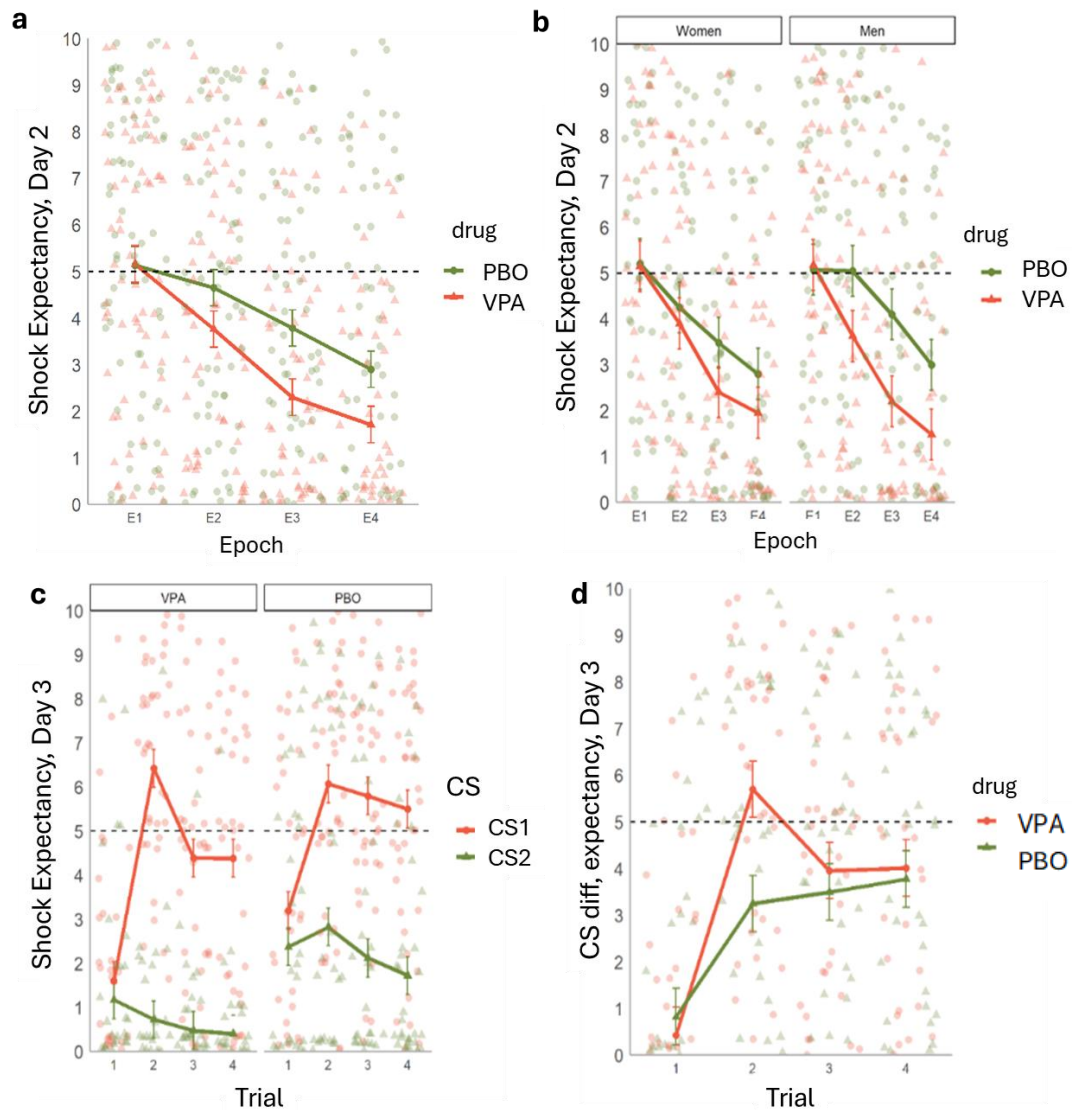


Figure 2.7 Drug effects over extinction and reinstatement trials. *a)* Drug on day 2; Over extinction there was a main effect of Drug on mean expectancy scores ($\chi^2 (1) = 5.52, p=0.02, \eta^2 = .071$) as well as a two-way interactions Epoch x Drug ($\chi^2 (4) = 16.03, p=.002, \eta^2 = .074$) resulting in a significantly accelerated reduction in extinction learning in the drug group (red line, triangle) compared to PBO (green line, circle). *b)* shows significantly accelerated reduction in expectancy scores in response to drug effects compared to placebo in men during extinction (Drug ($\chi^2 (1) = 7.60, p < .001, \eta^2 = .17$; Epoch x Drug ($\chi^2 (3) = 4.363, p < .017, \eta^2 = .11$)). this reflects a similar pattern to that

seen in the pre-planned analysis without sex as a moderator while drug effects were non-significant in women ($p > .05$) **c)** Drug, on day 3, Reinstatement; Trial x Drug x CS interaction on mean expectancy ratings during reflecting a significant effect of VPA (red, circle) to enhance reduction in explicit memory to the conditioned fear stimuli and the safety stimuli after test ($\chi^2(3) = 8.90, p < .001, \eta_p^2 = .02$) but not to protect against immediate expectancy of return of fear after representation of US at Trial 2. **d)** Showing significantly higher differential (CS1-CS2) in VPA than PBO (green, triangle) at Trial 2 due to potentiated attenuation effect of drug to safety signal compared to PBO [$F(1,78) = 7.87, p = .006, \eta_p^2 = .09$]. Dotted line represents chance rating score. Error bars = S.E.M.

No correlation was found between shock expectancy rating during retrieval in retrieval-extinction (a putative index of prediction error) and shock expectancy during recall and reinstatement.

2.5.2.4.i Exploratory analysis of Sex differences in shock expectancy, reinstatement

The best model fit when including sex into the model to predict expectancy scores over the four reinstatement trials included Trial, CS, Intervention, Drug and sex. The significant interactions with sex are detailed in Table 2.6.

Table 2.6: Reinstatement, Day 3: Sex, Intervention and Drug effects

<i>Model Term</i>	<i>Statistics</i>	<i>Effect description</i>
CS x Intervention	$(\chi^2(1) = 6.43, p = 0.01, \eta_p^2 = .01)$	CS1: PRE < EXT CS2: PRE > EXT
Trial x Sex x Drug	$(\chi^2(3) = 9.04, p = .03, \eta_p^2 = .01)$	Expectancy scores VPA <PBO across trials significant
CS x Intervention x Sex	$(\chi^2(1) = 18.09, p < .0001, \eta_p^2 = .034)$	Only in men CS2: PRE > EXT CS Diff: PRE < EXT
CS x Intervention x Sex x Drug	$(\chi^2(1) = 11.44, p < .001, \eta_p^2 = .22)$	
total model conditional $R^2 = 0.57$; marginal fixed effects $R^2 = 0.4$ -2LL = -1462.70 AIC = 2905.40		

2.5.2.4.i.a Analysis in men only

In the analysis of men only of mean expectancy scores over the four reinstatement trials, there was a significant main effect of drug (VPA<PBO) but no interaction effects with CS and/or Trial as seen in the pre-planned non stratified analysis above.

Table 2.7 Reinstatement, Day 3: Drug and Intervention effects in Men

<i>Model Term for Men alone</i>	<i>Statistics</i>	<i>Effect description</i>
Trial	(χ^2 (3) = 26.93, $p < .001$, $\eta_p^2 = .11$)	Expectancy to CSs increased at test (T2) reducing over subsequent trials
CS	(χ^2 (1) = 120.25, $p < 0.001$, $\eta_p^2 = .49$)	CS1 > CS2
CS x Trial	(χ^2 (3) = 33.65, $p < 0.001$, $\eta_p^2 = .13$)	CS1>CS2 post T2
Intervention	(χ^2 (1) = 6.32, $p < 0.01$, $\eta_p^2 = .03$)	PRE > PBO
Drug	(χ^2 (1) = 18.56, $p < .001$, $\eta_p^2 = .10$)	VPA < PBO
CS x Intervention	(χ^2 (1) = 21.32, $p < .0001$, $\eta_p^2 = .14$)	CS2: PRE > EXT CS Diff: PRE < EXT'
total model conditional $R^2 = 0.57$; marginal fixed effects $R^2 = 0.4$ -2LL = -1462 AIC = 2994		

In men, mean expectancy scores in the intervention groups followed a similar pattern to those at extinction (Table 2.3) but a main effect of overall mean PRE expectancy ratings greater than EXT reaching statistical significance at reinstatement ($p = .01$, $\eta_p^2 = .03$) due to a significant CS x Intervention effect, ($p < .0001$, $\eta_p^2 = .14$) that reflected significantly higher ratings to CS2 in PRE (M= 2.45 (3.24) compared to EXT(M= 0.625(1.72): $p_{adj} = .003$, $d = .70$) and therefore a larger difference between CS1 >CS2 in EXT than seen in PRE (although both ($p_{adj} < .001$). However, this was due to the potentiating effect of PRE to CS2 rather than an effect on CS1 and the effect of PRE to

significantly reduce CS differential response (CS1-CS2, Figure 2.8a) compared to EXT [$F(1,38) = 5.73, p < .03$] (PRE<EXT) in a way similar to during extinction (Figure 2.6) although with no effect on CS1 at reinstatement.

At test of reinstatement at trial 2, the enhancing effect of PRE on CS2 resulted in the PRE men who received PBO having a non-significant CS1>CS2 difference at test of explicit fear memory at test ($p_{adj} = .97$) (Figure 2.8b) driven by significantly higher ratings to CS2 than all other groups ($p_{adjs} < .0001$) rather than a significant reduction in response to CS1 (Figure 2.8b).

2.5.2.4.i.b Analysis of women only

In the analysis of women only, mean expectancy scores over the four reinstatement trials (Table 2.8) the three way interaction of CS x Intervention x Drug did not survive simple analysis and the CS x Trial interactions reflected similar patterns as describe for non-stratified analysis of reinstatement described above (Table 2.5).

At test of reinstatement at trial 2, there was a significant reinstatement of differential fear (CS1>CS2) in both PRE groups (VPA ($p_{adj} = .003, d = 3.01$) and PBO ($p_{adj} = .013, d = 1.89$), Figure 2.8a). In the EXT group there was a significant CS1>CS2 difference at test with VPA ($p_{adj} < .001, d = 3.5$) but PBO women in EXT (Figure 2.8c), had no significant CS1>CS2 post UCS at trial 2 ($p_{adj} = .95$). Descriptively, CS1>CS2 in EXT with VPA but not EXT with PBO was due to lower expectancy ratings to CS2 in the VPA compared to PBO.

Table 2.8 Reinstatement, Day 3: Drug and Intervention effects in Women

<i>Model Term for women alone</i>	<i>Statistics</i>	<i>Effect description</i>
Trial	$(\chi^2 (1) = 31.64, p < 0.001, \eta_p^2 = .11)$	Expectancy increased at test T2 reducing over subsequent trials
CS	$(\chi^2 (1) = 200.65, p < 0.001, \eta_p^2 = .67)$	Expectancy CS1 > CS2
CS x Trial	$(\chi^2 (3) = 37.10, p < .001, \eta_p^2 = .12)$	Expectancy CS1 > CS2 at test (T2)
CS x Intervention x Drug	$(\chi^2 (1) = 8.68, p < .003, \eta_p^2 = .01)$	Effect did not survive simple analysis

total model conditional $R^2 = 0.57$; marginal fixed effects $R^2 = 0.4$)-2LL = -

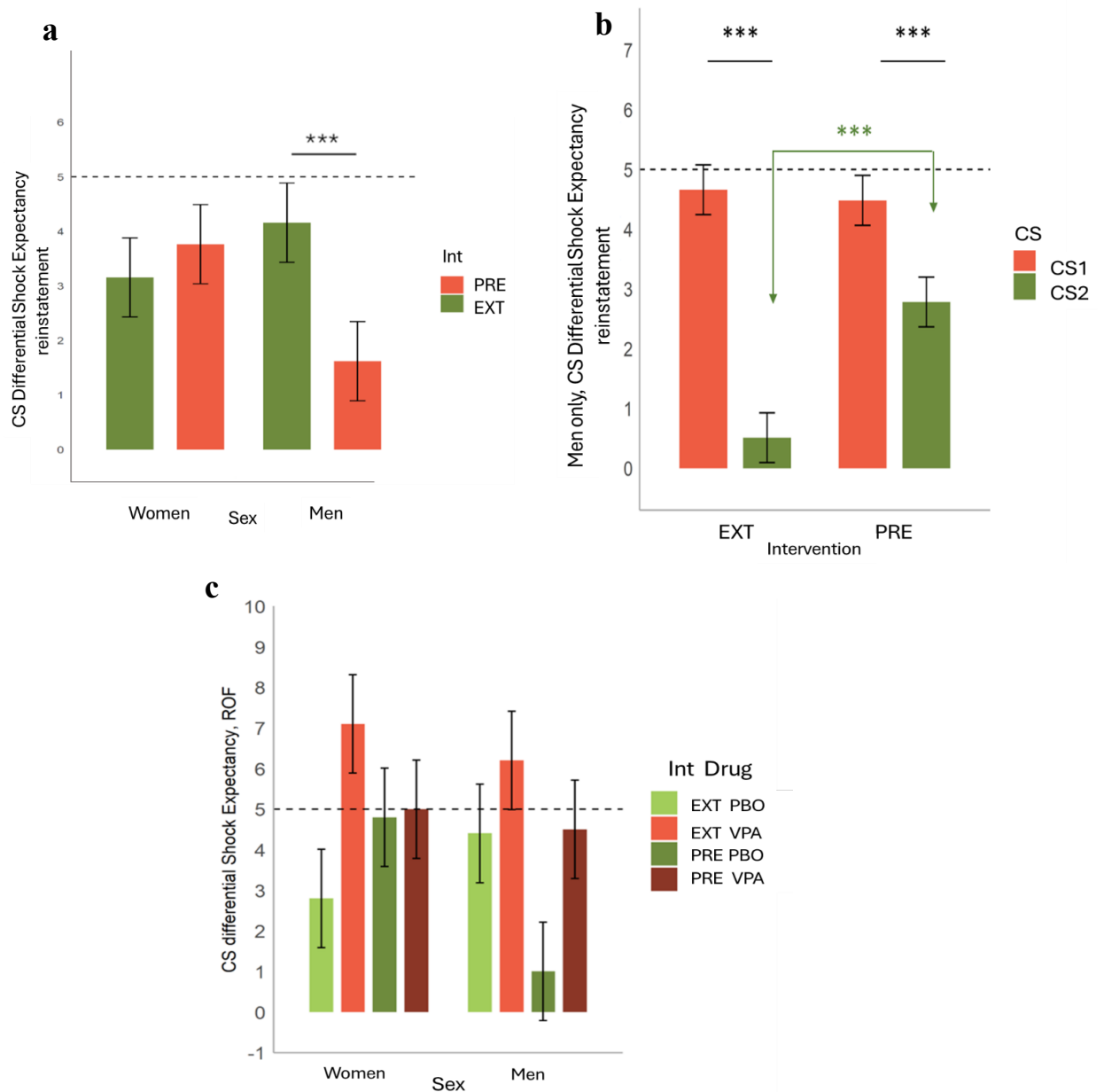


Figure 2.8 Sex dependent effects on mean shock expectancy scores during reinstatement trials. a) Potentiated reduction in CS2 responses in men in PRE (CS x Intervention [$F(1,275) = 17.10, p = <.001, \eta_p^2 = .14$]) led to a significantly reduced CS differential (see text) in PRE vs EXT not seen in women. b) in men, there was significantly higher ratings to CS2 in PRE ($M = 2.45 (3.24)$) compared to EXT ($M = 0.625 (1.72)$): $p_{\text{adj}} = .003, d = .70$) c) In men, at trial 2, in those in PRE given PBO was there a non-significant CS1 > CS2 in shock expectancy ($p = .97$) at test of ROF, all other groups ($p < .05$) due to significantly higher CS2 shock expectancy scores of PRE in PBO compared to all other Int x Drug groups ($p_{\text{adjs}} < .0001$) In women, those in the EXT group with PBO had a non-significant CS > CS2 ($p_{\text{adj}} = .95$) at test; all others ($p_{\text{adjs}} < .02$). Dotted line represents chance rating score. Error bars = S.E.M.

2.5.3 Skin Conductance responses

2.5.3.1 Acquisition (Day 1)

The final model over acquisition included fixed effects of CS, Epoch and an Epoch x CS interaction (table 2.9). SCRs to CSs did not significantly differ during Epoch 1 ($p_{adj} = .60$, $d = .06$) but that at Epoch 2 ($p_{adj} < 0.001$, $d = .51$) and subsequent epochs, SCR was greater on CS1 trials than CS2 trials ($p_{adj} < .001$), indicating successful differential conditioning of autonomic arousal. (Figure 2.5b). However, overall SCR did not significantly change over acquisition session, Epoch ($p = 0.06$).

Table 2.9 Acquisition (Day1), Extinction, (Day2) Recall/Reinstatement (Day3) – Intervention, and Drug and Sex effects on Skin conductance response

<i>Model Term</i>	<i>Statistics</i>	<i>Effect description</i>
Acquisition		
CS	$\chi^2 (3 = 43.13, p < .001, \eta_p^2 = .338)$	CS1 > CS2
Epoch	$\chi^2 (3 = 7.30, p = 0.06)$	No overall mean increase in SCR over Epochs
CSxEpoch	$\chi^2 (3 = 4.71, p = .030, \eta_p^2 = .039)$	>CS1 over Epochs <CS2 over Epochs Successful conditioning
total model conditional $R^2 = 0.23$; marginal fixed effects $R^2 = 0.06$)-2LL = 150.24 AIC = 327.82		

Extinction		
Epoch	$\chi^2 (3 = 57.54, p < .001, \eta_p^2 = .217)$	SCR Reduction over epochs
Drug	$\chi^2 (1 = 5.83, p = .018, \eta_p^2 = .07)$	VPA > PBO
CS	CS1>CS2 ($\chi^2 (1 = 16.36, p < .001, \eta_p^2 = .12)$)	CS1 > CS2
CS x Intervention	$\chi^2 (1 = 10.03, p = .002, \eta_p^2 = .08)$	Potentiated early < in PRE to CS1 vs EXT Gradual < CS2 in both groups (see text)
total model $R^2 = 0.44$, fixed effects marginal $R^2 = 0.10$)-2LL = 347.6267 AIC = 735.25		

Recall		
CS	$F(1,36) = 5.33, p = .027, \eta_p^2 = .13)$	CS1> CS2
Drug	$F(1,36) = 7.735, p = .009, \eta_p^2 = .18)$	VPA > PBO
TrialxDrugxSex	$F(1,36) = 6.341, p = .016, \eta_p^2 = .15)$	Drug (VPA >PBO) effect only on Trial 1 and in men only
Reinstatement		
Trial	Trial $\chi^2 (3) = 22.83, p < .001, \eta_p^2 = .092)$	Decrease in SCR from T2 to T4
CS	CS ($\chi^2 (1) = 5.90, p < .001, \eta_p^2 = .288)$)	CS1>CS2
Recall; total model $R^2 = 0.11$, marginal R^2 of fixed effect = 0.001 Reinstatement: total model conditional $R^2 = 0.18$; marginal fixed effects $R^2 = 0.08$		

2.5.3.2 Extinction learning (Day 2)

There was no difference between drug groups on the retrieval trial [$F(1,38) = 0.313, p = .579$]. Between the retrieval trial and first trial of extinction, a significant reduction in SCR to CS1 was seen in both groups [$F(1,38) = 12.826, p = .001, \eta_p^2 = .253$], (Table 2.9).

During the extinction session we found main effects of Epoch, Drug and CS, plus an interaction between CS and Intervention. SCRs reduced overall from Epoch 1 to 3 ($p_{adj} = .003, d = .40$), and more to Epoch 4 ($p_{adj} < .001, d = .63$). Contrary to expectancy ratings, the main effect of drug indicated *higher* overall SCR response following VPA than PBO (Mean (SD); VPA = .74(.69), PBO = .58(.47)). Valproate itself may therefore have had a general arousal enhancing impact on tonic (non CS-specific) autonomic activity levels.

The CS x Intervention interaction reflected a significant CR over extinction in EXT [$F(1,39) = 20.38, p < .001, \eta_p^2 = .03$] but not in PRE [$F(1,39) = .224, p = .64, \eta_p^2 = .006$] (Figure 2.9a). This may be indicative of rapid extinction induced by the retrieval trial; supported by a significant SCR reduction from reactivation trial to Trial 1 of extinction proper ($t(39) = 3.60, p < .001, d = .57$). Interestingly, PRE experienced higher mean SCR response to the reactivation trial than EXT at the first trial of extinction (the first CS presentation for these groups day 2) [$F(1,38) = 8.39, p = .006, \eta_p^2 = .18$]; PRE CS1-R, $M = 2.02$ (SD = 2.10), EXT Trial 1, $M = 0.83$ (SD = 0.66). Descriptively, SCR response to CS2 was higher in PRE than EXT, but, unlike for expectancy of shock rating, not significantly so ($p = .13$). Both Intervention groups evidenced gradual reduction in SCR response to CS2 with significant difference in CS2 SCR responses between start and end of extinction ($p_{adj} = .015$). Importantly, a CR remained in the standard Extinction group at Epoch 4 ($p_{adj} = .01, d = .06$), but not in the PRE group ($p_{adj} = .69$) and the

overall SCR CR over the extinction session was higher in EXT compared to PRE [$F(1,338) = 9.33, p = .002, \eta^2 = .03$] (Figure 2.9.b), suggesting more complete extinction of autonomic conditioned responding by PRE.

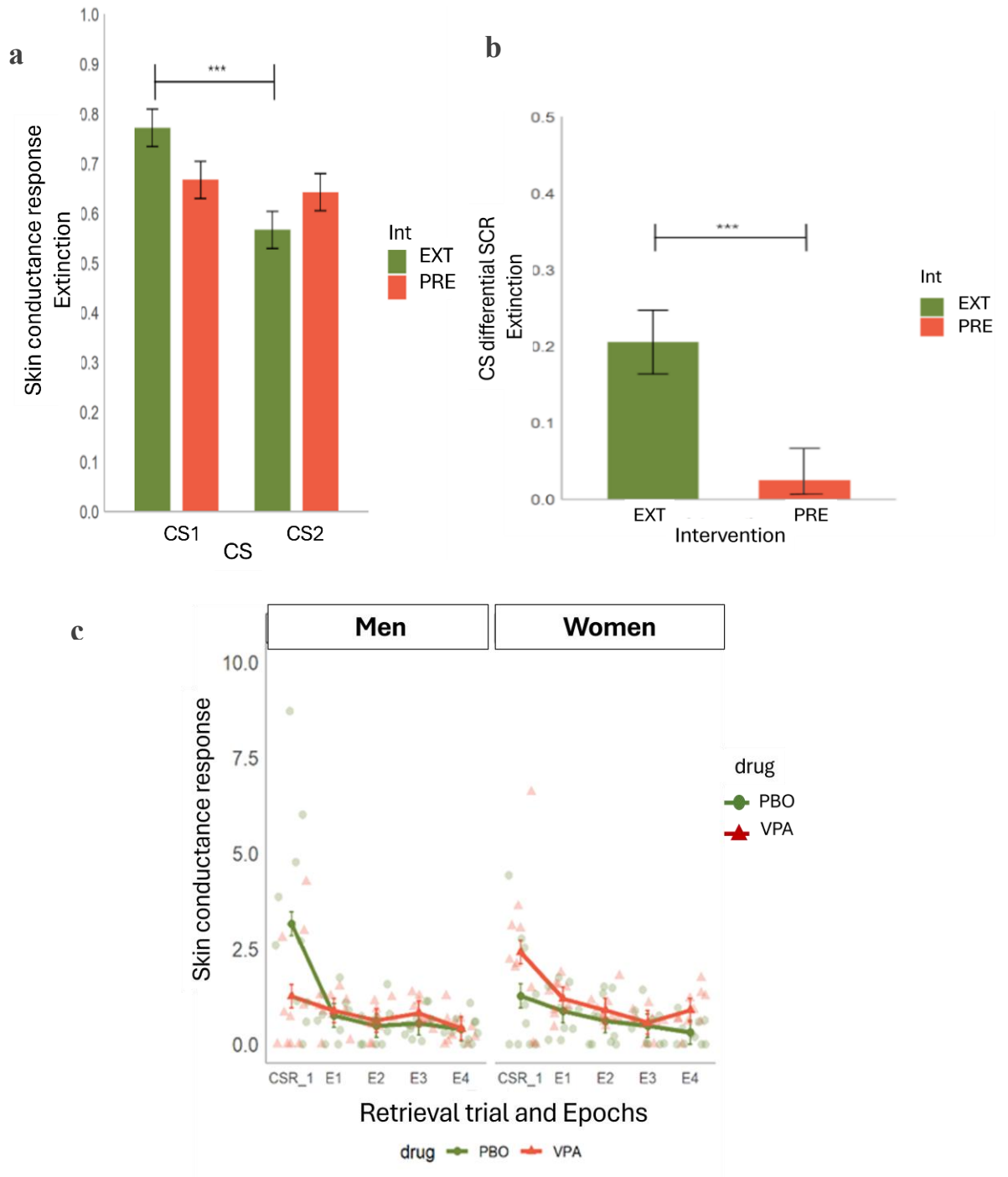


Figure 2.9 Skin conductance response (SCR) during extinction session on Day 2.
a) There was a significant SCR difference during extinction between CSs (CS1 > CS2) in EXT (green) $p < .001$, $\eta_p^2 = .03$ but not in PRE (Red) ($p = .64$) during extinction session on day 2. b) The differential responding between CSs between the Intervention groups resulted in significantly higher SCR CS differential (CS1 – CS2) over extinction session in EXT compared to PRE ($p = .002$, $\eta_p^2 = .03$). c) Sex and drug dependent effects on mean skin conductance responses (SCR) at reactivation trial (PRE) with opposite response patterns between men and women dependent on drug group (placebo green line/circle; sodium valproate red line, triangle) at presentation of the retrieval trial of CS1 (CS1R) without presentation of UCS ($p = .021$, $\eta_p^2 = .14$) before extinction epochs (E1-E4) at start of day 2. Error bars S.E.M.

2.5.3.2i Exploratory analysis of sex differences in SCR during extinction

Reactivation trial on Day 2: exploratory analysis of SCR responses by Sex during the single reactivation trial prior to extinction revealed a significant Sex x Drug interaction [$F(1, 39) = 5.869$, $p = .021$, $\eta_p^2 = .14$] (Figure 2.9c). Females had lower SCR than males in PBO [$F(1, 158) = 31.81$, $p < .001$, $\eta_p^2 = .17$] but higher SCR than men in VPA [$F(1, 158) = 19.72$, $p < .001$, $\eta_p^2 = .11$]. Men had significantly different SCR response between the drug groups ($p = .040$, $d = .89$) with lower SCR in VPA ($M(SD) = 1.25(1.46)$) compared to PBO ($M(SD) = 3.14(2.62)$) whereas there was no significant difference in the women's SCR response between drug groups at retrieval.

Extinction epochs: By the final trial of extinction, while there was no Drug x Sex x CS interaction in the EXT group ($p = .3$), in the PRE group, there was a Drug x Sex x CS interaction effect [$F(1, 72) = 12.3659$, $p < .001$] including a main effect of Drug [$F(1, 72) = 12.3659$, $p = .001$]. A CS x Drug interaction was only evident in women [$F(1, 36) = 5.3121$, $p = 0.03$] and now reflected VPA > PBO expectancy scores in PRE women to CS1 (VPA, $M = 0.8(0.6)$; PBO, $M = 0.08(0.18)$) but no significant difference in SCR in

PBO women. In neither sex was there a main effect of CS at the end of extinction in the PRE group confirming the complete extinction of autonomic conditioned responding by PRE seen in the pre-planned analysis above.

2.5.3.3 Skin conductance response during Recall and Recovery (Day 3)

Recall: Following Schiller et al (2012b) comparing SCRs from last trial of extinction to first trial of recall, showed a main effect of Trial, with higher SCR at first trial of recall, [$F(1, 38) = 7.61, p = .006, \eta_p^2 = .101$], but with no differential recovery between CSs. There was a main effect of Drug [$F(1, 76) = 5.893, p = .018, \eta_p^2 = .072$] which, contrary to what was observed in expectancy of shock but the same as overall SCR responding during extinction session, there was higher SCR responding in VPA ($M = .58 (.64)$) than PBO ($M = .40 (.52)$).

Recovery: Over the four trials of recovery itself (Table 2.9) there were no significant differences on SCRs between CSs, nor any interactions with intervention or Drug (formula: $\sim 1 \mid id$, total model $R^2 = 0.11$, marginal R^2 of fixed effect = 0.001).

2.5.3.3.i Exploratory analysis of sex differences in skin conductance response during recall and recovery

Considering the extinction and retrieval effects above analysis of Drug and Sex in the PRE group was conducted to explore a possible interaction effect on recovery at first trial of day 3 compared to last individual trial of extinction session, to capture the ‘setting effect’ that can recover return of fear (Schiller et al. 2012). Main effects of CS x drug, as well as a three-way interaction between trial x drug x sex (Table 2.9) was observed. Post hoc analysis indicated that there was a significant effect of drug at the first trial of recovery ($p_{adj} = .031, d = .28$) with a drug effect (VPA > PBO) only in men ($p_{adj} = .012, d = .68$) not in women ($p_{adj} = .20$) resulting in a significant difference between sexes at start of

recovery in the VPA group only ($p_{adj} = .032$, $d = 1.0$). In contrast, no main effects or interactions were observed in EXT at recovery.

2.5.3.4 Skin conductance response during Reinstatement (Day 3)

Over the four individual trials of reinstatement, the model of best fit included fixed effects of Trial and CS and included participant as random effect (Total model conditional $R^2 = 0.18$; marginal fixed effects $R^2 = 0.08$) and, as for recovery trials there were no drug or intervention effects or interaction (Table 2.9)s. The main effects of Trial ($\chi^2(3) = 22.83$, $p < .001$, $\eta_p^2 = .092$) and CS (CS1 > CS2; ($\chi^2(1) = 5.90$, $p < .001$, $\eta_p^2 = .288$). Interestingly, however, in contrast to what was observed with explicit expectancy of shock an increase in SCRs was not seen between Trial 1 (pre reinstating shock) and Trial 2 (following the reinstating shock; $p > 0.99$), but a decrease in SCRs was seen between Trial 2 and 4 ($t(79) = 3.96$, $p = .001$, $d = .558$), indicating that the absolute level of SCR reinstatement was relatively modest.

2.5.6 Correlation between reactivation trial (Day 2) and at test trial on Day 3

As for analysis of US expectancy, the pre-planned analysis found no significant correlation between SCR during retrieval (first trial on day 2) in retrieval-extinction (a putative index of prediction error) and SCR CS1 at test of reinstatement trial 2 on day 3.

2.6 Discussion

Currently, exposure based therapy, a form of extinction learning, is often used as a first-line behavioural intervention in phobia, stress and anxiety disorders but often produces partial or time-limited, attenuation of cued-induced fear responses. An important improvement to this therapy would be to protect the fear reductions achieved via current methods from reinstatement and renewal effects. In this study, Sodium valproate, an anxiolytic and an HDAC inhibitor, accelerated acute extinction learning, as well as delayed (re)-extinction of experimentally induced fear responses (explicit ratings) 24 hours later. This effect may have been mediated through HDACi-related increases in transcription and synaptic plasticity-related proteins. HDAC activity (or inhibition) has direct consequences on learning dependent neural activity and the protein synthesis required to drive (or not) LTP and neural plasticity in the amygdala (Bredy et al., 2007; Gräff et al., 2014).

Furthermore, epigenetic alteration to the expression of stress-related genes has been proposed as the mechanism underlying a pre-disposition (Yehuda and LeDoux, 2007) to stress related disorders. Epigenetic alterations of the BDNF gene known to be involved in extinction learning (Roth and Sweatt, 2009) and an accumulation of dysregulating epigenetic marks over a lifetime may drive some of the hippocampal and amygdala dysfunction seen in PTSD patients (Zovkic and Sweatt, 2013).

Contrary to previous research (Ecker, 2021), but see Luyten and Beckers (2017) and Chalkia et al. (2020), no benefit of retrieval prior to extinction in preventing the return of fear was found in non-sex-stratified analyses, although both drug and behavioural interventions had sex-dependent acute and lasting effects on safety signal (CS2) processing. Specifically, the attenuation of fear responding described by the pre-planned

analysis and an effect of PRE to reduce differential fear responding in a recall test vs. extinction was *only* observed in men.

HDAC inhibition via sodium valproate (VPA) administration prior to extinction learning

The accelerated rate of extinction learning in presence of VPA observed here is in line with enhanced extinction acquisition seen with Yohimbine (Cain et al., 2004). Individuals with a slower rate of extinction may be more vulnerable to relapse (King et al., 2018b) although accelerated extinction learning is an under-reported effect of pharmacological ‘extinction boosters’ (Cahill and Milton 2019). Faster extinguishers, but no slower extinguishers, were shown to benefit from adjunctive D-cycloserine during extinction, and in men this translated into enhanced re-extinction (King et al., 2018a) although it is unclear if accelerated extinction acquisition as distinct from enhanced extinction consolidation translates into enhanced long term protection against renewal effects (Cahill and Milton, 2019).

The enhanced extinction following VPA seen here is in line with the animal literature investigating epigenetic moderation of fear memory (Bredy et al., 2007; Bredy and Barad, 2008). Bredy et al. (2007) suggest that these effects on extinction learning are the result of BDNF acetylation upregulation, NMDA receptor upregulation and subsequent increased LTP in fear extinction-related brain regions during extinction enhancing extinction learning (fig 2.2). The results here are also, in part, in line with the limited human literature (Kuriyama et al., 2011; Kuriyama et al., 2013). Although, notably, our results did not support previous studies reporting protection against fear reinstatement 24 hrs later (Kuriyama et al., 2011). Instead, contrary to Kuriyama et al. there was an enhanced response to the previously extinguished conditioned stimuli (CS1) immediately following the test of reinstatement test trial compared to placebo, despite

overall lower responding in drug group compared to placebo across the four reinstatement trials due to enhanced re-extinction. The enhanced CS1 responding at test highlights the complex and sometimes paradoxical effects of HDAC inhibition on fear learning (Bredy and Barad, 2008) possibly due to HDAC inhibition promoting *activity-dependent* learning. VPA effects are highly sensitive to factors such as context, inter-trial training interval, and the use of massed versus spaced extinction training (Bredy and Barad, 2008).

Sodium Valproate effects on declarative fearful responses and influence on ‘safety signal’

It is important to note that enhanced discrimination between truly aversive (i.e. shock-paired) and ‘safe’ stimuli (CS2 here at test) could be seen as highly adaptive response following trauma but become maladaptive if fear overgeneralization and impaired safety signal learning continue long term, characteristics of PTSD. Indeed, these maladaptive processes are thought to perpetuate symptomatology.

Importantly, in this study we found effects on declarative memory (i.e. expectancy of shock) in contrast to much of the human reconsolidation interference literature, where participants have usually been found to retain their declarative memories of CS-US contingencies (Soeter and Kindt, 2010a). The results here also indicate profound effect of VPA in reducing fearful responses to the ‘safety signal’ (the stimuli never associated with a shock, CS2) across all post-drug sessions. The accelerated reduction in fear responding described in this study cannot be attributed solely to general anxiolysis or fear blunting via the GABAergic action of VPA, (de los Ángeles Cintado et al., 2024), as the response to the fearful stimulus (CS1) was *enhanced*, state anxiety measures showed no drug effect and SCR levels overall were *higher* following VPA than placebo during extinction.

HDAC inhibition as a pharmacological adjunct to novel behavioural manipulation of aversive memories

HDAC inhibitors have been proposed to enhance memory destabilisation potential (Bonomi et al., 2022). However, contrary to what has been demonstrated previously (Gräff et al., 2014) no interactive effect of VPA given prior to reactivation trial in preventing the return of fear was found here. Whether a memory is destabilized, and in such a way to prevent return of fear to the conditioned stimulus with or without pharmacological adjunct, is exquisitely sensitive to boundary conditions (Box 1) via mechanisms not fully characterized (Merlo et al., 2014; Milton et al., 2023). However, age and intensity of fear memories are important conditions (Gräff et al., 2014; Silva and Gräff, 2023). Here, the memories were only 48hrs old and may have been too ‘young’ to observe clear benefits. Young and old memories are known to rely on different brain regions for their maintenance (Frankland and Bontempi, 2005; Clem and Huganir, 2010) and so in this case epigenetic priming might have made no observable beneficial effect on these memories either alone or in combination with engagement of reconsolidation mechanisms.

Retrieval prior to extinction (PRE) effects on aversive memory without pharmacological adjunct

Retrieval prior to extinction (PRE) has been reported to be effective when used as a standalone behavioural intervention to moderate aversive memories (Monfils et al., 2009a; Schiller et al., 2009; Kredlow et al., 2016; Ecker, 2021) however there are a number of inconsistent findings, null results and methodological challenges in using the paradigm (Auber et al., 2013; Schroyens et al., 2017b; Chalkia et al., 2020; Schroyens et al., 2021). The pre-planned, non sex-stratified analysis here found PRE alone did not

protect against return of fear although PRE did produce more rapid early reduction in expectancy of shock to CS1 compared to standard extinction. Interestingly, this effect was also reported in a reactivation plus in-vivo exposure therapy trial that resulted in enhanced reduction of fear >1mth later (Telch et al., 2017). However, as with VPA alone discussed above, early rapid extinction in the current study did not translate to significantly lower differential ‘occasion setting’ or in fear responding compared to placebo at test of renewal or reinstatement (24 hours after extinction) in either memory domain.

Exploratory sex dependent effects of both PRE manipulation and drug.

Exploratory analysis revealed key insights into sex dependent effects of both PRE manipulation and drug. In men, PRE, but not standard extinction, prevented differential reinstatement of explicit conditioned responses and produced non-significant conditioned responding in SCR over extinction trials. Notably, the patterns of drug effects from the overall analysis were only present (and stronger) in men but were largely absent in women. As with pre-planned non sex stratified analysis, in men, VPA significantly attenuated the CS2 while it was enhanced in PRE men compared to control conditions.

It should be noted that in the current study, the return of a differentiated response between CS1 and CS2 to pre-acquisition/chance-level in males undergoing PRE *could* be attributed to the putative ‘overwriting’ of the original memory contingencies that PRE is supposed to induce. However, because this reduced discrimination was largely due to potentiated response to CS2 rather than attenuation of response to CS1, instead likely points to generalization of fear responding rather than successful updating.

While exploratory, these results add to a burgeoning body of literature highlighting the importance of considering sex differences in emotional learning and extinction processes and has implications for development and treatment of stress-related disorders

(Lebron-Milad et al., 2012; Inslicht et al., 2013; Lonsdorf et al., 2015; Ramikie and Ressler, 2018). However, compared to fear extinction learning (Garcia et al., 2018) less research has been conducted on sex differences in fear and safety signal discrimination in both human and animal models (Day and Stevenson, 2020) but research so far in humans suggests sex may play an important discriminating role (Gamwell et al., 2015). Additionally, there is some evidence for role of sex in moderating pharmacological manipulation of reconsolidation of reactivated aversive memories (Meir Drexler et al., 2015; Meir Drexler et al., 2016).

Stress modulates memory in both sexes not just via interactions with and between neuroendocrine responses but also via different epigenetic mechanisms (sections 1.1.7 and 1.1.8) resulting in different patterns of memory related gene expression between men and women (Keiser and Wood, 2019; Hiscox et al., 2023). Environmental challenges (such as a noxious stimulus exposure), recruit epigenetic mechanisms to produce gene expression changes in the brain (Chandramohan et al., 2007; Reul and Chandramohan, 2007). Previous studies, report gene level sex differences in a range of fear and memory related brain mechanisms, including effects of histone modifying enzymes, (Tsai et al., 2009). Differing effects are observed in male and female rodents following VPA specifically (Murray et al., 2009) with BDNF expression in the dorsal hippocampus observed in male but not female rodents during consolidation (Mizuno et al., 2007). Additionally, any sex differences in recruitment of neural ‘priming’ during extinction learning may speculatively hint at an explanation for the sex dependent fear memory response to VPA in this study. However, no neuroimaging was conducted in this study to explore this possibility and previous research in VPA in humans, while including both sexes, did not report separate analysis by sex (Kuriyama et al., 2011; Kuriyama et al., 2013) with which to compare these exploratory sex dependent effects. Together, the

current and recent findings suggest that much more attention needs to be paid to sexual divergence in the effects of interventions leveraging fear memory mechanisms.

2.6.1 Limitations

The limitations of this study are consistent with the criticisms made of reconsolidation literature in general (Lee et al., 2017; Elsey et al., 2018). The inconsistency of engaging reconsolidation mechanisms in the absence of recognised biomarkers in humans is likely a significant factor in inconsistency of the results in the literature. The behavioural index of PE proposed (Sevenster et al., 2013) was not found to be associated with subsequent fear responding at test in this study for either implicit or explicit responding. This highlights the need for further research to define reliable protocols and identify biomarkers of successful memory destabilization.

Additionally, the complexity of real-life fear memories, which involve multiple associations beyond simple CS-US pairings, presents a challenge for translating laboratory findings to clinical applications. We acknowledge that two days old experimentally generated associative ‘fear’ memories are not comparable to long held real life phobic or trauma memories. Here, the entire conditioning procedure consisted of only 8 CS+ trials (only 5 of which were reinforced). Relative to naturalistic memory, this constitutes a very ‘weak’ form of fear learning and it is entirely possible that the effects observed here would not generalise to more extensive fear conditioning, let alone to naturalistic fear memories. The conditioning protocol was based on prior research for the sake of comparability, but future research must employ more extensive conditioning protocols and naturalistic learning paradigms.

The potential for generalization of fear to safety signals following PRE, as observed in this study, further complicates the interpretation of results and the

development of effective interventions. This also highlights a serious issue when the only metric of reporting in conditioning studies is the differential CR. An apparently beneficial effect of reduced fear CR could simply reflect fear generalisation, which would be considered a highly undesirable outcome (Elsey et al 2018). As seen in this study, when differential CRs are compared to trial-by-trial individual responses to each CS, much more informative patterns of results can be found. It is thus imperative that researchers report more than just differential CRs in conditioning papers.

The fact that in this study exploratory analysis found sex and drug dependent effects and sex dependent responses to reactivation prior to extinction to moderate fear responses in both memory domains does emphasise the importance of exploring the ‘noise in the signal’ in fear conditioning studies specifically (Lonsdorf and Merz, 2017) as well as in research on brain processes more generally (Cahill, 2012). However, the small sample sizes and exploratory nature of the findings must be clearly noted. In addition to the exploratory nature of the analysis on sex, there are limitations in analysing sex as a dichotomous variable (Garcia et al., 2018; Hsu et al., 2021). Future studies might gain greater insight by recording menstrual phase and, to more accurately determine biomarkers that determine sex differences in associative fear (un)learning include assay of stress and gonadal hormone levels, particularly estradiol, in both sexes (Arnegard et al., 2020).

Finally, while the findings have been interpreted through the lens of epigenetic priming and reconsolidation, there are multiple alternative interpretations of pattern of results. Valproate is by no means a ‘selective’ HDACi. Its full pharmacodynamics are not well understood, but it has impacts on GABAergic signalling through a variety of mechanisms, blocks voltage gated sodium channels (Zanatta et al., 2019) and, perhaps most importantly, antagonises androgen and progesterone receptors, causing an

upregulation of androgen synthesis in the gonads and functions as an aromatase inhibitor (Verrotti et al., 2011). These latter effects particularly might explain the sex-dependent effects of VPA observed here, given the different androgen and progesterone receptor density found in males and females and the interaction between fear, stress, gonadal hormones and memory (Toufexis et al., 2006; Maeng and Milad, 2015) (discussed further in *Chapter 1.2* and *Chapter 5*). Further work will be required to tease apart these hormonal and epigenetic effects.

2.7 Conclusions

In conclusion, Sodium valproate can accelerate extinction of explicit fear responding and may attenuate fear responses globally but in this study these effects operated differently in men and women and obscured, rather than enhanced, any benefit of reconsolidation vs standard extinction, primarily through its effects on safety signal processing compared to those following reactivation. More research is needed to understand the hormonal and epigenetic mechanisms underlying these effects and contributing to the persistence and re-experiencing of maladaptive, emotional memory. Future well-powered studies including sex and sex hormones as *a priori* moderators seem warranted to disentangle the sex differences in the effect of engaging reconsolidation mechanisms relating to both safety and aversive stimuli to enhance extinction of aversive memories.

Chapter 3

Hydrocortisone administered immediately after a trauma-like event accelerates the reduction of aversive intrusive memories in women and men

3.1 Introduction

An adaptive fear response provides the ability to respond quickly in the face of life-threatening events to promote survival when encountering a current environmental or endogenous threat and by promoting adaptive learning, it increases the chances of survival when encountering future similar challenges (Sapolsky, 2005). However, the wisdom of this response can break down and become maladaptive due to the formation of overly consolidated aversive memories (Schwabe et al., 2022). This leads to the spontaneous recall of elements of the trauma in response to non-threatening environmental cues due to habitual rather than flexible behavioural responses to such triggers (Hermans et al., 2014a; Smeets et al., 2019). Spontaneous involuntary ‘intrusive’ memories impair contextualized, narrative details of the trauma event and, as in those with PTSD, are associated with long-term, negative consequences for both mental and physical health (Kessler et al., 2017). As discussed in *Chapter 2*, there are challenges in both therapeutically and experimentally moderating long held, robust post-trauma memories (Kessler et al., 2022; Silva and Gräff, 2023). Additionally, there are limited resources available to effectively and affordably treat PTSD using current treatment options resulting in a significant burden (Watson, 2019b) on global health resources due to stress related disorders. An ability to provide effective, accessible, and affordable secondary preventative measures could significantly reduce this burden as well as ease the life-limiting suffering of victims of trauma (Howlett and Stein, 2016).

Glucocorticoids, stress and moderation of aversive memory consolidation

PTSD can be viewed as a disorder of memory. During a life-threatening event adrenal and glucocorticoid responses enhance arousal and memory encoding. While (nor)-adrenaline levels drop quickly glucocorticoid levels rise to high-stress levels over the

following hour before gradually returning to baseline to constrain the stress response (Schwabe et al., 2022). Advances in understanding of how the stress response acts on glucocorticoid receptor-rich brain regions influencing the initial stages of memory consolidation (McEwen et al., 2016; Meir Drexler and Wolf, 2017) and on subsequent retrieval (Tollenaar et al., 2009; Wolf, 2017) suggest an opportunity to pharmacologically intervene at the early stage of the consolidation period.

An intervention administered during the brief (< 6 hour) period post encoding when protein synthesis and synaptic changes are occurring before a memory becomes fully developed (Zohar et al., 2011; Dudai, 2012) can prevent the formation of maladaptive fear memories (Sijbrandij et al., 2015; Wright et al., 2019; Astill Wright et al., 2021). Because synaptic consolidation is a protein synthesis-dependent process, commonly used drugs that inhibit down-stream protein synthesis, such as NMDA receptor antagonists or beta-blockers could be used as secondary preventive measures. However, currently neither meta-analyses of clinical trials (Astill Wright et al., 2021; Florido et al., 2023) nor experimental studies (Astill Wright et al., 2019) support use of beta-blockers, although see Kamboj et al. (2020), and only indirect experimental (Das et al., 2016) or cross-sectional (Deforges et al., 2021) evidence on NMDAR antagonists currently exists. However, experimental evidence (Iyadurai et al., 2019; Kamboj et al., 2020), and observations in clinical settings (Schelling et al., 1999; Weis et al., 2006; Zohar et al., 2011) suggest that altering the glucocorticoid response to stress by directly targeting the HPA axis using a stress level dose of the drug hydrocortisone can prevent the development of involuntary recall of trauma memories and reduce phobic fear (Soravia et al., 2018), although see Graebener et al. (2017), in a way designed to protect against persistent intrusive memory formation.

Role of HPA axis dysregulation in PTSD; The HPA axis has been shown to be dysregulated in PTSD (Dunlop and Wong, 2019) indeed low baseline cortisol levels have been shown to be a risk factor for rather than because of PTSD development. (Steudte-Schmiedgen et al., 2016; Olff and van Zuiden, 2017). There is evidence for stress levels of cortisol post trauma constraining the stress response (de Kloet and Joëls, 2023) to promote return to pre-stress neuronal and neural network functioning (Henckens et al., 2010; Soravia et al., 2018). Utilizing exogenous administration of glucocorticoids shortly after a trauma event aims to promote timely allostasis of the stress system and appropriate contextualized learning of the event to reduce likelihood of maladaptive memory formation.

Mechanistic evaluation of post-trauma hydrocortisone as a preventative strategy to reduced trauma memory re-experiencing; Many of the small-scale trials conducted so far have been in clinical settings and while preliminary evidence for reduced PTSD symptomology in GC treated patients is encouraging (Sijbrandij et al., 2015, Wright et al., 2019) there are limitations to clinical pilot trials. Small sample sizes, heterogeneity of dosages and/or multiple dosing of hydrocortisone commonly in severely physically ill individuals (Astill Wright et al., 2021) make mechanistic assessment more difficult than can be attempted in controlled experimental settings. For effective use within the short secondary preventative window, it is important to clarify which memory phase is being targeted by the exogenous administration of glucocorticoids as well as to conduct research that has the statistical power to robustly analyse and identify individual differences and biomarkers of drug response while limiting the potential influence of statistical ‘noise’ that can be associated with clinical studies that understandably prioritise clinical outcomes over experimental control.

Within the existing research literature inconsistencies have been reported in the effects of glucocorticoids levels on memory formation and retrieval (de Quervain et al., 2017). Administration of exogenous cortisol has been shown to enhance (Buchanan and Lovullo, 2001) or have no effect (Rombold et al., 2016b) on intrusive, aversive memories. Stress effects on memory are complicated with differential effects on distinct memory phases and domains such that memory can be both enhanced or impaired by either endogenous or exogenously administered (e.g., hydrocortisone) increases in glucocorticoid levels. Hydrocortisone effects on memory are time (Het et al., 2005) and dose dependent (Schwabe and Wolf, 2014) such that both high and low levels of glucocorticoids can impair, enhance or have no effect on memory if acting prior, at or after encoding, consolidation or during recall (Baldi and Bucherelli, 2005; Schilling et al., 2013). Additionally, cortisol effects are themselves dependent on concurrent levels of noradrenergic activity (Roosendaal et al., 2006) and other neuromodulators (Olff and van Zuiden, 2017) and these interactions will have differential effects in different memory domains (Schwabe and Wolf, 2012). Considering these issues, experimental medicine studies are critical for identifying and optimising PTSD prevention strategies (Iyadurai et al., 2019). Such studies allow aversive memory symptomology (e.g., distressing involuntary memories) to be modelled and assessed, while limiting the influence of confounds common in clinical studies (James et al., 2016).

Sex and sex hormone interactions with stress on frequency and affective quality of aversive memory moderation; Although there are sex differences in rates of exposure to different trauma types, men and women have similar exposure to traumatic events in their lifetimes (Olff, Langeland, Draijer, & Gersons, 2007). However, despite this, women are twice as likely to develop PTSD than men (Haskell et al., 2010). Stress and sex differences are reported in all phases of memory formation resulting in different effects on fear

memory recall performance (Garcia et al., 2018). Women are also disproportionately affected by severity of symptoms (McLean et al., 2011) of PTSD, by peritraumatic symptoms such as dissociation (Fullerton et al., 2001) and anxiety (Rattel et al., 2019b) as well as rumination styles (Zetsche et al., 2009) and subjective interpretation of trauma (Christiansen and Hansen, 2015).

It is perhaps not surprising that to date there is mixed evidence for the effects of hydrocortisone on memory performance between men and women (Felmingham et al., 2012b; Meir Drexler et al., 2015; Meir Drexler et al., 2016). However, there is increasing evidence that the large monthly fluctuations in gonadal hormones across the natural menstrual cycle in women influences vulnerability or resilience to the development of intrusive memories (Kornfield et al., 2018; Maddox et al., 2019). Estradiol and progesterone levels, or their ratio (Soni et al., 2013), have been shown to interact with stress hormone response (Herrera et al., 2016) to have a profound influence on subsequent emotional memory (Ney et al., 2019) and in intrusive memory formation specifically (Ferree et al., 2011; Soni et al., 2013) possibly due to alterations in associative/extinction learning depending on these gonadal hormone levels (Garcia et al., 2018).

There are also complex individual differences in cortisol reactivity following a range of stressors even within women depending on hormone status (Kuhlmann and Wolf, 2005). Hormone contraceptives increase cortisol-binding globulin concentrations that lead to a blunted free cortisol response (Kirschbaum et al., 1999) and lower basal cortisol levels are a risk factor for PTSD development (Lehrner et al., 2016). There is considerably less research looking at the role of gonadal hormone moderation of intrusive memory in men (Garcia et al., 2018; Hsu et al., 2021). Despite men not having the large gonadal hormone fluctuations associated with female menstrual cycle there is considerable variability in basal and stress-induced gonadal hormone level changes between

individuals and there is evidence to suggest that men exhibit extinction recall similarly to high estradiol women (Milad et al., 2010) although see (Milad et al., 2006).

Previous experimental administration of hydrocortisone given at stress-levels (30mg) shortly *after* (Kamboj et al., 2020) an analogue trauma (a highly distressing film designed to elicit PTSD-like intrusive memories) demonstrated a reduction in intrusion frequency compared to placebo an effect not seen with 20mg *before* trauma (Rombold et al., 2016b). Kamboj et al (2020) study purposefully controlled for the monthly fluctuation in sex hormone levels by excluding women not taking a hormone-based contraceptive; they also excluded men from this study. It was therefore not clear whether hydrocortisone would have a similar effect in men.

The current study

This study aimed to extend the findings of Kamboj and colleagues (2020) to determine if hydrocortisone (30mg, oral) administration (immediately after an analogue trauma) would be associated with lower frequency, vividness, and distress ratings of intrusive memories in a sample of healthy men and women recorded using an online diary over 7 days relative to placebo. Specifically, the pre-registered hypothesis of a differential time course in reduction in intrusion frequency, following hydrocortisone and reduced average vividness and distress ratings (Hennessy et al., 2019). Declarative memory (free and cued recall) for trauma film contents tested 7 days later was hypothesised would be unaffected.

Additionally, it was hypothesised that men and women would respond differently to the effects of hydrocortisone on emotional memories. Given evidence of differences in men and women in the effects of hydrocortisone on memory performance (e.g., (Felmingham et al., 2012b; Drexler et al., 2016) time- and sex dependent effects of

hydrocortisone on intrusive memories following analogue trauma were explored. However, since there was uncertainty regarding sex effects of hydrocortisone specifically on intrusive memories, no directional predictions were made regarding the underlying relationships. Although not pre-registered, sex- and drug-dependent influence of progesterone and estradiol on intrusions was investigated.

3.2 Methods and Materials

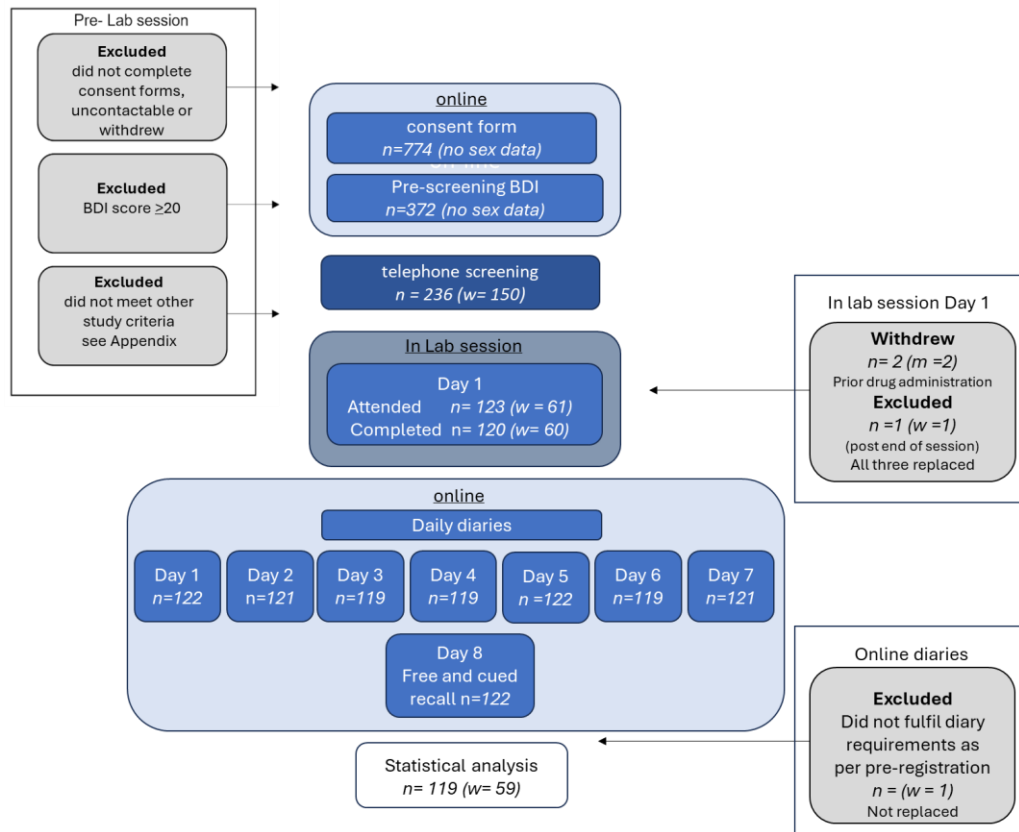
The study was pre-registered on the Open Science Framework at osf.io/76yvk. Ethical approval was received from the UCL Ethics Committee, and all procedures were conducted in accordance with the Declaration of Helsinki, participants provided informed consent (see appendix).

3.2.1 Participants

Healthy men and women (18 – 35 years, $n = 120$), were recruited via online participant recruitment platforms and campus-wide advertisements. Eligibility was assessed via online survey and telephone screening interview. Participants were excluded for any psychiatric disorder that currently or had previously required treatment, if they had experience of an event or events judged at interview to put the volunteer at increased risk of PTSD. Women were additionally required and verified to be taking a hormone-based contraceptive (>1month). This requirement was to control for the fluctuations in sex hormones accompanying the female menstrual cycle by maintaining them at levels comparable to the follicular phase and so limiting fluctuating hormone level influences on intrusive memory formation (Soni et al., 2013). This criterion additionally sought to keep female estradiol and progesterone levels similar to those of the male participants (Howlett and Stein, 2016). Full list of eligibility criteria are available in the appendix and at osf.io/76yvk.

Exclusions and withdrawals (see Figure 3.1); An equal number of men and women completed Day 1 in the lab ($n = 120$). Subsequently, four participants were excluded; two men in the hydrocortisone group withdrew from the study before drug administration due to finding the contents of the trauma film too distressing, and one woman (placebo group) participated in a similar pharmacological experiment during the following week (between

day 1 and 7) which was judged to potentially interfere with the results of this study; they were replaced. The fourth exclusion (woman; placebo) failed to complete the diary entries as required by pre-registration criteria for inclusion. She was not able to be replaced due to the COVID-19 restrictions then in place. Therefore, the final (non-replicated) analysis was conducted on sample $n=119$ (women; $n=59$).



BDI = Beck Depression Inventory, W = Women

Figure 3.1. Participant flow diagram depicting participant recruitment from response to advertisement to inclusion in final data analysis. Participants were not included if they did not consent to or complete the Beck Depression Inventory online (Beck et al. 1996) or if their BDI score was ≥ 20 . All participants were spoken to on the telephone ($n=236$ (Women (W)=150)) and excluded if they did not meet the inclusion criteria (see appendix and osf.io/76yvk). On Day 1 in lab session, 123 participants took part, 2 men withdrew prior to drug administration and were replaced, one woman was excluded due to concurrent participation in a study deemed to confound the results and was replaced. One woman completed the study, but incomplete diary entries led to her exclusion as per registration requirements; she was not replaced. Final inclusion into statistical analysis was $n=119$ (Women=59)

3.2.2 Drugs

Both participant and experimenter were blind to drug condition. Envelopes containing either hydrocortisone or placebo capsules were pre-prepared and labelled only with participant id by external experimenter. Placebo (skimmed milk powder) and Hydrocortisone (Auden Mckenzie Pharma Division, Ruislip, UK) were prepared in identical gelatine capsules (two capsules per participant), Drug (30mg hydrocortisone or matched placebo, following Kamboj et al. (2020)). The capsules were taken with water under supervision of the experimenter immediately following viewing the distressing film (analogue trauma). Post drug measurements were taken after a 1hr drug absorption period (at T60, see Figure 3.2). At the end of Day 1 of the study a ‘treatment guess’ was made by participants (hydrocortisone or placebo). Their entry was not visible to the researcher and the researcher made their guess subsequently. Both guesses were made directly into the computerized survey. Blinding was maintained until all data had been collected from every participant.

3.2.3 Trauma Film

Participants experienced analogue trauma by watching a ‘trauma film’ (~14 minutes) that contained two scenes from the publicly available film ‘*Irreversible*’ (120 Films, Studio Canal +, France, 2002) designed to be distressing and to induce involuntary memories (Weidmann et al., 2009). Each scene was preceded by a voiceover describing the characters and context of the scenes and is described in detail elsewhere (Das et al., 2016; Kamboj et al., 2020). Participants watched the film on a Tobii T60 (Tobii AB, Stockholm, Sweden) eye-tracker monitor in a darkened lab, with audio presented through

headphones. A chinrest was used to minimize head movements during eye tracking. The exact start and finish time of the film was event-marked.

3.2.4 Self-report measures

Self-reported measures of general psychological characteristics and stable mental health were assessed before the start of the study, Beck Depression Inventory, BDI, (Beck et al., 1996), to confirm depressed mood <20 . Then on Day 1, before the film procedure and drug administration (Figure 3.2), anxiety was assessed with the Spielberger Trait Anxiety Inventory, STAI, (Spielberger, 2010), dissociation using the Dissociative Experiences Scale-II, DES, (Carlson and Putnam, 1993), impulsivity with the Barrett Impulsiveness Scale, BIS, (Patton et al., 1995), and habitual responses to emotion using the Emotion Regulation Questionnaire-9, ERQ, (Spaapen et al., 2014). Sleep characteristics (average hours of sleep/night) were assessed with the Pittsburgh Sleep Quality Index, PSQI, (Buysse et al., 1989). Women stated the type, duration and pattern of hormone-based contraceptive use and menstrual cycle pattern. On Day 8 general trauma-like symptoms (previous 7 days) were assessed using the 22-item Impact of Events Scale, IES, (Weiss, 2007) adapted for the trauma-film (Holmes et al., 2009).

Changes in *state* subjective affect from pre- to post-film and post-drug were assessed using the Positive and Negative Affect Schedule, PANAS, (Watson et al., 1988). The Bodily Symptoms Scale, BSS, (Bond and Lader, 1974) was used to assess a range of subjective cognitive and physical sensations (anxiety, depression, memory impairment, palpitations, nausea, emotional numbness, euphoria, drowsiness, muscle tension, headache, concentration, tremor, vertigo, confusion).

3.2.5 Physiological and endocrine measures

At the same time-points as the state measures, cardiovascular and endocrine (saliva) samples were taken. Immediate post-film (t_0 ; BP, salivary hormones, subjective state and HR from final 5 min of film) measures were intended to capture the acute stress response to the film (see Figure 3.2).

3.2.5i Cardiovascular measures

Sympathetic activation was monitored in response to the film via changes in blood pressure (BP; BM40 XL, Beurer, UK) and heart rate (HR; BodyGuard-2, FirstBeat Technologies, Jyväskylä, Finland) using ECG electrodes attached to the torso, either side of the midline (Bodyguard 2, Firstbeat), HR was recorded continuously and subsequently sampled pre-, and post-film (as well as post-drug) as described previously in Kamboj et al. (2017). Event markers were added to correspond to the relevant time points; for example, at the start ($t -15$) and end of the film (i.e., t_0), and end of the 1 hr drug absorption period ($t+60$) to identify 5-min epochs for use in obtaining average HR.

3.2.5ii Endocrine (saliva) measures

Steroid hormone levels were determined as part of a hormone panel from saliva samples taken pre-film (t_{-15} ; see Figure 3.2), immediately post-film (t_0), 15-min post film (t_{+15}) and 1-hour post drug (t_{+60}). Saliva was collected in salivettes as passive drool (see appendix for details) and stored at -80°C . Cortisol, cortisone, and progesterone levels were analysed using liquid chromatography-tandem mass-spectrometry (LC/MS) as described in detail elsewhere (Gao et al., 2015). Estradiol and salivary amylase levels were measured using a commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). The intra and inter-assay coefficients for estradiol were below 11%.

EEG. A simple EEG headset with dry electrodes (DREEM band, Rhythm, Paris, France) was worn during the procedure, although this data was not used.

3.2.6 Memory assessments

3.2.6.i. Intrusive memories

After viewing the film, participants were given a description of the nature of intrusive memories following Kamboj et al. (2020) with full details available at osf.io/76yvk and appendix 2.2. Participants recorded momentary intrusions for 60 min on an E4 device (Empatica, Boston, MA, USA), starting after the 1 hr drug absorption period (i.e. t_{+60} to t_{+120}) during a second filler (music) task period. Thus, while listening to a standard set of classical music pieces and periodically rating these for pleasantness, they were instructed to press the response button on the E4 once for every occurrence of an involuntary memory relating to the film. This acute period of memory monitoring was intended to capture post-encoding memory retrieval which may contribute to early consolidation differently in the two drug groups. Any early difference in groups would be expected to reflect non-genomic effects of hydrocortisone/cortisol (Joëls, 2008).

Before they left the laboratory, participants were given detailed instructions on how to record intrusive memories over the following week (Das et al., 2016; Kamboj et al., 2020) as described in appendix 3.2. Participants received daily reminders at 9pm to record (as close to bedtime as possible) the number of intrusions that occurred together with a brief description (to verify the relevance of the memory to the contents of the trauma film). The definition of intrusive memories provided to participants emphasized their involuntary nature and distinguished between sensory and verbal mental events. Based on the descriptions provided in the memory diaries, all recorded intrusions conformed to the definition of sensory-perceptual memories. Additionally, participants

recorded how distressing and vivid they found each intrusive memory using a 5-point scale (1 = ‘not at all’; 5 = ‘extremely’). The average number/day and associated average distress and vividness over 7 consecutive days per participant was calculated.

3.2.6.ii Voluntary (recall) memory (Day 8)

Voluntary memory was assessed on one occasion only (day 8). For free recall, participants were asked to recall “as much information and detail as possible including information about where things happen, when they happen, who they happen to, what the people and scenes look like, etc.,” typing this directly into a text box with no time limit. Performance was determined by counting the number of recalled ‘idea units’. After the free recall task, participants provided open text responses to 19 questions about events in the two clips (cued recall). These questions related to contextual aspects of the narrative (i.e., where events take place; what happens to the actors; who does what to whom, and when). There was no time limit for either recall task. Every participant’s free and cued recall task performance was independently scored either 0 (inaccurate), 0.5 (partially accurate), or 1 (accurate) by two treatment-blind researchers. High interrater agreement was achieved (>90%) in scoring both tasks with disagreements resolved through discussion. Positive and negative affect, PANAS, (Watson et al., 1988) ratings were taken before and after the memory recall tasks.

3.2.7 Procedure summary

The study protocol is depicted in Figure 3.2. Following acceptance into the study participants were invited to the lab on Day 1 with testing commencing between 13.00 and 18.00 to limit the effects of diurnal variation in cortisol levels (Walker et al., 2010). Upon arrival, participants confirmed fasting (except for water) for the past ≥ 2 hr, and abstinence from alcohol/recreational drugs ≥ 24 hr (and intention to do likewise for the 24 hr after the

session). After attaching the ECG device, participants completed an inhibitory control task followed by the self-report measures of 'trait' and 'state' mental health and general psychological characteristics (above and Table 3.1). Baseline BP, HR, endocrine (saliva), and state subjective affect, PANAS, (Watson et al., 1988), cognitive and physical characteristics, BSS, (Bond and Lader, 1974) were then sampled pre-film (t_{-15} ; see Figure. 3.2), after which they viewed the trauma film (~14mins). Drug capsules were then administered, and the state and physiological pre-film measures repeated at the post-film (t_0) time-point. Participants sat quietly while completing a 2 hour 'filler' music task to allow for a 1 h drug absorption period followed by a second 1 h period (60 – 120 min post drug) when they were asked to record acute intrusive memories (full details of instructions available in the appendix 3.2).

The day 1, in-lab session was followed by seven consecutive daily online diaries (starting at the end of day 1), reporting intrusion frequency, content, vividness and distress. Voluntary memory assessment and trauma symptoms using Impact of Events Scale (Weiss, 2007) modified for the trauma film (Holmes et al., 2009) were assessed remotely only on one occasion (day 8). All self-report measures including intrusion variables were recorded using the online survey tool, Qualtrics (Provo, UT, USA). Once completed, participants were debriefed and compensated (£37).

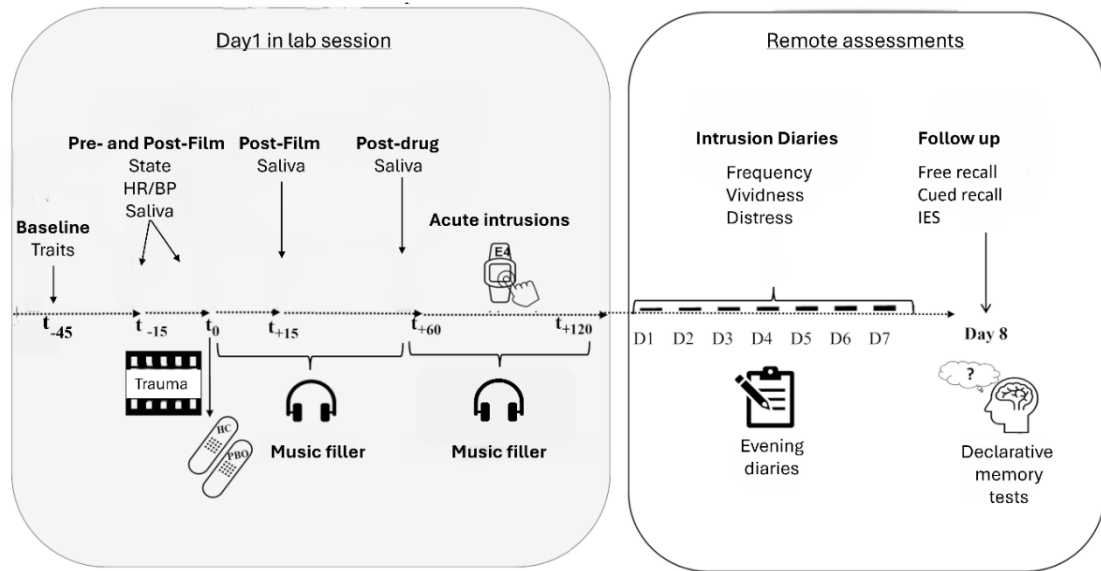


Figure 3.2 Schematic depicting study protocol. Day 1 was conducted within the lab. After baseline trait questionnaires (see in text) were completed, measurements taken at four time points (T1 – T4) for blood pressure (BP) stress and sex hormones (saliva). State measures (self-reporting of mood and behavioural responses using Positive and Negative affect scale and Bodily Symptoms Scale) were taken at T1, T2 and T4. Additionally, heart rate (HR) was continuously measured. Participants recorded time and frequency of spontaneous (intrusions) about the analogue trauma (Trauma film, see in text) during and after peak drug absorption. Following daily recording of intrusions over next 7 days (7 day diary) Impact of Events, (IES), declarative memory (Free and Cued Recall) and the state measures as for Day 1 were assessed online on Day 8. Adapted from (Hennessy et al., 2022)

3.3 Statistical analyses and power calculations

Statistical analyses were conducted using R (R Development Team, 2015) and Stata (Version 17). Figures are depicted with either standard errors or 95% confidence intervals and descriptive statistics reported in the text are means with standard deviations. As expected, there was very little missing data on the main (intrusion-related) outcomes (two missing entries of 833 possible entries for the included $n=119$ participants; $<0.3\%$ missing). The missing counts were replaced by the value for that participant from the next day's diary entry (referred to as 'next observation carried back' in the *missing data* section of the preregistration document). Per the pre-registered analysis plan, outliers were defined as having a standardized absolute value $z > 3$. These values were winsorised to $1+$ largest non-outlier. This applied to 11 individual data points for the intrusion frequency data (1.3%), seven of which came from a placebo participant, and four from hydrocortisone participants. Eight of these data points were from days 3-7. Baseline characteristics were compared between men and women and between different contraceptive using women, using independent samples t-tests, with Benjamini-Hochberg false discovery rate (FDR) adjustment applied to p values. Equality of variance assumptions were found to hold and therefore no adjustments to dfs or p values were made. Generally, men and women had similar baseline characteristics, except for years of education, BMI, systolic BP and suppression, as outlined in Table 3.1.

In all analyses, the within-subjects factor was either 'timepoint' (with 3 or 4 levels, depending on the variable) or 'day' (7 levels: days 1-7). Drug group was the key between-subjects independent variable and sex, the primary between-subjects moderator. Differences in cell counts in a 2 x 2 Table of Assigned Condition x Guessed Condition were analysed using a Chi squared test.

Within-session changes in BP, HR, cortisol/cortisone and PANAS ratings were analysed using repeated measures ANOVAs. Violations of sphericity were corrected by adjusting *dfs* and *p* values. *P* values associated with post hoc pairwise comparisons are reported with Bonferroni correction. For intrusion outcomes where day was a factor, general or generalized linear mixed models (GLMM) were used to estimate fixed effects of drug, sex, day and their interactions (Zuur et al., 2009) using the glmmTMB package. To model the serial dependency from the same person, participant was included as a random factor in the analyses. As is typical in trauma film research (Das et al., 2016; Kamboj et al., 2020), intrusion data were over-dispersed (dispersion statistic: 1.18, $p < 0.0001$). There was also a preponderance of zeros (65%), suggesting that the appropriate modelling approach was the zero inflated Poisson (ZIP) GLMM. Simulation with 1000 datasets (Hartig, 2021) confirmed overdispersion ($p < 0.0001$) and zero inflation (>95% of simulated samples contained fewer zeros than the data; $p = 0.032$), validating the ZIP model. Model assumptions were checked by plotting residuals against predicted values. No problematic residuals were detected. Confidence intervals for estimates presented for the mixed effects ZIP analysis were obtained using the procedure outlined by Brooks et al., (Brooks et al., 2017).

Linear mixed models were used to model fixed effects and interactions of drug, sex and day for vividness and distress ratings. Mixed effects models were used to analyse the main hypotheses to account for serial dependencies in the data arising from repeated within-participant assessment. Model selection was based on the Akaike Information Criteria (AIC), a function of model fit and complexity. Where AICs of two models were similar, the more parsimonious model was selected.

Exploratory analysis: The nature of the interaction between drug, sex and the sex hormones estradiol and progesterone on total diary intrusion counts were explored

separately for each hormone as there was no four-way Drug x Sex x Estradiol x Progesterone interaction ($p=.372$). This analysis must be considered exploratory as it was not specifically detailed in the pre-registration documents.

3.3.2 Power calculations and randomisation to group

Power calculation for a simple between groups (Sex x Drug=4 groups) comparison on total number of intrusions, with an assumption of a large effect size ($f=0.4$, based on similar pharmacological and behavioural studies (Sijbrandij et al., 2015; Astill Wright et al., 2019) and $\alpha=0.05$ and $\beta=0.95$ suggested that a total of $n=112$ was adequate. Group allocation was achieved using a random number generator (<https://www.random.org/sequences/>) produced a randomly ordered and non-repeating sequence of integers (1-120) which corresponded to a unique id. Each number in the random sequence was assigned alternatively to either hydrocortisone or placebo and separately to male or female with equal number of men and women before the sequence was ordered from 1-120. On Day 1 participants were assigned the next sequential id depending on their sex.

3.4 Results

3.4.1 Participant Characteristics

Participant characteristics (Table 3.1) were in the expected range for normative samples, with average values close to published averages in similar studies (Rombold et al., 2016b; Kamboj et al., 2020). In general, men and women showed similar baseline characteristics although there were significant differences in number of years of education (men: $M = 15.9$, $SD = 2.4$, women: $M = 17.2$, $SD = 2.3$; $p = 0.014$) and BMI (men: $M = 24.4 \text{ kg/m}^2$, $SD = 3.2$, women: $M = 22.6$, $SD = 2.9$; $p = 0.014$). There were also differences found on systolic BP (men: $M = 113.1$, $SD = 13.0$; women: $M = 103.6$, $SD = 12.3 \text{ mmHg}$, $p = 0.001$ (Wright et al., 2011), and on the tendency to suppress emotions (ERQ-suppress (Spaapen et al., 2014); men $M = 16.0$, $SD = 5.2$; women: $M = 12.3$, $SD = 5.1$; $p = 0.001$). The inclusion of these latter variables as post hoc covariates in the analysis of intrusions did not affect the results substantially. Other baseline variables—including estradiol and progesterone levels did not differ between men and women (Table 1b). Baseline estradiol levels were 3.53 ± 1.47 (range: 0.59-6.70) pg/ml in men and 3.32 ± 1.83 (range: 0.11-8.26) pg/ml in women ($t(113)=0.663$, $p=0.509$). Baseline progesterone levels were 8.38 ± 11.13 (range: 0.00-64.90) pg/ml in men and 12.99 ± 17.26 (range: 0.00-72.43) pg/ml in women ($t(110)=1.68$, $p=0.095$). Among women, $n=17$ used a progesterone-type, and $n=42$, a combination-type (estradiol + progesterone) contraceptive. Contraceptive type did not affect baseline estradiol or progesterone levels (p values >0.7) or amylase, cortisol or cortisone levels (p values >0.4). Other baseline variables did not differ among women using progesterone-only or combination estrogen-progesterone contraceptive using women (p values ≥ 0.72).

Participants and experimenters guessed treatment assignment at chance levels (both 55%; $\chi^2(1) \leq 1.31$, $ps \geq 0.253$) indicating successful blinding.

Table 3.1 Participants characteristics by drug.

	Placebo (n=59)	Hydrocortisone (n=60)
Sex (m:w)	30:29	30:30
Age (yrs)	24.8 (4.5)	24.8 (4.5)
Education (yrs)	16.7 (2.4)	16.5 (2.5)
BDI	4.6 (4.8)	5.1 (4.8)
STAI	32.9 (7.8)	35.4 (7.9)
BIS	36.0 (6.5)	36.7 (6.5)
DES	9.5 (9.2)	9.0 (7.0)
ERQ (reapp)	26.5 (4.7)	25.7 (5.5)
ERQ (suppress)	13.9 (5.6)	14.5 (5.4)
Sleep (hr)	7.6 (0.9)	7.4 (0.9)
BMI (kg/mm2)	22.8 (3.3)	23.9 (3.2)
Systolic BP (mmHg)	108.6 (12.1)	108.2 (14.0)
Diastolic BP (mmHg)	68.9 (10.7)	69.2 (10.8)
HR (BPM)	72.3 (10.3)	72.5 (9.1)
Cortisol (nmol/L)	3.95 (2.77)	3.63 (2.23)
Cortisone (ng/ml)	4.68 (2.23)	5.59 (2.16)
Progesterone (pg/ml)	9.65 (15.73)	11.68 (13.36)
Estradiol (pg/ml)	3.39 (1.74)	3.47 (1.58)

The final sample was $n = 119$ with men($n=60$) and women($n = 59$) BDI Beck Depression Inventory, STAI Spielberger Trait Anxiety Inventory, BIS Barrett Impulsiveness Scale Sleep, DES Dissociative Experiences Scale, ERQ Emotion Regulation Questionnaire (ERQ-cognitive reappraisal, ERQ-expressive suppression). ‘Sleep’ refers to average hours of sleep per night. Sex differences: Between men and women there were significant differences in number of years of *education, *BMI, *systolic BP and on the tendency to suppress emotions (*ERQ-suppress (all $ps < .015$). However, the inclusion of these latter variables as post hoc covariates in the analysis of intrusions did not affect the results substantially. Other baseline variables including estradiol and progesterone levels did not differ between men and women (FDR corrected $ps > 0.05$). (Hennessy et al., 2022)

3.4.2 Measures from Day 1 lab session

3.4.2.1 Blood pressure and heart rate

There was no main effects or interactions involving drug (F values <1) involving cardiovascular measures (systolic or diastolic or HR) nor on measures of affect (PANAS scores). However, time (pre-film: $t-15$, post-film: t_0 , post-drug: $t+60$) by drug (placebo, hydrocortisone) repeated measures ANOVAs indicated that changes in diastolic and systolic BP over time were not moderated by drug; there was also no main effect of drug (interaction and main effect F values <1). Main effects of time were found for both systolic ($F(2, 234)=7.05$, $p=0.001$) and diastolic BP ($F(2, 234)=5.48$, $p=0.005$). This reflected an increase in systolic BP from pre- to post-film (Δ Systolic $t-15 \square t_0 = +3.2$, $p = 0.001$), followed by recovery within an hour (Δ Systolic $t_0 \square t+60 = -2.8$, $p=0.005$). A similar pattern (increase BP followed by recovery) was found for diastolic BP (Δ Diastolic $t-15 \square t_0 = +2.7$, $p=0.009$; Δ Diastolic $t_0 \square t+60 = -2.0$, $p=0.041$).

HR (Figure 3.1C) also showed a main effect of time ($F(2,224)=38.93$, $p<0.001$; $\eta^2=0.26$), and no main/interaction effect of drug (F values <1). The time effect for HR, resembled the pattern for systolic BP: a $t-15$ to t_0 increase (Δ HR $t-15 \square t_0 = +2.2$, $p<0.001$), followed by recovery between (Δ HR $t_0 \square t+60 = -4.6$, $p<0.001$; Figure 3.1C).

3.4.2.2 Subjective measures

Paralleling the changes in the above indices of sympathetic arousal, negative affect (Figure 3.3 D) showed a main effect of time ($F(1.3, 155.8)=130.32$, $p<0.001$), but no main/interaction effects involving drug (F values <1). This reflected an increase in negative affect from pre- to post-film (Δ PANAS(neg) $t-15 \square t_0 = +6.8$, $p<0.001$) followed by recovery at $t+60$ (Δ PANAS(neg) $t_0 \square t+60 = -7.1$, $p<0.001$). The time effect (and absence of drug effects $F<1$) on positive affect ($F(1.3, 155.8)=64.95$, $p<0.001$; Figure

3.3B) was in the opposite direction from pre- to post-film (Δ PANAS(pos) $t-15 \square t0 = -4.9$, $p < 0.001$) but unlike negative affect and the cardiovascular indices, there was on recovery within 1 hr (Δ PANAS(pos) ($t0 \square t+60$): -0.1 ($p > 0.99$); Figure 3.3C).

Supporting the notion that changes in cardiovascular activity and negative affect had a common underlying cause, Δ PANAS(neg) $t-15 \square t0$ was positively correlated with Δ Systolic $t-15 \square t0$ ($r(117) = 0.24$, $p = 0.009$), Δ Diastolic $t-15 \square t0$ ($r(117) = 0.31$, $p = 0.001$), and Δ HR $t-15 \square t0$ ($r(112) = 0.22$, $p = 0.020$). Although there was no control condition for the trauma-film, these analyses were generally consistent with a time-limited stress response (increase in physiological and subjective arousal/negative affect) in response to the film, followed by recovery within an hour (Figure 3.3).

There were also time effects found for some items of the Bodily Symptoms Scale (Bond and Lader, 1974). These effects showed either a V-shaped time ($t-15$, $t0$, $t+60$) effect (Figure 3.3A), with lowest scores at $t0$ (euphoria (Figure 3.3A), drowsiness, concentration, $ps \leq 0.003$) or an inverted-V, i.e., peaking at $t0$ (anxiety, depression, palpitations, nausea, emotional numbness, muscle tension, tremor; main effects of time, all $ps < 0.001$). The memory impairment item showed a Time x Drug interaction. However, this reflected a non-significant (post hoc $p = 0.102$) 2-point larger increase (on a 0-100 scale) between $t-15$ and $t0$ in the placebo versus hydrocortisone group. Confusion, headache and vertigo showed no time or drug effects (p values of main effects and interactions ≥ 0.150).

3.4.2.3 Sex effects on affective measures

Overall women reported lower positive affect than did men ($F(1,116) = 5.211$, $p = 0.029$) but no significant (Δ PANAS(pos) between the sexes ($p = .062$) and no significant drug/time interactions with sex for reported positive or negative affect. However, the

changes in HR over time reported earlier, were different for men and women ($F_{1,92}, 212.51 = 3.590, p = 0.031$) with significantly higher HR t_0 ($p = 0.019$) and HR $t+60$ ($p = 0.004$) in women than men although there had been no significant difference in HR at baseline $t-15$ ($=.25$). A main effect of sex on systolic BP ($F_{1, 116} = 22.061, p < 0.001$) reflected lower systolic BP in women at all time points ($p_s < 0.001$). Diastolic pressure followed a similar pattern.

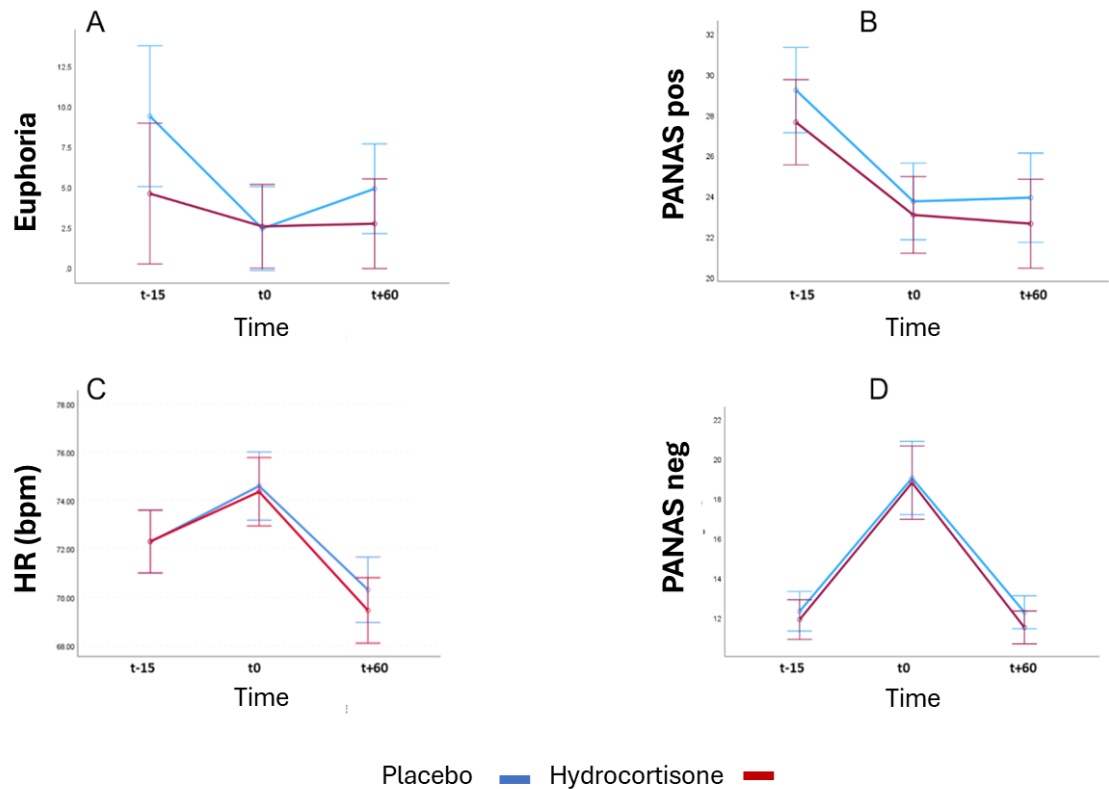


Figure 3.3 Cardiovascular and subjective changes during lab session (Day 1). Mean heart rate (bpm) (C) as well as systolic and diastolic BP (not depicted) and PANAS (negative) (D) all increased from pre- (t_{-15}) to post-film (t_0 ; $ps=0.001$) and recovered to approximately baseline levels by the post-drug timepoint (t_{+60} ; $ps\leq 0.041$) and similar time effects were found for some items of the Bodily Symptoms Scale, BSS, (Bond and Lader, 1974)). These effects showed either a V-shaped time (t_{-15} , t_0 , t_{+60}) effect, with lowest scores at t_0 (e.g. euphoria, (A)) or as for PANAS negative (D) an inverted-V, i.e., peaking at t_0 (anxiety, depression, palpitations, nausea, emotional numbness, muscle tension, tremor; main effects of time, all $ps<0.001$, not depicted). The time effect on positive affect (B) was in the opposite direction to negative effect from pre- to post-film but unlike negative affect and the cardiovascular indices, there was no recovery within 1 hr (see text). No main effects or interactions involving drug for any of these variables were found (F values <1). (Adapted from Hennessy et al., 2022).

Table 3.2. Salivary hormones cortisol and cortisone, values are means $\pm$ SDs. (Hennessy et al., 2022).

Cortisol nmol/L	t-15	t0	t+15	t+60
Placebo (n=50)	2.99 (2.94)	10.99 (28.20)	4.41 (5.15)	2.97 (3.59)
Hydrocortisone (n=47)	3.59 (2.27)	22.31 (19.88)	5.95 (4.19)	86.46 (73.96)
Cortisone (ng/ml)				
Placebo (n=50)	4.69 (2.33)	3.52 (1.18)	4.66 (2.66)	4.36 (2.73)
Hydrocortisone (n=47)	5.43 (2.08)	3.85 (1.62)	4.70 (2.08)	28.35 (16.23)

3.4.2.4 Salivary hormones: cortisol and cortisone

Table 3.2 shows the effects of Time and Drug on levels of cortisol and cortisone. Hydrocortisone administration resulted in a robust time-dependent effect of drug on salivary cortisol levels as evidenced by a time (4 levels; t-15, t0, t+15 and t60) x drug interaction: $F(1.3, 123.1)=47.32, p<.001$). This reflected supra-physiological levels of cortisol at the peak absorption timepoint i.e., 60 min post drug (t₊₆₀; 86.46 ± 73.56 nmol/L), which were significantly higher than all previously measured timepoints ($ps<.001$) whereas the t+60 cortisol levels in the placebo group did not significantly differ from any previous timepoints (t-15, t0, t+15; all $ps >.99$). The peak post hydrocortisone levels of cortisol we are reporting here as measured by liquid chromatography-tandem mass-spectrometry (LC/MS) are consistent with previous hydrocortisone studies measured using immunoassay techniques and the drug group levels of cortisol in this study are many times larger than would be expected following the use of social psychological stressor (Dickerson and Kemeny, 2004) or a physical, cold pressor, stress induction protocols (Schwabe and Schächinger, 2018). The accuracy of the cortisol response was supported by a similar salivary cortisone response (considered a more

accurate measure than free serum cortisol as it is unaffected by oral hydrocortisone) which was confirmed by a similar Time x Drug interaction effect ($F(1.1,101.6)=102.04$, $p<0.001$; $\eta^2=0.52$) as seen with cortisol i.e., significantly higher cortisone levels at $t+60$ than at any other time point in the drug group ($ps<0.001$) but no difference between that time point and any other in the placebo group ($ps>.99$).

3.4.2.5 Acute and sub-acute post-film intrusions (Day 1)

Instantaneously recorded intrusions reported during the 60 min period starting 60 min after drug administration (t_{+60} to t_{+120} ; see Figure 3.2) did not differ significantly between the two drug groups: $IRR=1.19$, $SE=0.30$, $z=0.69$, $p=0.491$ (coefficient for reference (placebo) group =7.38, $SE=1.31$) despite the difference in cortisol levels between the hydrocortisone drug ($t+60$ 86nmol/L (73.56) group and the placebo group ($t+60= 2.97$ nmol/L(3.59)) at the start of this period. This suggests that involuntary retrieval of trauma-like memories in the post-encoding period was unaffected by (rapid, non-genomic effects of) hydrocortisone.

The time delay between the end of the in-lab session and completing the day 1 diary at bedtime that evening was similar in the placebo (10.8 ± 2.7 hr) and hydrocortisone groups (11.3 ± 3.4 hr; $t(117) = 0.86$, $p=0.393$). As per previous findings (Kamboj et al., 2020) and stated at pre-registration the possible association between the reported sub-acute intrusions and $t+60$ (post cortisol) levels in the hydrocortisone group reported during this time interval was explored. However, unlike previous findings (Kamboj et al., 2020) no significant linear or quadratic relationship in the hydrocortisone group as a whole ($ps>.3$) was found. However, the previous study had observed a significant correlation in their all-female sample (Kamboj et al., 2020) correlations were examined separately in

men and women in the current sample. However, there was no significant association in either sex.

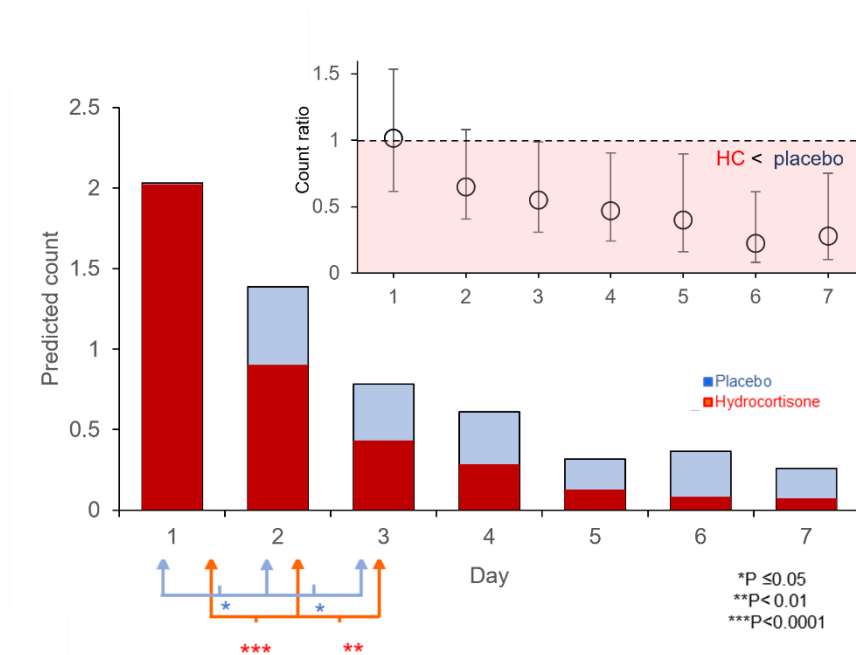


Figure 3.4. Intrusive memories reported across days 1 – 7. The optimal ZIP GLMM model indicated a faster reduction in intrusions in the hydrocortisone group (Figure x; Day x Drug interaction: ($\chi^2(6)=27.40$, $p<0.001$) and was not moderated by main effect of sex. Specifically, there was a larger mean difference between days 1 and 2 in the hydrocortisone group ($b=0.81$, $SE= 0.13$, $t(817)=6.20$, $p<0.001$) relative to placebo ($b=0.38$, $SE= 0.13$, $t(817)=3.01$, $p=0.043$). Similarly, larger reductions between days 2 and 3 were found with hydrocortisone ($b=0.73$, $SE= 0.208$, $t(817)=3.52$, $p=0.008$) relative to placebo ($b=0.57$, $SE= 0.17$, $t(817)=3.40$, $p=0.0123$). No other sequential day effects were found in either drug group (all $ps>0.3$). Figure 3.4 inset. on day 2 the ratio of intrusions in the hydrocortisone to intrusions in the placebo group showed a non-significant difference relative to a 1:1 ratio (the dashed line) but by day 3 there was an at trend difference between drug groups ($p=0.05$) and from day 4 onwards there was a significant difference between those having received hydrocortisone and those in the placebo group ($ps\leq 0.0362$). (Hennessy et al., 2022) reproduced with permission.

3.4.4 Measures across the week. Day 1 – 8 (Diary records)

3.4.4.1 Intrusion frequency

Model selection: In line with the pre-registered hypotheses, the effects of time, sex and drug on intrusion counts were tested. Backwards selection based on AIC values showed that neither the three-way interaction, nor the two-way interactions involving sex (Sex x Drug and Sex x Time) were important. Although there was a main effect of sex ($\chi^2(1)=4.95, p=0.024$) with women reporting greater number of intrusions over the week (Mean = 1.3 ± 3.04) than did men (Mean = 1.04 ± 2.77), in the optimal model, as judged by AIC, sex had minimal effect on model fit. Since the models with and without the sex main effect had similar AIC values, the sex effect was omitted. Therefore, the optimal ZIP model contains a Drug, Time and Drug x Time interaction, plus a random intercept for participant.

Accelerated reduction in intrusion frequency: There was the expected reduction in intrusion frequency over the week in both drug groups. However, the optimal ZIP model indicated a faster reduction in intrusions in the hydrocortisone group (Figure 3.4; Day x Drug interaction: ($\chi^2(6)=27.40, p<0.001$). Specifically, there was a larger mean difference between days 1 and 2 in the hydrocortisone group ($b=0.81, SE= 0.13, t(817)=6.20, p<0.001$) relative to placebo ($b=0.38, SE= 0.13, t(817)=3.01, p=0.043$). Similarly, larger reductions between days 2 and 3 were found with hydrocortisone ($b=0.73, SE= 0.208, t(817)=3.52, p=0.008$) relative to placebo ($b=0.57, SE= 0.17, t(817)=3.40, p=0.0123$). No other sequential day effects were found in either drug group (all $ps>0.3$). Note, the difference (*beta*) values were calculated from log coefficient

estimates (see appendix) rather than the original count units and hence do not correspond to differences between bars displayed in Figure 3.4.

Differential effect of drug across days: between group comparisons at each level of day confirmed a differential effect of drug across days. As shown in the inset of Figure 3.4, on day 2 the ratio of intrusions in the hydrocortisone to intrusions in the placebo group showed a non-significant difference relative to a 1:1 ratio (the dashed line) ($b=-0.43$, $SE=0.25$, $t(817)=1.72$, $p=0.087$). By day 3 there was an at trend difference between drug groups ($p=0.05$) and from day 4 onwards there was a significant difference between those having received hydrocortisone and those in the placebo group ($ps \leq 0.0362$).

3.4.4.2 Intrusion related Distress and Vividness

3.4.4.2.i Distress rating

Average distress ratings (2.7 ± 1.04) related to intrusions experienced on day 1 on Likert scale from 1 ('not at all') to 5 ('extremely') suggested intrusions generated at least moderate levels of distress. Distress across the week was analyzed using a linear mixed effects model with Day as a fixed factor with random intercepts for participant to partition the variance. There was a significant amount of participant-level variance justifying the need for the multi-level model. The best fitting model included the main effect of Day and Drug and a Day x Drug interaction. This model was not substantially improved when sex was included as a factor. Moreover, sex was not a significant moderator, so as when modelling intrusion counts, sex was excluded from the final model. There was a significant Drug x Day interaction ($\chi^2(6) = 16.58$, $p = 0.011$). A complex pattern emerged across the week (Figure 3.5), which did not match that found within the intrusion count effects (Figure 3.5) with both drug groups seeing a reduction in distress but at different

points in the week. In the placebo group there was a small but significant reduction in distress early in the week ($\Delta\text{Distress}_{(\text{Day } 1\text{--}2)} = 0.4$, $SE=0.2$, $z=2.21$, $p=0.027$), but no subsequent sequential difference between days from then on. In contrast, no significant reduction in distress was reported in the hydrocortisone group until between days 5 to 6 when reported distress dropped sharply ($\Delta\text{Distress}_{(\text{Day } 5\text{--}6)} = 0.9$, $SE=0.4$, $z=2.40$, $p=0.017$).

When distress ratings were weighted by the number of intrusions (distress load), there was no indication of a Day x Drug interaction ($\chi^2(6)=1.72$, $p=0.94$). However, such weighting may have been strongly influenced by the large number of zero counts later in the week, diluting any group differences.

3.4.4.2.ii Vividness ratings

Based on the same model selection processes as for distress the best fitting model included a random per participant intercept with Day, Sex, Drug and a Day x Drug interaction. Similarly, to distress there was a significant Day x Drug interaction for vividness ($\chi^2(6)=14.35$, $p=0.0259$) which also reflected a similar uneven reduction in vividness ratings over the week in both drug groups (Figure 3.5 bottom panel).

Mirroring the pattern of distress, there was an early (day 1–2) significant reduction in vividness in the placebo ($\Delta\text{Vividness}_{(\text{Day } 1\text{--}2)} = 0.6$, $SE=0.2$, $Z=3.44$, $p=0.001$) but not in the hydrocortisone group but unlike distress there was a subsequent significant, if moderate, reduction in vividness later in the week between days 6 and 7 in the placebo group, ($\Delta\text{Vividness}_{(\text{Day } 6\text{--}7)} = 0.7$, $SE=0.3$, $Z=2.11$, $p=0.035$). Like that for distress there was also a larger reduction in the hydrocortisone group from day 5 to 6 ($\Delta\text{Vividness}_{(\text{Day } 5\text{--}6)} = 1.1$, $SE=0.4$, $Z=2.54$, $p=0.011$). However, in contrast to distress, there was a main effect of Sex, women showed higher overall vividness effect ($\chi^2(1)=5.24$, $p=0.022$) than men.

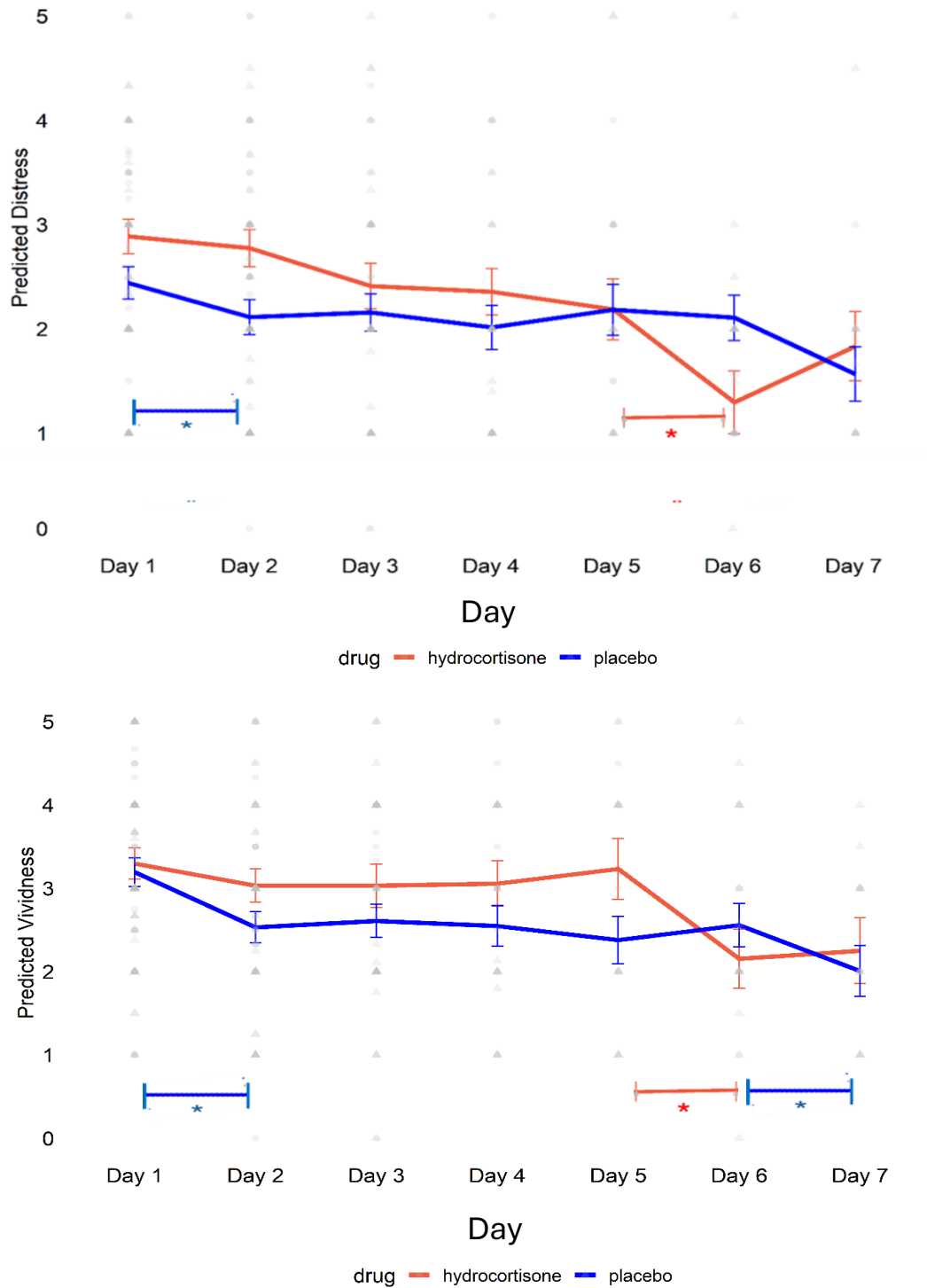


Figure 3.5. Distress and vividness ratings in response to intrusions. *Estimated marginal means ($\pm$ SE) day by drug intrusion-related distress (top panel) and vividness (bottom) ratings for placebo (blue lines) and hydrocortisone (red lines) * $p < 0.05$. Raw data grey circle (hydrocortisone); grey triangle (placebo). (Hennessy et al., 2022).*

3.4.4.3 Voluntary recall of trauma film and symptoms of PTSD (Day 8)

There was no statistically significant difference between the two drug groups in their free recall ($F(1,117)=0.075$, $p=0.785$). or cued recall ($F(1,116)=0.057$, $p=0.812$) performance on day 8. On the free recall test those in the hydrocortisone condition recalled $M=37.4$ ($SD=14.9$) 'idea units' whereas $M=36.6$ ($SD=18.3$) were recalled in the placebo group. During cued recall those in the hydrocortisone group recalled ($M=8.9$, $SD=2.2$) compared to ($M=9.0$, $SD=2.4$) for those in the placebo group. No differential increase in negative affect ($F(1,116)=2.85$, $p=0.094$), or decrease in positive affect ($F(1,116)=0.02$, $p=0.898$) was evident from pre- to post recall in the two drug groups. There was also no effect of hydrocortisone on total IES scores (placebo: $M=14.3$, $SD=11.9$; hydrocortisone: $M=15.12$, $SD=12.8$; $F(1,117)=0.128$, $p=0.721$). None of the subscale differed between the two groups (F values ≤ 0.41 , $ps \geq 0.521$).

3.4.6 Relationship between estradiol and progesterone on intrusive memory frequency

At mean levels of progesterone and estradiol, intrusion frequency did not differ significantly between men and women. However, there was a sensitivity to the effects of hydrocortisone depending on the interaction between sex hormone (estradiol and progesterone) levels and sex. *Estradiol*: the number of intrusions was co-determined by a three way interaction between baseline levels of estradiol, and sex and drug group ($b=0.420$, $SE=0.085$, $z=4.96$, $p<0.001$; Figure 3.6). Men administered hydrocortisone reported fewer intrusions with increasing levels of estradiol, whereas women showed the reverse pattern (Sex x Estradiol, $b=0.455$, $SE=0.065$, $z=7.00$, $p<0.001$) while this effect was not seen in the placebo group (Sex x Estradiol: $b=0.036$, $SE=0.054$, $z=0.67$, $p=0.504$; Figure 3.6). *Progesterone*: while less pronounced than for estradiol, the relationship of baseline progesterone with intrusions was still significant (Progesterone x Sex x Drug

interaction: $b=0.028$, $SE=0.011$, $z=2.61$, $p=0.009$). The effect was again seen only in the hydrocortisone group (Figure 3.6 ($b= -0.017$ $SE=0.007$ $z=2.28$, $p=0.022$) and not in the placebo group ($b=0.011$, $SE=0.008$, $z=1.41$, $p=0.158$; Figure 3.6). No moderated relationship between progesterone and intrusions existed in the hydrocortisone-treated women while, in contrast to estradiol, in men more intrusions were associated with higher levels of progesterone (Figure 3.6).

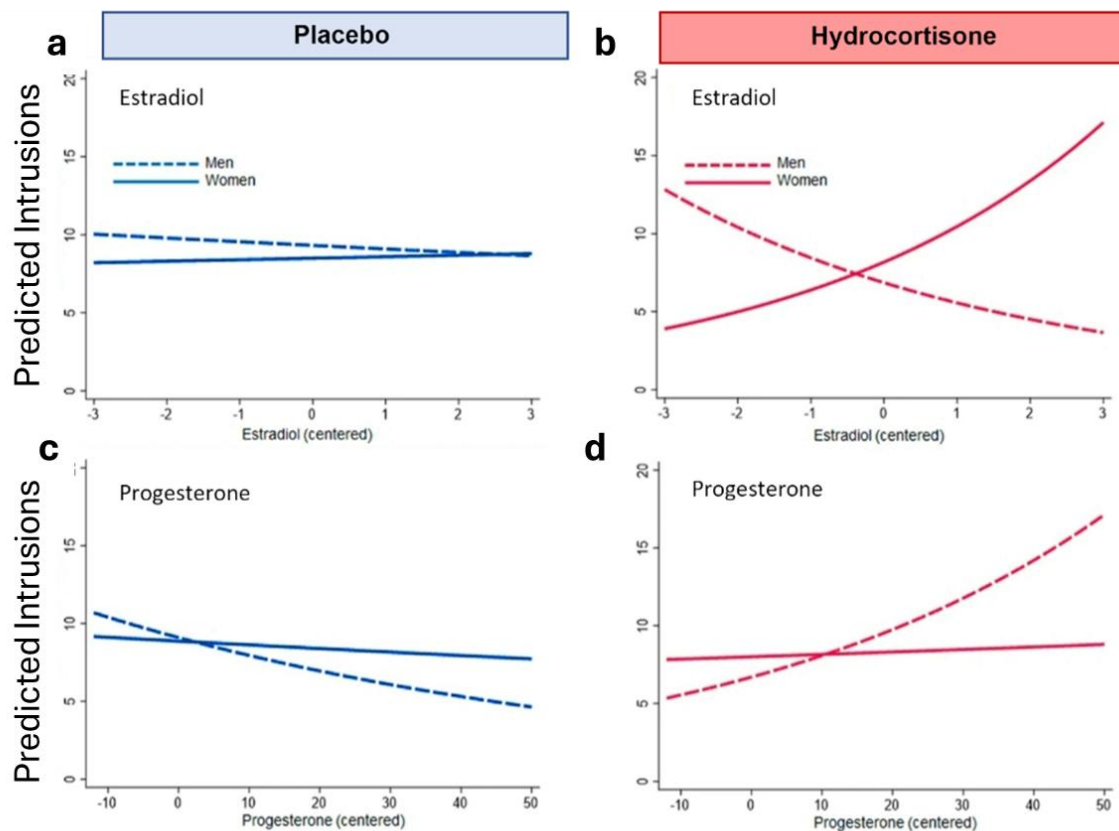


Figure 3.6. Associations between intrusions and baseline gonadal hormones (a,b) Association between intrusions and estradiol levels (mean-centred) and (c,d) between intrusions and progesterone levels (mean-centred). Solid lines = women, dashed lines = men. Red = hydrocortisone, Blue = placebo. Adapted from (Hennessy et al., 2022).

3.5 Discussion

The aim of this study was to examine the effects of a single 30mg dose of hydrocortisone on intrusive memory, a transdiagnostic symptom of psychopathology and the cardinal symptom of PTSD, while probing other predictors that might influence the efficacy of such treatment for preventing the onset of PTSD using an experimental model of trauma memory.

In line with the pre-registered hypothesis, and in support of previous pre-clinical research in hydrocortisone and propranolol (Kamboj et al., 2020) and in nitrous oxide (Das et al., 2016), although see Graebener et al. (2017), compared to placebo, hydrocortisone produced a different (accelerated) decline in intrusive memories (Hennessy et al., 2022). This was reflected in larger reductions in intrusion counts in the hydrocortisone group day 1->2 and day 2 ->3, as well as different placebo vs. hydrocortisone effects on each day, starting on day 2 and becoming (marginally) significant from day 3. The pattern of effects of hydrocortisone on intrusions differs from its effects on subjective reports of distress and vividness where differences between groups were observed at the beginning of and end of the week. Additionally, voluntary memory recall of narrative details of the film measured at the end of the study was neither enhanced nor impaired in either drug group. Hydrocortisone's differential effects on intrusions, vividness and distress and declarative memory reported here have been noted

in previous studies with nitrous oxide (Das et al., 2016), propranolol and hydrocortisone (Kamboj et al., 2020). The results potentially reflect dissociative effects of hydrocortisone on memory, affective and visuospatial cognitive systems in line with previous results (Kamboj et al., 2020).

Previous studies in clinical settings suggest that early post trauma treatment with hydrocortisone results in lower incidence in PTSD symptomology (Schelling et al., 1999; Schelling et al., 2001), reviewed in Astill Wright et al. (2021), rather than intrusive memory development specifically. The findings here suggest a more specific effect of hydrocortisone on post trauma memory processes, specifically the sensory perceptual memories that underly re-experiencing symptoms in PTSD. In the current study, hydrocortisone administration had an impairing effect on intrusive memories while the ability to voluntarily recall contextualised verbal memory was left unaffected consistent with dual representation theory (Brewin et al., 2010). Selectively preventing the hyper-consolidation of sensory perceptual memory and sparing voluntary contextualized memory would be the ideal in any potentially therapeutic preventative strategies for PTSD.

Dual representation theory: this influential clinical theory of PTSD proposes that sensory-perceptual involuntary memories on one hand, and context-rich voluntary memories on the other, represent the expression of dissociable memory systems; the Sensory Representational System and the Contextualized Representational System (Brewin et al., 2010; Brewin and Burgess, 2014). The theory implies that the two dissociable memory systems interact such that adaptive functioning of the contextualising memory system facilitates recovery after a trauma event and promotes more complete and/or coherent memories of the event (Brewin, 2001) due to successful integration of the more fragmented and valanced representations stored in the sensory-perceptual memory

system (Ehlers and Clark, 2000). Stress-related disorders are associated with an information processing bias that results in robust response to sensory information (Miller et al., 2017) fragmented incomplete representation of the traumatic event. This is seen as the result of disrupted functional connectivity between hippocampal and amygdala activity (Bisby et al., 2016) that disrupts adaptive associative learning and contextual processing to contribute to the encoding of stronger sensory memories and weaker contextualized episodic memories in individuals with PTSD (Brewin and Burgess, 2014).

Glucocorticoid moderation of sensory-perceptual memory consolidation:

However, in the experimental literature, there is mixed evidence for a selective impairment of sensory-perceptual memory consolidation by glucocorticoids. Much of glucocorticoid research has focused on cued-associative memory, i.e. fear conditioning/extinction (Brueckner et al., 2019) or voluntary episodic-verbal (word lists) memory (Buchanan and Lovallo, 2001) and this literature, focused on explicit memory consolidation, demonstrates a heterogeneity within the results with timing of exogenous administration a key determinant (Het et al., 2005).

Therefore, there is a complicated relationship that exists between glucocorticoid levels and cognitive performance and their potential role in psychopathology (Diamond et al., 2007; Kim and Kim, 2023). Glucocorticoid effects are determined temporally and in relation to other proximately co-activated neurochemicals particularly (nor) adrenaline at initial encoding in combination with mineralocorticoid receptor (MR) activity to affect single memory systems as well as large scale network changes (Schwabe, 2017). The following glucocorticoid driven ‘recovery’ phase post stress (glucocorticoid receptor (GR) mediated) is slower, longer lasting. Alterations in response levels across this coordinated stress response results in differential effects on dissociable memory systems during encoding, consolidation and retrieval (Schwabe et al., 2010). Below, is discussed

how the findings here might be reconciled with the complex and varied effects of glucocorticoids on human memory.

The larger reduction in intrusions from day 2 (i.e., the day after the trauma video) reported here may partly reflect a non-linear relationship between cognitive performance and cortisol levels, rather than there being a straightforward association between high levels and enhanced or low levels and impaired cognitive function. In particular, the supra-physiological levels of cortisol at t+60 might represent the rightmost part (i.e., the ‘impairment arm’) of an inverted U-shaped dose-response curve. Indeed, studies that examined varying levels of endogenous cortisol/doses of hydrocortisone have demonstrated impairment at high levels/doses (Lupien et al., 1999; Andreano and Cahill, 2006; Schilling et al., 2013).

Glucocorticoid moderation of sensory-perceptual memory retrieval: Hydrocortisone’s well-established impairing effects on retrieval of long-term memories (Het et al., 2005) have been observed shortly after encoding (i.e. before the memory was fully consolidated (Kirschbaum et al., 1996; Kuhlmann et al., 2005) and this could therefore represent an indirect route to impaired early (synaptic) consolidation. In a previous study (Kamboj et al., 2020) hydrocortisone’s delayed effects on intrusions were suggested as reflecting earlier acute effects on the spontaneous retrievals that support consolidation (Lupien et al., 2002). To test this possibility in this study, intrusions were assessed acutely (i.e., >1 hr after the trauma video), but no group differences were found. However, short-term memory systems have also been shown to act as a buffer for internally generated visual (iconic) memories and hydrocortisone has been shown to accelerate the decay of these early memory traces (Miller et al., 2015). This plausibly could be a route by which hydrocortisone could similarly disrupt the sensory perceptual memory traces that lead to intrusions and *indirectly* impair early consolidation to

accelerate reduction intrusion frequency over subsequent days (i.e. from day 2 in this study).

Broader acute glucocorticoid effects on emotional regulation: The delayed emergence of hydrocortisone's effects could have relied upon systems-level reorganization of memory rather synaptic consolidation processes. The presence of and timing between the adrenal and the initial GR response via mineralocorticoid receptors has been shown to be key to enhanced *encoding* of fear memories (Schwabe et al., 2022) while the strength of the subsequent, slower, long lasting glucocorticoid activity via GRs is key in moderation of fear memory in the aftermath of trauma to support neuronal plasticity required for adaptive memory storage.

Therefore, elevated cortisol levels can have generalised acute effects on emotional and cognitive processing that occur over several hours and are involved in emotional learning (Zohar et al., 2011; Chakraborty et al., 2020). The post trauma, genomic GRs activity aims to constrain the stress response to restore homeostasis of the stress system (Sapolsky et al., 2000). The suppressive actions of slow genomic GR activity regulate and constrain the salience (stimulus- response) network as extended amygdala activity if uncoupled from medial prefrontal cortex can lead to hyper-consolidated sensory/perceptual features of the trauma event (Maddox et al., 2019). It is therefore possible that hydrocortisone's effects on intrusions reflects the delayed protective effects of normalizing brain regions involved in memory contextualization, while constraining plasticity in structures involved in maladaptive emotional learning (Merz et al., 2018). Glucocorticoid-treated rodents in an animal model of traumatic stress also show delayed effects on plasticity in structures involved in emotional learning (Zohar et al., 2011; Chakraborty et al., 2020). Given the >24 hr delay before group differences were evident,

sleep might also have played a role in determining hydrocortisone's effects on intrusions (Plihal and Born, 1999).

Therefore, increased cortisol levels can lead to broad acute effects by influencing emotional regulation and facilitating cognitive processing, which gradually unfold over several hours. These effects are evident in improved prefrontal functioning during neutral cognitive tasks (Henckens et al., 2011) and they may also extend to enhanced prefrontal activation during emotion regulation after exposure to negative stimuli (Jentsch et al., 2019). As such, hydrocortisone's effects on intrusions might be an *indirect* consequence of enhanced and temporally extended emotion regulation.

Sex dependent effects of hydrocortisone: Despite the above discussion of the role of glucocorticoids on memory, it should be noted that unlike previous work in a women-only sample (Kamboj et al., 2020) the observed hypothesised negative association between post-hydrocortisone cortisol levels and intrusion frequency on day 1 was not observed in this study. It is unclear if the relatively minor methodological differences between the study here and previous study (Kamboj et al., 2020) are sufficient to explain the delay in effects. One potential methodological factor was the use of a mixed sample of men and women in the current study. However, the cortisol-intrusion association on day 1 was also non-significant when women were analysed separately, although the direction of the effect was as predicted. It is possible that this association is only present in women and the true effect too small to have been observed in the current, relatively small sample.

In addition to the effects of hydrocortisone to accelerate the reduction in intrusive memory frequency across the following week described above, it was hypothesised that its effects would be dependent on sex. Sex is an established determinant of the effects of

stress on memory (Cornelisse et al., 2011b; Felmingham et al., 2012b) and the effects of hydrocortisone on emotional memory (Merz and Wolf, 2017). Sex differences in response to stress have implications for understanding the disparity between the sexes in vulnerability to development of and persistence of PTSD (Ramikie and Ressler, 2018) as well as response to potential novel strategies to prevent intrusive memories.

Previous research suggests that men and women might respond differently to the effects of moderating stress on emotional memories due to interactions between stress and gonadal hormone systems under acute and chronic stress due to feed-forward and feedback links within and between the HPA and HPG (Barel et al., 2018; Sze and Brunton, 2020). These systems interactions contribute to sex differences seen in stress reactivity (Goldstein et al., 2010) as well as differences in their memory processing styles under stress (Beck and Luine, 2010) and on emotional learning (Toufexis et al., 2006). However, the results do not support the pre-registered hypothesised Sex $\times$ Drug interaction, although exploratory analyses did show associations between baseline gonadal hormone levels (i.e., estradiol and progesterone) and total intrusions that were dependent upon drug condition and sex.

Hydrocortisone dependent sex $\times$ gonadal hormone interaction on intrusive memory: Despite limited previous relevant research on sex-by-sex-hormone interactions in intrusive memory moderation specifically, the literature does provide evidence of individual variation in sex hormone profiles both between and but also within both sexes in their interaction with the stress response resulting in differential effects on all fear memory phases (Garcia et al., 2018) with implications for moderation of intrusive memories (Hsu et al., 2021). While estradiol/stress interactions are more widely examined, Ney and colleagues (Ney et al., 2019) suggest both direct and indirect enhancing effects of progesterone on fear learning at the time of trauma. Additionally,

interactions between peri-trauma levels of cortisol levels and progesterone (Herrera et al., 2016) and implications for intrusion frequency dependent on the ratio of gonadal hormones in women (Soni et al., 2013) justifies the reasoning behind extending the analysis to include sex x gonadal hormone x drug effects in both sexes in this study.

Existing research on the effect of estradiol and progesterone on intrusive memories specifically is limited (Hsu et al., 2021) with inconsistencies in results conducted in clinical and pre-clinical studies (Garcia et al., 2018). One study (Cheung et al., 2013), but also see (Lalonde et al., 2021), has specifically examine the effect of sex and associations between estradiol and progesterone levels and recall of negative images in both sexes. Cheung et al. (2013) report that in men, cortisol levels moderated recall of negative images but found no relationship with gonadal hormone levels and recall was observed in men whereas a positive association between estradiol and negative recall was reported in women, a similar effect in women observed in the current study with hydrocortisone. Comparisons between the two studies are complicated by different methods of analysis of intrusion frequency. Additionally, the previous study does not report any details on interactions between their ‘arousal group’ factor (i.e. high vs low endogenous cortisol groups) with gonadal hormone levels. Pharmacologically induced high arousal/ cortisol levels was a moderator of the gonadal hormone x intrusion interaction in the current study.

A better understanding of these stress/sex/hormone interactions can provide a basis for future confirmatory studies that could improve our understanding of individual differences in memory effects of glucocorticoids. This in turn has implications for how sex x gonadal hormone differences may drive response to any proposed novel preventative strategies (Shansky, 2015; Arnegard et al., 2020). Despite only recruiting contraceptive-using women, the study here found considerable variations in background estradiol levels

in these participants (Table 3.1). This might suggest that naturally cycling women, who show large intra-individual variations in oestrogen levels, would exhibit differential sensitivity to hydrocortisone dependent on menstrual cycle phase. In addition, the results suggest that, in men, it may be possible to pharmacologically augment the effects of hydrocortisone on intrusive memories by co-administering estradiol.

3.5.1 Limitations

There are limitations that need to be acknowledged in the current study. The study recorded intrusive memories through momentary assessments of 'acute' intrusions immediately after the analogue trauma, with memories of intrusions over the following week collected retrospectively. Although retrospective recording may introduce reporting biases and involve meta-memory processing, evidence suggests that few differences exist between memory recording methodologies (Rattel et al., 2019a) intrusions themselves were a result of the trauma film paradigm. The trauma film intends to serve as an analogue procedure to elicit intrusions in a manner ethically acceptable for healthy individuals. However, resulting intrusions may not fully reflect those observed after real-life traumas, particularly in terms of their frequency. Although intrusion counts were relatively low in the study, they were consistent with similar experimental studies and not significantly different from clinical participants' intrusion frequency (Porcheret et al., 2020). Despite its limitations, implementing this paradigm allows for methodological control while investigating hydrocortisone's effects on memory in a broader clinical context. However, the extent to which findings from this analogue 'secondary prevention' study in healthy participants generalize to clinical populations remains uncertain and will require well-powered clinical trials to determine. Nonetheless, the results align with meta-analyses of existing small-scale clinical trials investigating hydrocortisone treatment in PTSD.

3.6 Conclusion

In conclusion, the effects reported here support some previous experimental work (Kamboj et al., 2020) and the results are consistent with an attenuation (faster reduction) of intrusive involuntary memories following administration of hydrocortisone via its effects on the sensory perceptual representations that underlie these types of memories seen the small-scale clinical trials of hydrocortisone on the prevention of PTSD conducted so far. Hydrocortisone is a safe, widely available drug and these findings have potentially important implications for the development of its use as a secondary preventive strategy for trauma-related disorders. However, as mentioned above, the effects on memory are varied and still not fully explained. From these results it is not clear whether the observed day-dependent effects of hydrocortisone are acting specifically on sensory-perceptual involuntary memories. This study found no effect of HC on voluntary memory but might an effect be observed on this memory if it were measured matched to involuntary memory at measurement? The length of time-from-trauma-to when hydrocortisone can be given needs to be accurately determined as does dose dependency in relation to both baseline glucocorticoid levels and glucocorticoid interaction with (nor)-adrenaline) to moderate memory under acute stress and mechanistically how such preventative strategies moderate the relative influence of the two types of glucocorticoid receptors, i.e. mineralocorticoid and glucocorticoid (de Kloet and Joëls, 2023) within these timeframes. The sex hormone observations in this study highlight growing (if still limited) evidence of the importance of sex hormones in how memory functions in PTSD (Hsu et al., 2018; Ney et al., 2019) and it may be most valuable to assess sex differences not just as a dichotomous variable

but together with assessment of gonadal hormone levels, in both women and men, to better understand their importance as potential indicators of vulnerability to stress but also as biomarkers of response to treatment (Garcia et al., 2018). It will be important to remember that neuromodulators of the stress response do not work in isolation (Joëls and Baram, 2009; de Kloet and Joëls, 2023) and with the paucity of past studies it will be important to replicate and extend the current findings via examination of simultaneous manipulation of stress and gonadal hormone levels in both sexes to help the advancement of personalised preventative strategies for stress related disorders such as PTSD .

Chapter 4

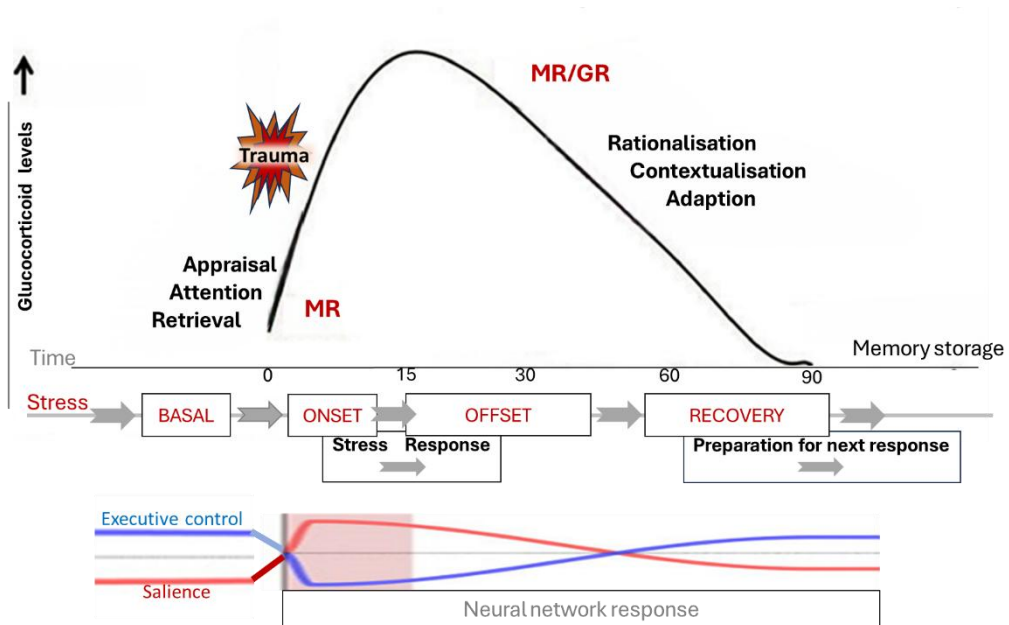
Reducing distress and vividness of aversive memories of a trauma-like event: the effects of spironolactone as a psychopharmacological preventative strategy for PTSD

4.1 Introduction

Post-traumatic stress disorder (PTSD) is a persistent and debilitating psychiatric condition affecting ~4% of the general population globally (Koenen et al., 2017). In addition to the immense personal suffering it causes, PTSD is associated with high levels of psychiatric and physical comorbidity, placing a substantial burden on healthcare systems (Watson, 2019a). The core symptom of PTSD is the reliving of spontaneous, highly distressing intrusive memories of an experienced, or witnessed, traumatic, usually, life-threatening event triggered by cues associated with the trauma (American Psychiatric Association, 2013). As described in *Chapter 2*, such memories once established are persistent and difficult to alleviate with current methods. They are characterized, not just by their frequency, but also by their vivid, ‘nowness’ leading to a traumatic event appearing to be repeatedly ‘relived’ resulting in extreme distress (Kleim et al., 2013), hypervigilance and avoidance of environmental triggers. The development of a preventative strategy against the development of, and reduce the distress associated with, these types of hyper-consolidated sensory-perceptual memories could increase resilience against long-term psychological harm associated with experiencing trauma.

Role of glucocorticoids in moderation of aversive memories; Exposure to a traumatic event is a necessary but insufficient condition for the development of PTSD. Psychosocial risk factors play a pivotal role in determining an individual’s response to traumatic events. In addition, as described in previous chapters, numerous biological (neuroendocrine and (epi)genetic) variables are associated with PTSD risk and are targets for reliable measurement, experimental (pharmacological and/or behavioural) manipulation, and potentially, to clinical intervention (Astill Wright et al., 2021). The cellular and molecular processes identified in the formation of durable memories of

emotional events are an especially relevant target for PTSD prevention efforts. Such secondary prevention strategies are likely to be most effective if deployed within a critical window of opportunity before the labile memory of the traumatic event undergoes early (synaptic) consolidation (Nader et al., 2000).



Adapted from de Kloet et al. (2019)

Figure 4.1 Mineralocorticoid receptor (MR) activity in appraisal of a threatening event Mineralocorticoid receptor (MR) activity in acute stress response to a traumatic event prior to the glucocorticoid receptor (GR) mediated recovery phase, adaptation and preparation for future responding. MR stress induced activity upregulates neural connectivity within the salience network, as well as attentional and information processing. MR engagement promotes retrieval of threat-relevant information while impairing non-threat information from memory for enhanced early appraisal of the event, to assess threat in the defense of the 'self'. (de Kloet et al., 2019).

Adaptive modification of memory under stress is partially mediated by the coordinated genomic and non-genomic effects of glucocorticoids (see Glossary) at mineralocorticoid (MR) and glucocorticoids receptors (GR) in the brain (Joëls and Baram, 2009; de Kloet and Joëls, 2020). When bound to cortisol, intracellular MR and GR act as transcription factors, mediating slow genomic responses during stress adaptation.

Alternatively, membrane-bound versions of MR/GR mediate rapid, non-genomic effects on neuronal excitability (Groeneweg et al., 2012).

Cortisol's actions at MRs during acute stress may play a role in rapidly shifting cognitive resources away from hippocampal and prefrontally-mediated complex information processing that is involved in 'contextualizing' memories, and towards the formation of basic, rigid stimulus-response representations of the traumatic episode (Schwabe and Wolf, 2012). Adaptively, this acts to enhance swift appraisal of the event to detect threat to the 'self' (de Kloet et al., 2019).

However, if extended or sustained, upregulated salience network activity at the expense of top down executive control and the resulting information processing bias, can contribute to the formation of poorly contextualized and fragmented declarative (voluntary) memory and promote prolonged simple stimulus-response learning (e.g. perceptual priming) that is thought to underlie intrusive memories, on the other (Ehlers et al., 2004). This shift could contribute to impoverished contextual representations of the traumatic event and a tendency for the episode to be reexperienced as fragmented. MR functioning can be probed in humans using the MR antagonist, spironolactone, a potassium sparing diuretic (Schwabe et al., 2013; Vogel et al., 2017). Pharmacological studies provided evidence suggesting that MR antagonism may modulate neural processes contributing to memory formation (Karst et al., 2005; Groc et al., 2008; Karst et al., 2010; Vogel et al., 2016), and therefore also pathogenic memory processes (Wingenfeld and Otte, 2019).

Testosterone, modulation of stress response and aversive memory; Testosterone plays a role in modulating stress responses, and memory processes (Chen et al., 2014; McHenry et al., 2014) with higher testosterone levels being associated with lower anxiety

via metabolite modulation of on GABA_A receptors. Other affective behaviours are also affected by testosterone (Hermans et al., 2006; Hermans et al., 2007). Further, some work points to a potential interaction between cortisol and testosterone (Terburg et al., 2009) and indirect suppression of HPA activation (Reul and Holsboer, 2022). Considering that spironolactone possesses anti-androgenic properties and can affect testosterone levels (Burinkul et al., 2021) we explored whether pre-drug testosterone levels would moderate the effects of spironolactone on intrusive memories. Although this aspect was not part of the pre-registered hypotheses in this study, investigating this interaction may provide insights into the neuroendocrine mechanisms underlying individual differences in responses to traumatic stress.

The potential role of MRs in intrusive memory formation was investigated using the ‘trauma film’ paradigm, which reliably elicits intrusive memories (James et al., 2016). The trauma film offers an experimental model for evaluating the effects of pharmacological interventions (Das et al., 2016; Rombold et al., 2016a; Rombold et al., 2016b; Graebener et al., 2017; Elsey et al., 2020; Kamboj et al., 2020; Hennessy et al., 2022). Per the preregistration, <https://osf.io/698q7/>, it was hypothesized that spironolactone administered *before* a simulated traumatic event (the trauma film) would be associated with a larger and/or faster reduction in intrusive memories (occurrence and/or associated vividness and/or distress). Given the proposed cognitive mechanism of action described above (Schwabe and Wolf, 2012), it was not predicted that there would be an impairment in *declarative* memory for the trauma film following spironolactone. There is a growing body of literature suggesting the importance of considering sex as a biological variable in fear and aversive memory disorders (Kornfield et al., 2018; Hiscox et al., 2023) and in investigating their treatment (Hsu et al., 2018; Hsu et al., 2021). An additional aim of this study was to extend the previous findings on moderation of

hydrocortisone's effects on trauma-related intrusions by gonadal hormones (Hennessy et al., 2022) by examining the interaction between testosterone and spironolactone treatment on intrusive memories.

4.2. Materials and Methods

4.2.1 Pre-registration and Ethical approval

The study protocol and analysis plan were pre-registered on the Open Science Framework (<https://osf.io/py9n5>) on 14 July 2021, and an addendum was posted on 13 August 2021(<https://osf.io/698q7/>). The latter described the rationale for removing one of the originally planned tasks. The focus of the current paper is on the effects of spironolactone and the potential modulating influence of gonadal hormones on intrusive memories. Other exploratory measures outlined in the pre-registration (EEG, oculomotor responses during film viewing; <https://osf.io/698q7/>) are intended to be the subject of a future report and will not be described further here. All procedures were approved by University College London ethics committee and conducted in accordance with the Declaration of Helsinki.

4.2.2 Experimental design

This was a randomized, double-blind, placebo-controlled study. The main explanatory variables were the between-subjects factor, drug (spironolactone or placebo) and the within-subjects factor, day (day 1 to day 7). An additional time factor, timepoint (T1 to T3), was relevant for effects measured during the lab session on day 1.

4.2.3 Participants

Participants (n=78) were healthy men (rationale for the male only sample outlined below). The sample size was determined by assuming at least a moderate-large effect ($f=0.245$, equivalent to $\eta^2=0.057$) of drug in a repeated measures analysis, with $\alpha = 0.05$, $1-\beta = 0.80$, and $\rho=0.5$ between repeats. Two participants attended the first session but did not complete/fulfil study requirements (appendix item 4.1) and were therefore replaced. One of these (the first participant to be tested) provided feedback about the duration of the protocol that resulted in one of the tasks being dropped; this participant was withdrawn after their lab session (they did not complete memory diaries). Another participant failed to complete the minimum number of intrusion diaries required and was excluded based on *a priori* (pre-registered) criteria (Figure 4.2).

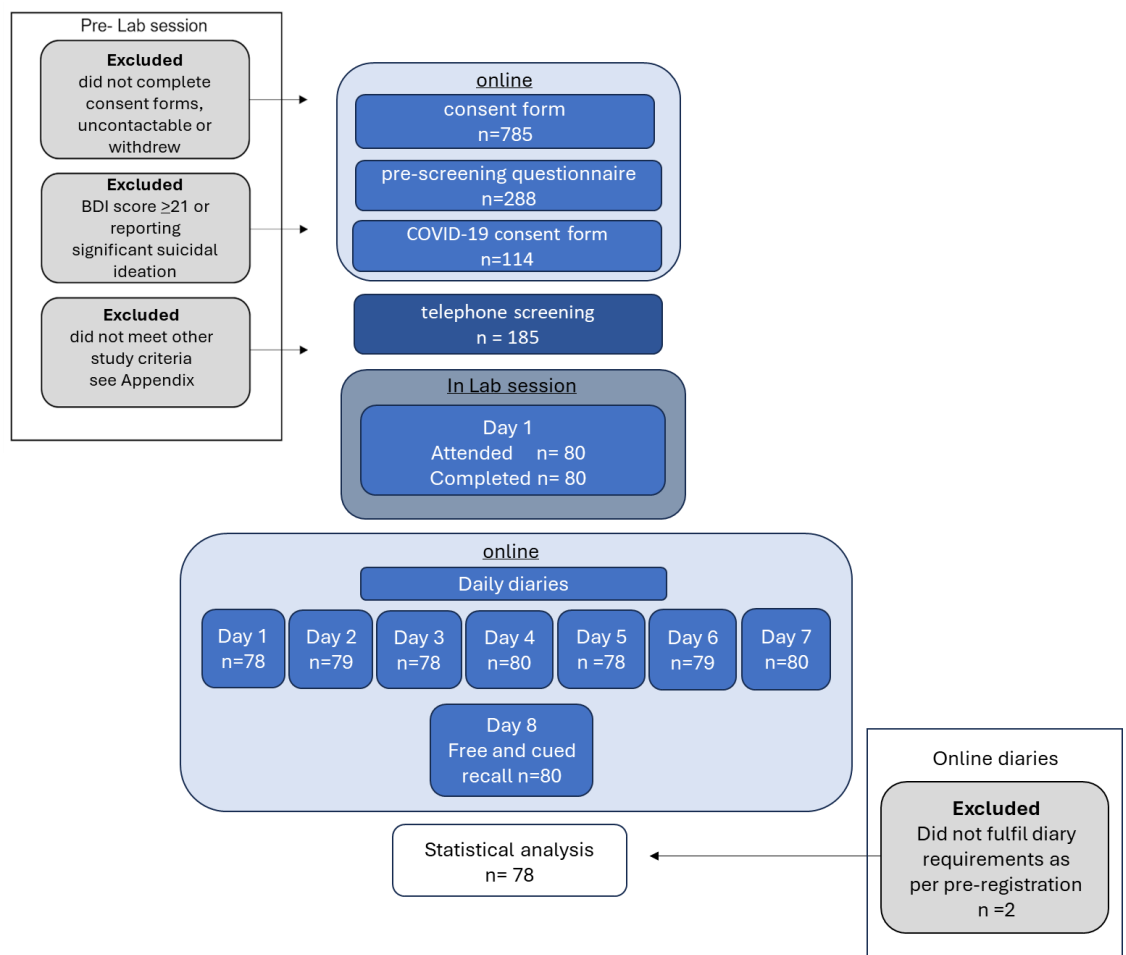


Figure 4.2. Participant flow diagram depicting participant recruitment from response to advertisement to inclusion in final data analysis. All participants eligible after completing online pre-screening questionnaire were spoken to on the telephone (N= 185) and excluded if they did not meet the pre-registered inclusion criteria <https://osf.io/698q7/> (see appendix item 4.2) including having a score ≥ 21 on Beck Depression Inventory (BDI) (Beck et al., 1996) or reporting significant suicidal ideation at time of screening. On Day 1 of study, 80 men attended and completed the within lab session. Two men were subsequently excluded through not completing the diary requirements as specified in pre-registration document. Statistical analysis was conducted on 78 participants.

Eligibility was assessed via an online questionnaire and telephone screening interview. Participants were required to be in good general health and aged 18-35 years, and not have any of the following exclusions (non-exhaustive): historical or current diagnosis of a psychiatric disorder or score ≥ 21 on the Beck Depression Inventory-II, BDI-II; (Beck et al., 1996) indicating at least moderate depression at screening; current suicidal ideation; excessive use of alcohol or recreational drugs, history of blood/injury phobia, experiences of relevant stressful life events judged during screening to be sufficiently intense as to have led to increased risk of PTSD; any contraindications to use of spironolactone; currently use of cardiovascular, anti-inflammatory or psychiatric medication or medication. Full inclusion/exclusion criteria are outlined at <https://osf.io/py9n5> and in appendix 4.2.

All participants provided informed consent prior to any experiment procedure. Upon completion of the study, they received a £60 honorarium at the end of the study.

Recruitment of only male participants. We had originally planned (and received IRB approval) to recruit a balanced sample of men and women. However, when the study eventually started (in July 2021) - after significant COVID-related delays - it was evident that recruitment was likely to be significantly slower than expected. As such, to complete the study within a timeframe constrained by funding, we decided pragmatically to only recruit male participants (per our pre-registration), thereby

removed sex and (menstrual cycle-related fluctuations in) ovarian hormone levels as a source of variability (see *Chapter 5* for general discussion on research limitations in this regard).

4.2.4 Trauma film

We used an ethically acceptable, though intentionally distressing procedure to simulate a traumatic event. This ‘trauma film paradigm’ is widely employed in experimental medicine studies of PTSD treatments because it reliably induces the defining reexperiencing symptom found in PTSD patients, namely emotional memories that are both *involuntary* and *distressing*. participants watched a distressing film containing acts of extreme violence. The film comprised two clips from the commercial film ‘Irréversible’ (Studio Canal+) and included commentaries that linked the scenes to create a coherent narrative, (see Das et al., 2016; Kamboj et al., 2020; Hennessy et al., 2022).

4.2.5 Drugs

To minimize differences in absorption, eligible participants were instructed to avoid food/drink except water for 2 hours prior to the lab session. Spironolactone tablets (400 mg = 4 x 100 mg; Accord-UK Ltd, Barnstaple, UK) or closely matched placebo Tablets (vitamin C, 1000 mg = 4 x 250 mg) were taken orally with water 100 minutes before the trauma film. Participants and experimenters were blind to drug condition by envelopes containing either spironolactone or placebo capsules pre-prepared and labelled only with participant id by external experimenter. The integrity of double blinding was assessed at the end of the lab session.

4.2.6 Trait and State Self-Report Measures

Relevant baseline traits were assessed once, at the beginning of the lab session on day 1 using the Černis Felt Sense of Anomaly, CEFSa, a measure of dissociation (Černis et al., 2021), State-Trait Anxiety Inventory, STAI trait form (Y-2) (Spielberger, 2010), Emotion Regulation Questionnaire-9, ERQ (Spaapen et al., 2014), and Childhood Trauma Questionnaire, CTQ, short form (Bernstein et al., 2003). Demographic information was also recorded (age, ethnicity, highest level of education).

Subjective state measures included the Positive And Negative Affect Schedule, PANAS (Watson et al., 1988), and a Bodily Symptoms Scale, BSS, created to capture potential subjective bodily sensations produced by spironolactone. In addition, ‘peri-traumatic’ dissociation was assessed using a modified version of the Clinician-Administered Dissociative States Scale, (CADSS) (Bremner et al., 1998) that referred to participants’ experiences while watching the film and Revised Impact of Events Scale, IES-R (Weiss, 2007) adapted for use with the trauma video given at the end of the week, see Figure 4.3.

4.2.7 Memory Measures

Intrusive memories: Intrusive memory occurrences during the music filler period (acute intrusions’) were recorded via button-presses on an E4 wrist-worn device (Empatica, Boston, MA, USA). At the end of the music filler, participants additionally guessed the number of button presses made (these ‘guesses’ were highly correlated with the actual number of button-presses; $r=0.93$). The relevance of these acute intrusions to the film was verified by asking participants to describe the contents and rate the intrusions on the level of vividness and distress on a 7-point Likert scale (1 = not at all, 7 = extremely). Prior to leaving the lab on D1, participants were provided with detailed instructions on how to complete the online intrusive memory diaries. They were asked to

record the number of times they experienced each thematically distinct film-related intrusive memory, provide a brief description of the memory and the accompanying distress and vividness rated on the Likert scale above. Throughout the experiment (including the initial information sheet, screening interview and at the end of the lab session), the importance of the intrusion data to the validity of our study was stressed to participants. An email reminder to complete the diary was sent at 9 pm to all participants on days 1-7. These steps appeared to be an effective means of increasing compliance, with only 3 missing entries out of 546 data cells across all participants and days (<0.6%). Further details on the instructions provided to participants regarding intrusive memories and the diary are provided on the pre-registration document (<https://osf.io/698q7/> and in appendix item 3.2).

Voluntary memory recall: Free and cued recall assessment occurred on day 8. For the free recall tasks, participants wrote as much as they could recall about the film in a free-text box. The cued recall task contained 19 items prompting participants to recall detail (rather than gist) aspects of the film.

4.2.8 Physiological and Biological Measures

Heart rate was assessed using an ECG-like device (Bodyguard 2, Firstbeat Technologies, Jyväskylä, Finland) using a modified lead II configuration, that records interbeat (RR) interval at 1 kHz. Blood pressure using a wrist-worn monitor (RS1, Omron Corporation, Kyoto, Japan).

Circulating neurosteroid levels were estimated from saliva samples (1 ml passive drool) obtained at T1-T3, which were stored at -80°C for subsequent analysis. The analyzed neurosteroid hormone panel included cortisol and cortisone, and the gonadal hormones estradiol, progesterone, and testosterone. Liquid chromatography-tandem

mass-spectrometry (LC/MS) was used to analyse cortisol, cortisone, progesterone and testosterone. Estradiol levels were determined separately using chemiluminescence immunoassay kits (IBL International, Hamburg, Germany).

4.2.9 Study procedure

In order to limit the influence of diurnal cortisol fluctuations (Lightman and Conway-Campbell, 2010), the lab session (day 1; D1, Figure 4.3) commenced between 13:00-14:30. All self-report and memory measures were recorded (in lab and remotely) using the online survey platform, Qualtrics.

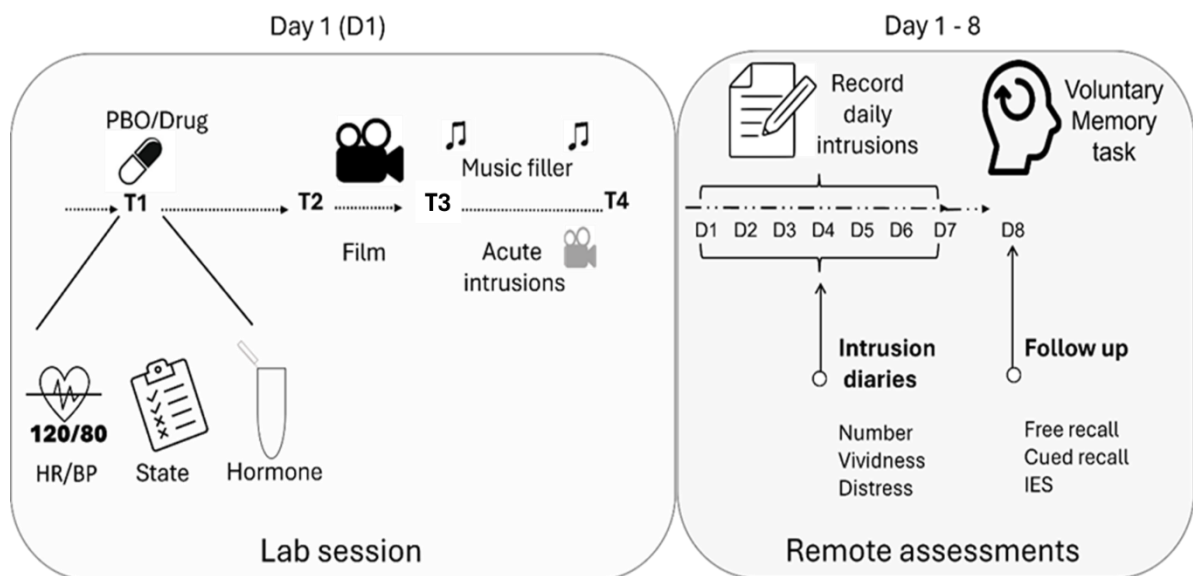


Figure 4.3: Timeline of relevant experimental procedures. The timeline of day 1 (D1) lab session is displayed in the left box. There were three or four ‘blocks’ of state, physiological and salivary-endocrine (T1-T3) measurements. At T1 (pre-drug), heart rate (HR) and blood pressure (BP), subjective state measures (see main text) and the first saliva sample were taken. After spironolactone / placebo administration, there was a 90

min drug absorption period after which, at T2 (post-drug), the T1 measures/samples were repeated. After the film, the third set of state/physiological data/saliva samples were taken (T3) and participants began recording post-film intrusions during a music filler task (acute intrusions'), using a wrist-worn monitoring device. Remote assessments of intrusive memories (right box) started on day 1 using an intrusion diary and continued daily until day 7 (D1-7). On day 8 (D8), participants completed free and cued recall tasks (voluntary recall) and the IES.

After the consent procedure, height and weight were recorded and the physiological devices attached (see below). Participants then provided demographic details and completed 'trait' baseline measures followed by the first, pre-drug block of self-report subjective 'state' questionnaires, heart rate (HR) and blood pressure (BP) measures, and the pre-drug saliva sample. After drug tablets were administered, there was a 90-minute drug-absorption period during which participants engaged in self-selected emotionally neutral activities.

At the end of the absorption period, the T2 block of subjective state and physiological (HR and BP) measures were taken, alongside the second saliva sample. Participants were then seated at a standardized distance of 50 cm from a 17-inch monitor/eye-tracker with head movements minimized using a chinrest, while viewing the trauma film. The T3 block of state and physiological measures and the final *analyzed* saliva sample was taken at T3 (note: an additional sample taken at T3+15 min was not retained for subsequent hormone analysis). Participants then listened to a series of standardized musical clips (rated for pleasantness; music filler, Figure 4.3), during which they were instructed to record the occurrence of intrusive memories via button press response (acute intrusions, see below). After the music filler, participants completed an additional measure of state affect (T4 + 60 min PANAS and BSS only; no saliva or cardio-physiological measures).

Starting from the end of the day of the lab session (day 1), participants completed daily online memory diaries for a week (up to day 7), recording intrusive memory frequency, content, and accompanying distress and vividness of intrusive memories. On day 8 free and cued recall relating to the film, and the Impact of Event Scale-Revised, IES, (Weiss, 2007) were completed online. Participants were debriefed at the end of day 8.

4.3 Statistical Analysis

Statistical analyses were performed in Stata (version 17) and SPSS (version 28). According to the pre-registered analysis plan, the outcomes of interest were intrusion frequency and intrusion-related distress and vividness. Mixed effects models were used to analyze these outcomes to account for the serial dependence of observations across days. Mixed models are also superior to ANOVA in their handling of missing data. Drug and day were treated as fixed effects, and participant, a random factor. In addition, intrusion-by-day slopes were allowed to vary between groups (i.e. Drug x Day was an additional fixed factor). As expected, random intercept models displayed better fit relative to models containing only fixed effects (day and drug and Day x Drug), although inclusion of additional random slopes did not improve model fit in any of the analyses, regardless of the selected covariance structure. As such, the models described below only contain participant as a random effect.

Intrusion frequencies: numbers of intrusions exhibited distributional properties typical of count data, and hence, a generalized linear mixed modeling approach was appropriate. There was a high degree of dispersion (intrusion frequency variance >10 x mean) and a preponderance of zeros across all participant-by-day cells (65.8%). Despite the large proportion of zeros, model comparisons using the *countfit* command from the SPost13 Stata package (Long and Freese, 2006) indicated strong evidence favoring negative binomial distributional assumptions rather than zero-inflated (Poisson or negative binomial) models, based on AIC and BIC fit indices. As such intrusion count data was modelled using mixed effects negative binomial regression. In analyses examining moderation of drug effects by pre-drug gonadal hormone levels, the latter were mean centered, and, as described previously (Hennessy et al, 2022), their effect was examined on total intrusion counts (across the week) in single-level negative binomial models with Drug as fixed part of the model and participant (id) as random effect with mean-centered pre-drug levels of testosterone, estradiol and progesterone added as continuous moderators.

Parameter estimates and *p* values for all regression models are based on robust standard errors calculated using a sandwich estimator (Acock, 2008). Although the resulting SEs and *p* values are more conservative, the use of robust SEs reduces the risk of misspecification due to heteroskedasticity.

One-way ANOVAs were used to analyze continuous variables assessed at a single timepoint (e.g. IES, voluntary memory performance). Other continuous repeated measures data acquired within the lab session on day 1 and therefore less likely to contain missing values (HR, BP, PANAS, BSS) were analyzed using repeated measures ANOVAs with Drug as the-between-subjects factor, and Timepoint, the within-subjects factor. Post

hoc tests were adjusted for multiple comparisons and all p values associated with such tests are Bonferroni corrected.

4.4 Results

4.4.1 Descriptive characteristics

Table 4.1. Participant's characteristics at baseline. Mean ($\pm$ SD)

	<i>Placebo (n=39)</i>	<i>Spironolactone (n=39)</i>
Demographics		
Age (years)	23.51 $\pm$ 4.73	24.82 $\pm$ 4.98
BMI (kg/m ²)	23.05 $\pm$ 2.45	24.19 $\pm$ 3.20
Education (years)	16.10 $\pm$ 1.43	16.54 $\pm$ 1.80
Psychological characteristics		
Depression	3.85 $\pm$ 4.76	4.87 $\pm$ 6.02
Dissociation	11.72 $\pm$ 13.25	14.90 $\pm$ 23.68
Anxiety	35.2 $\pm$ 8.6	36.4 $\pm$ 8.6
Childhood trauma	34.1 $\pm$ 10.6	37.6 $\pm$ 13.9
Emotion regulation (reappraisal)	26.3 $\pm$ 4.9	24.0 $\pm$ 6.0
Emotion regulation (suppression)	14.7 $\pm$ 5.0	14.7 $\pm$ 5.0
Baseline physiological/endocrine measures (T1)		
Systolic BP (mmHg)	112.77 $\pm$ 9.84	110.21 $\pm$ 10.32
Diastolic BP(mmHg)	70.33 $\pm$ 6.68	69.64 $\pm$ 8.47
HR (beats/min)	69.67 $\pm$ 11.76	69.79 $\pm$ 10.53
Cortisol (nmol/L)	2.37 $\pm$ 1.67*	1.95 $\pm$ 1.15
Progesterone (pg/ml)	1.97 $\pm$ 1.34 [†]	1.99 $\pm$ 1.22 [†]
Estradiol (pg/ml)	4.96 $\pm$ 1.61*	5.11 $\pm$ 1.19
Testosterone(pg/ml)	82.76 $\pm$ 31.28*	85.05 $\pm$ 29.54

All cells: n=39, except *n=37; [†]n=35, *p<0.001

Participant's characteristics in the two drug conditions to relevant psychological traits and history were assessed using the BDI (depression), Černis Felt Sense of Anomaly (dissociation), State-Trait Anxiety Inventory (anxiety), Childhood Trauma Questionnaire-Short Form (history of childhood trauma/adversity) and Emotion Regulation Scale-9 (reappraisal and suppression). Physiological/endocrinological measures were obtained pre- drug treatment; T1.

4.4.2 Drug guess

Forty-three participants (55%) correctly guessed their treatment assignment, which was not significantly different from chance (exact binomial test; $p=0.428$). Experimenters also guessed assigned drug condition of participants at chance levels (45 correct; 58%, $p=0.213$).

4.4.3 Physiological and endocrine effects of spironolactone

Blood pressure (systolic and diastolic) and heart rate increased and decreased respectively across the course of the experiment (Table 4.2) but were unaffected by drug condition (Drug x Timepoint interactions, p values > 0.5). The lack of effect of spironolactone on blood pressure is likely due to participant characteristics (health, young and normotensive).

Table 4.2. Mean ($\pm$ SD) participant blood pressure (systolic and diastolic) and heart rate

	T1	T2	T3	Time F(2,152)	Drug F(1,76)	T x D (2,152)
Systolic						
Placebo	112.77 (10.08)	112.49 (9.78)	118.49 (11.26)	27.00*	0.32	0.74
Spironolactone	110.21(10.08)	111.79 (9.78)	118.33 (9.78)			
Diastolic						
Placebo	70.33 (7.62)	71.18 (8.21)	75.64 (8.38)	15.80*	0.18	0.21
Spironolactone	69.64 (7.62)	71.21 (8.21)	74.46 (8.38)			
HR						
Placebo	69.67 (11.17)	60.64 (9.14)	62.85 (9.79)	61.15*	0.15	0.29
Spironolactone	69.79 (11.17)	61/95 (9.14)	63.82 (9.79)			
*p<0.001, The two drug conditions were assessed across three timepoints during the experiment (T1, (pre-drug). T2, (post-drug/pre-film), T3, (post-film))						

However, changes in cortisol level served as a positive control for acute spironolactone activity during the encoding / immediate post-encoding period. While there were no changes in salivary cortisol levels in the placebo group across timepoints

(all p values ≥ 0.7), there was a clear increase in the spironolactone group from T1 to T2 ($p=0.001$) and from T2 to T3 ($p=0.024$; Figure 4.4). Significant *between-drug* differences in cortisol were also evident at T2 ($p=0.003$) and T3 ($p<0.001$). Although the effect of drug at T3 (post drug; Figure 4.4) is obvious and large ($d=1.2$ based on $\log_{10}$ transformed values), the effect is still rather modest compared to cortisol levels after 30mg oral hydrocortisone ($d=3.3$, based on log transformation of data reported in (Hennessy et al., 2022)).

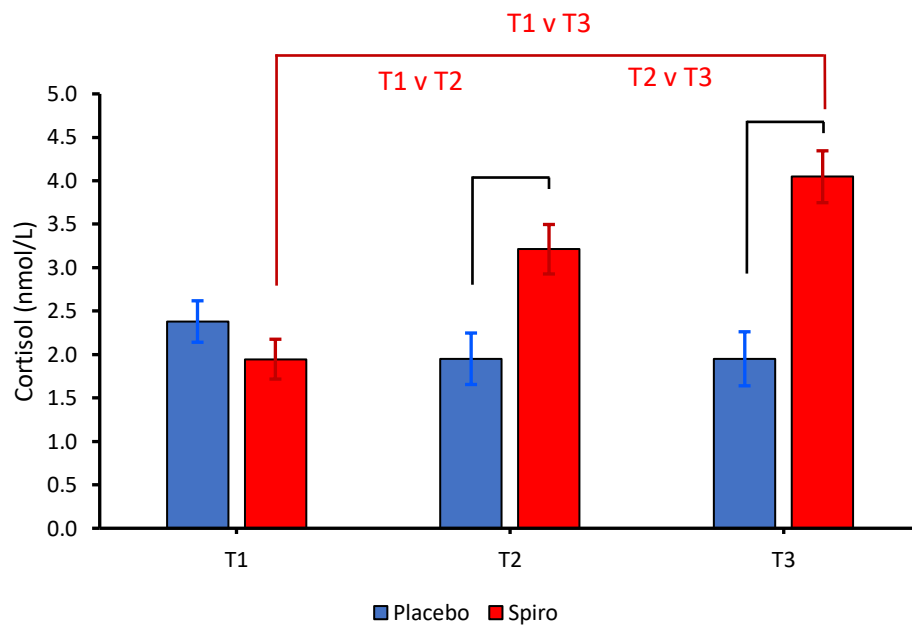


Figure 4.4. Cortisol levels (nmol/L) at three key stages of the experiment: T1: pre-drug, T2: 90 min post-drug; T3: post-film for the two drug conditions. Histograms represent means and error bars are standard errors.

As reported previously (Hennessy et al 2022), there was no effect of film viewing on cortisol levels (lack of timepoint effect in the placebo group between T2 and T3) consistent with the nature of the trauma film as a non-social-evaluative stressor (Dickerson and Kemeny, 2004).

This suggests that the T1→T2 increase in the spironolactone group reflects the effect of spironolactone on cortisol release (rather than film-related stress). The further increase between T2→T3 though significant, is very modest relative and suggests either that $C_{\max}$ was achieved after T2, or that T3 cortisol levels reflected a synergistic effect of film-induced- and spironolactone-induced increases in cortisol.

4.4.4 Measures from the Day 1 lab session

4.4.4.1 Blood pressure and heart rate

Time (three levels: pre-drug, post-drug/pre-film and post-film) x Drug (two levels: placebo, spironolactone) repeated-measures ANOVAs were carried out to evaluate the effect of film viewing across drug conditions for cardiovascular measures (BP, HR).

There was a significant main effect of Time for systolic BP ($F(2,152)=26.996$, $p<0.001$, $\eta_p^2=0.262$) and diastolic BP ($F(2,152)=15.800$, $p<0.001$, $\eta_p^2=0.172$) (Figure 4.5 (a and b.)). Simple contrasts revealed that although pre-film BP was not significantly higher than pre-drug for systolic BP ($F(1,76)=0.452$, $p=0.503$, $\eta_p^2=0.006$) or diastolic BP ($F(1,76)=1.694$, $p=0.197$, $\eta_p^2=0.022$), post-film BP was significantly higher than pre-drug for systolic BP ($F(1,76)=44.630$, $p<0.001$, $\eta_p^2=0.370$) and diastolic BP ($F(1,76)=31.306$, $p<0.001$, $\eta_p^2=0.292$). Given the extended period of BP stability between pre-drug and pre-film, and the clear increase between pre- and post-film (most obviously for systolic BP, Figure 4.5(a), it seems likely that this was due to viewing of the trauma film.

There was no significant main or interaction effect of drug on systolic or diastolic BP (F values ≤ 0.74 ; p values ≥ 0.474). There was a significant main effect of time for HR ($F(2,152)=61.148$, $p<0.001$, $\eta_p^2=0.446$). Simple contrasts revealed that pre-film ($F(1,76)=98.842$, $p<0.001$, $\eta_p^2=0.565$) and post-film HR ($F(1,76)=58.060$, $p<0.001$,

$\eta_p^2=0.433$)) were significantly lower than pre-drug. The main effect of Drug for HR was non-significant ($F(1,76)=0.148$, $p=0.701$, $\eta_p^2=0.002$), as was the Time x Drug interaction ($F(2,152)=0.292$, $p=0.747$, $\eta_p^2=0.004$)

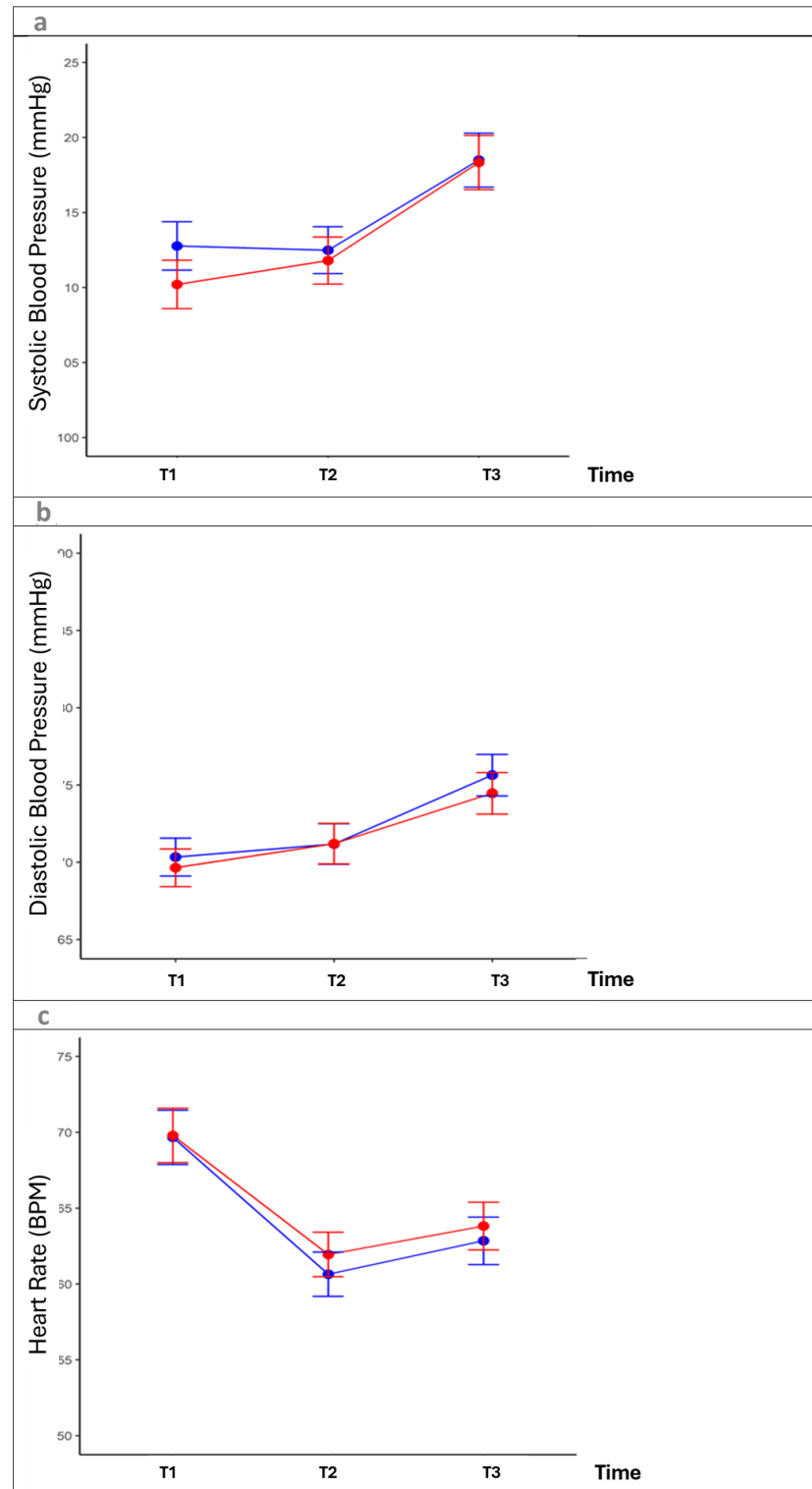


Figure 4.5. Intra-D1 changes in physiological measures (systolic (a) and diastolic (b) blood pressure (mmHg); heart rate (c) (BPM)) Estimated marginal means from RM-ANOVA models are displayed. Blue = placebo, red = spironolactone. Error bars = standard error of the mean (SEM).

4.4.4.2 Day 1 Repeated subjective state measures

Positive and negative affect (PANAS), in lab, Day1; Time (pre-drug: t₋₆₀, pre-film: t₊₇₀, post-film: t₊₁₁₅; post-filler: t₊₁₁₅) x Drug (placebo, spironolactone) repeated measures ANOVAs were carried out to evaluate the effect of film viewing across drug conditions for the positive and negative affect scales from the PANAS. There was a significant main effect of Time for positive affect ($F(3,228)=84.180$, $p<0.001$, $\eta_p^2=0.526$). Simple contrasts also revealed that pre-film ($F(1,76)=67.855$, $p<0.001$, $\eta_p^2=0.472$), post-film ($F(1,76)=198.235$, $p<0.001$, $\eta_p^2=0.723$), and post-filler ($F(1,76)=112.374$, $p<0.001$, $\eta_p^2=0.597$) positive affect scores were significantly lower than at T1(pre-drug). For positive affect, there was no main effect of Drug ($F(1,76)=0.043$, $p=0.836$, $\eta_p^2=0.001$) or Time x Drug interaction ($F(3,228)=0.982$, $p=0.402$, $\eta_p^2=0.013$). For scores from the negative affect scale of the PANAS, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated ($c^2(5)=123.001$, $p<0.001$), so Greenhouse-Geisser corrected degrees of freedom and p values are reported here for the main effect of Time and the Time x Drug interaction. .There was a significant main effect of time for the negative affect scale of the PANAS ($F(1.492,113.362)=74.315$, $p<0.001$, $\eta_p^2=0.494$). Simple contrasts revealed that pre-film ($F(1,76)=35.076$, $p<0.001$, $\eta_p^2=0.316$), post-film ($F(1,76)=54.964$, $p<0.001$, $\eta_p^2=0.420$) and post-filler ($F(1,76)=6.090$, $p=0.016$, $\eta_p^2=0.074$) negative affect scores were significantly higher than pre-drug. For negative affect, there was no main effect of Drug ($F(1,76)=1.818$, $p=0.182$, $\eta_p^2=0.023$) or Time x Drug interaction ($F(1.492,113.362)=1.171$, $p=0.302$, $\eta_p^2=0.015$).

Bodily Symptoms Scale (BSS), in lab, Day 1; Repeated ANOVA analyses were conducted on items of the Bodily Symptoms Scale (BSS) to assess potential changes in subjective bodily sensations produced by spironolactone at the four sampling time points ((pre-drug: t_{-60} , post-drug: t_{+70} , post-film: t_{+115} ; post-filler: t_{+115}). There were no significant reported effects of Drug or Time or their interaction on BSS items capturing sensations of spasm, stomach-ache, itching or dizziness ($p > .05$). There was a main effect of Time for items capturing sensations of nausea ($F(1,152)=7.295$, $p=0.002$, $\eta_p^2=0.048$), drowsiness ($F(1,152)=11.466$, $p<0.001$, $\eta_p^2=0.054$), confusion ($F(1,152)=4.758$, $p=0.22$, $\eta_p^2=0.032$) and concentration ($F(1,152)=7.854$, $p<0.001$, $\eta_p^2=0.042$). Post-hoc Bonferroni pairwise analysis revealed significant decreases in concentration from post-drug ($p=0.006$, $d=0.53$) levels to post-film which significantly increased ($p=.002$, $d=0.58$) back to pre-drug levels at post-filler but with no main effect of Drug ($F(1,76)=0.625$, $p=0.625$, $\eta_p^2=0.002$) or TimexDrug effects on concentration ($F(1,152)=0.638$, $p=0.58$, $\eta_p^2=0.004$). There was a reverse pattern of effects of Time reported for the other items with drowsiness levels increasing from pre-drug to post drug ($p = 0.001$, $d=0.48$) and then had significantly decreased by post film ($p<0.001$, $d=-0.56$) but with no main effect of Drug ($F(1,76)=1.469$, $p=0.23$, $\eta_p^2=0.012$) or TimexDrug effects on drowsiness ($F(1,152)=0.864$, $p=0.44$, $\eta_p^2=0.004$). Nausea levels increased from pre-drug to post film but did not survive Bonferroni correction ($p=0.09$, $d = 0.3$) and reduced significantly post-film returning to pre-drug levels post-filler ($p < 0.02$, $d=0.47$) but nausea was were not significantly influenced by Drug ($F(1,76)=0.755$, $p=0.39$, $\eta_p^2=0.005$) nor was there a TimexDrug interaction ($F(1,152)=0.966$, $p=0.37$, $\eta_p^2=0.007$). The main effect of Time on reports of confusion did not survive post-hoc analysis and, as for the other BSS items, there was no main effect of Drug ($F(1,76)=0.0001$, $p=0.996$, $\eta_p^2<.001$) nor Time x Drug interaction ($F(1,152)=0.325$, $p=0.63$, $\eta_p^2=0.002$) on reports of confusion sensations.

4.4.5 Measure across the week (Diary records)

4.4.5.1 Intrusion frequency

As expected, intrusion frequency declined across the week (main effect of day: $\chi^2(6) = 136.00$, $p < 0.001$). However, there was no effect of drug (main effect: $\chi^2(1) = 0.18$, $p = 0.673$; Drug x Day interaction: $\chi^2(6) = 6.07$, $p = 0.416$; Figure 4.6).

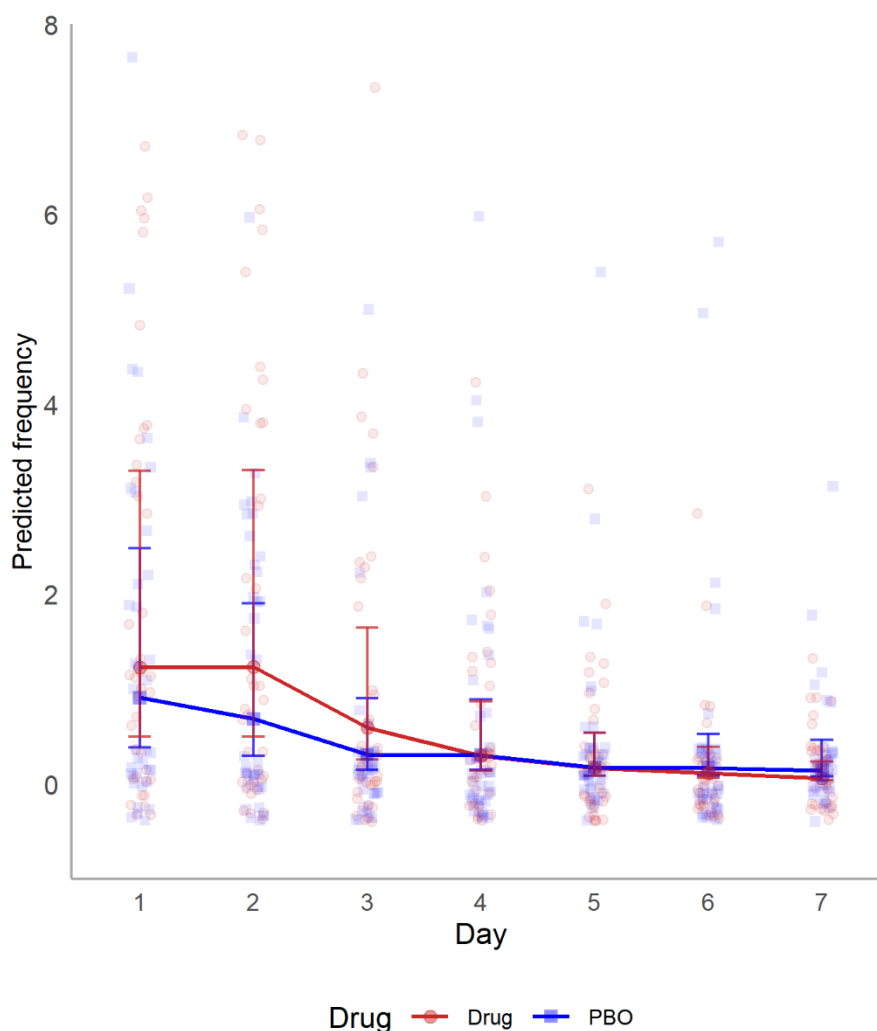


Figure 4.6. Predicted intrusive memory frequency (mean $\pm$ SE) for spironolactone (red, circle) and placebo (blue, square) For clarity, 3 datapoints ranging from 10-13 intrusions that contributed to the means/SE for days 1-3 for placebo and 1 datapoint contributing to the mean/SE for day 2 for spironolactone with a value of 15 intrusions are not shown.

4.4.5.2 *Intrusion-related distress and vividness*

A linear mixed effects model for distress with robust standard errors indicated significant effects of day ($\chi^2(6)= 50.46$, $p<0.001$) and critically, of drug ($\chi^2(1)= 3.88$, $p= 0.049$), but no Drug x Day interaction ($\chi^2(6)=5.85$, $p=0.440$). As shown in Figure. 4.7 (a) the spironolactone group showed lower levels of distress across days 1-7. Figure. 4.7 (b) shows the effects of spironolactone on vividness, with a similar critical effect of drug ($\chi^2(1)= 4.15$, $p=0.042$). The effect of day was also significant ($\chi^2(6)= 80.76$, $p<0.001$), but not of Drug x Day ($\chi^2(6)= 6.31=0.389$).

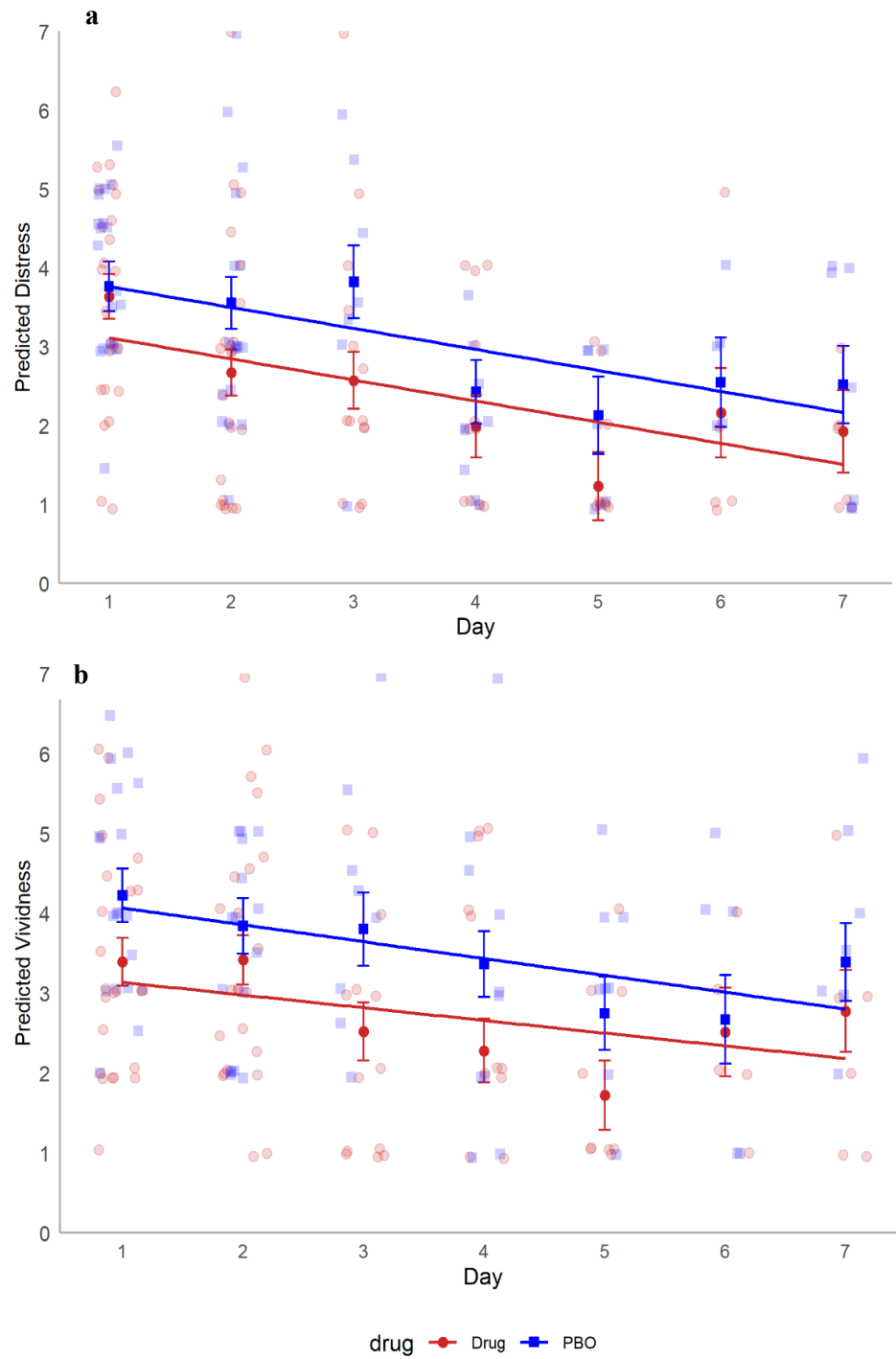


Figure 4.7. Intrusion-related (a) distress and (b) vividness across days 1-7 Error bars = standard error (SE). Blue (square symbols) = placebo, red (circles) = spironolactone.

4.4.5.3 IES score and voluntary memory on Day 8 (remote assessment)

There were no significant effects of drug on IES scores, free recall or cued recall tasks related to the film (F values ≤ 1 ; $p \geq 0.4660$).

4.4.5.4 Repeated subjective 'state' measures Day 8 (remote assessment)

Positive and Negative affect (PANAS), RM-ANOVA analysis was carried out for positive and negative affect scales from the PANAS (completed before and after cued and free recall for trauma film contents). This indicated that although there was a significant main effect of time for the positive ($F(1,76)=57.194$, $p<0.001$, $\eta_p^2=0.429$) and negative ($F(1,76)=18.635$, $p<0.001$, $\eta_p^2=0.197$) affect scales of the PANAS, there was no significant Time x Drug interaction for positive ($F(1,76)=0.989$, $p=0.323$, $\eta_p^2=0.013$) or negative affect ($F(1,76)=3.194$, $p=0.078$, $\eta_p^2=0.040$).

4.4.6 Association between intrusive memories and sex hormones

Hydrocortisone has been reported to differentially influence intrusion frequency following a trauma like event in men and women, depending on circulating levels of progesterone and estradiol (Hennessy et al (2022)). No effect of testosterone in either sex was reported in that study. Exploratory analysis in the current study revealed no moderating effect of pre-drug (T1) progesterone or estradiol (Drug x Hormone: $z \leq 1.20$, $p \geq 0.228$) However, there was an interaction between spironolactone and pre-drug testosterone levels ($Z=2.31$, $p=0.021$). However, the effect did not survive correction for multiple hormone comparisons ($n = 3$), not reaching the adjusted level of statistical significance ($p < 0.017$), and therefore requires a pre-registered, appropriately powered study to explore further. However, descriptively, Figure 4.8 shows the effects of drug at different levels of (mean centered) testosterone and suggests that the primary effect of reduced MR activity via spironolactone may in fact be to *increase* the tendency to

experience intrusions, at least relative to high testosterone. This effect appeared to be specific for the occurrence of intrusions rather than their associated vividness or distress, both of which were unaffected by pre-drug testosterone (Drug x Testosterone F values ≤ 1.2 , $p \geq 0.280$).

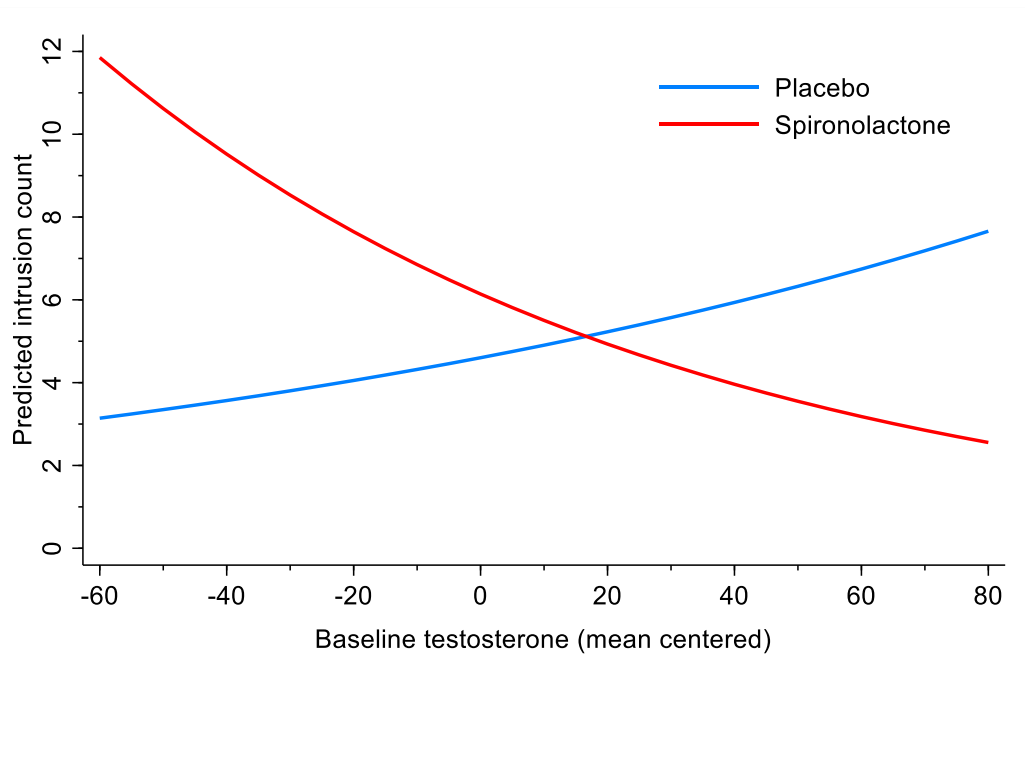


Figure 4.8. Hormone x Drug effect on predicted intrusions (summed across D1-7) for selected hormones. Blue = placebo, red = spironolactone.

4.5 Discussion

This study demonstrates a role for mineralocorticoid receptors in encoding / early consolidation of an emotionally arousing event intended to simulate traumatic stress exposure. Specifically, antagonism of MRs during these early phases of memory formation resulted in a subsequent memory effect reflected in reduced self-reported intrusive memory vividness and distress measured over the course of a week. Alternatively, *occurrence* of intrusions was unaffected by drug, although there is preliminary evidence for a negative association between intrusion frequency and testosterone levels in those receiving spironolactone.

The findings of spironolactone's effects on vividness and distress were in line with the pre-registered hypotheses and the proposed role of MR in the stress-induced shift from hippocampal to striatal-mediated cognitive processing (e.g., Vogel et al., 2015). These findings therefore extend the previous work by Schwab and colleagues showing that MR antagonism with spironolactone inhibits the shift from controlled to habitual S-R strategies (Schwabe et al., 2013). They also build on neuropharmacological research showing that a range of drugs with therapeutically important cardiovascular effects (propranolol, losartan, prazosin) can influence emotional memory and reexperiencing in a variety of ways that are relevant to the onset/maintenance of traumatic stress symptoms, (e.g., Kamboj et al., 2020; Shkreli et al., 2020). Confirming speculation that such a treatment would have potential value in PTSD prevention is important as, currently, many sufferers with established PTSD and stress-related disorders fail to respond to first-line psychological and pharmacological therapies (Kessler et al., 2017; Kessler et al., 2022). Prominent psychiatric researchers have decried the 'crisis' in the development of treatments for PTSD (Krystal et al., 2017). The crisis in the psychopharmacology of PTSD

is exacerbated by the well-known inefficiencies in the drug discovery process, as well as insufficient influence of pre-clinical human research findings in the drug discovery process, potentially leading to costly failures in early phase trials (e.g., Golier et al., 2023).

Distress and vividness of intrusive memories: It is therefore encouraging that the current study showed that pharmacologically downregulating MR activity during encoding or early consolidation of a simulated traumatic event selectively affected aspects of subsequent memory performance relating to the event. Specifically, and in partial support of the pre-registered hypotheses, spironolactone reduced the distress and vividness associated with involuntary memories of the simulated trauma but, and contrary to the *a priori* hypotheses, without reducing the frequency, or rate of reduction in frequency of involuntary memories (intrusions). These results suggest that in addition to the dissociation between voluntary and involuntary memory ‘performance’ (number of details/gist aspects recalled and number of intrusions respectively) demonstrated by the differential impact of both pharmacological and behavioral interventions on these two forms of memory in the literature generally (Soeter and Kindt, 2010a; Visser et al., 2018), interventions can also have differential effects on aspects of intrusions, namely their occurrence (frequency) and impact (distress and vividness). Indeed, the attenuation of the negative affective quality of intrusions might be thought to be the most therapeutically valuable.

These results resemble those found with behavioural strategies that specifically target the sensation of vividness using a visuospatial task that competes for information processing resources in working memory with the vivid imagery of the traumatic event (Stuart et al., 2006; Engelhard et al., 2011). However, the results here show that modality specificity - i.e., matching characteristics of the targeted symptom and characteristics of the intervention – are not required to observe reductions in that symptom.

Intrusion frequency: In contrast, and contrary to the pre-registered hypothesis, MR antagonism did not affect the size or rate of reduction in the *frequency* of intrusive memories. This is unlike the effects that have been observed using a similar paradigm with nitrous oxide, hydrocortisone, propranolol, and although these drugs were administered after trauma film-viewing (Das et al., 2016; Kamboj et al., 2020; Hennessy et al., 2022). Additionally, while the effect of spironolactone post drug (T3, Figure 4.3) is obvious and large ($d=1.2$ based on $\log_{10}$ transformed values), the effect is still rather modest compared to cortisol levels after 30mg oral hydrocortisone ($d=3.3$, based on log transformation of data previously reported (Hennessy et al., 2022)). While there was the hypothesized spironolactone led reduction in the *vividness* and *distress* associated with these intrusive memories, the Time x Drug interaction was not significant, indicating that there was not a differential effect of Drug on intrusion affect across days 1-7. However, in line with the secondary pre-registered hypothesis, measures of voluntary memory (free and cued recall) did not differ between spironolactone and placebo, suggesting that spironolactone has a selective effect upon the vividness and distress associated with involuntary intrusive memories, but not the detail of those memories recalled voluntarily.

There are multiple neural mechanisms by which MR antagonism may have reduced intrusive memory vividness and distress. Importantly, the amygdala is thought to play a role in orchestrating the MR-dependent shift in memory systems under stress (Vogel et al., 2016), implicating a possible role for the amygdala, particularly the basolateral amygdala (BLA). For example, BLA amygdala neurons *in vitro* demonstrate an MR-dependent increase in excitability after cortisol administration (Karst et al., 2010). Further, one study in humans demonstrated an enhancing effect of stress upon amygdala-dorsal striatum functional connectivity, and a decrease in amygdala-hippocampal functional connectivity – an effect that was blocked by spironolactone (Schwabe et al.,

2013). Spironolactone might, through its action on MRs in the amygdala, interfere with the shift in memory systems that occur under conditions of stress (Hermans et al., 2014a; de Quervain et al., 2017), see Figure 1.1.2, Box 1.1.1, and thereby reduce the vividness and distress associated with these memories. Dorsal striatal activity is thought to be involved in the encoding of simple habitual, stimulus-outcome associations (Wirz et al., 2018), rather than the more cognitively elaborate memories critically involving the hippocampus (Squire, 2004; Menon, 2011) and may contribute to intrusive memories and psychopathology. However, no neuroimaging data is presented in this study, so it remains unclear as to what the neural basis of the apparent reduction in intrusive memory vividness / distress in the spironolactone group may be, and further work should seek to explore this.

Beyond the possible effects of spironolactone in the modulation of the reported shift in neural memory systems under stress, it remains possible that spironolactone is interfering with memory consolidation. A high dose of spironolactone was given, and the active metabolites of spironolactone (e.g. canrenone) have a half-life of hours (Accord-UK.Ltd, 2022) so it is possible that the spironolactone may be mediating its effects through cytosolic MRs with slow, genomic actions upon memory consolidation (de Kloet and Joëls, 2020) rather than acutely via membrane bound receptors. Further work may look to administer spironolactone *after* trauma-film viewing to evaluate whether the apparent effects of this drug on intrusive memories act through memory consolidation processes, or information processing under stress.

Role of the gonadal hormone, testosterone, in moderation of intrusion frequency by spironolactone; While overall intrusion frequency was not affected by spironolactone, exploratory analyses revealed that baseline testosterone levels moderated this effect. Specifically, participants with higher testosterone levels had relatively fewer intrusions

with spironolactone, whereas low testosterone was a risk factor for *more frequent* intrusions. Although the mechanism of this interaction was not explored in detail, it is possible that testosterone influences emotional memory consolidation. For example, testosterone reduces fear and anxiety levels in humans and in non-human animal models (Aikey et al., 2002; Hermans et al., 2007) due to cross-talk between the end products of both the HPA and HPG axes under stress (McHenry et al., 2014). Specifically, testosterone may indirectly suppress HPA activity via moderation of CRH expression in the hippocampus (Reul and Holsboer, 2022) and rapidly modulate neuronal activity to protect against stress-mediated oxidative stress (McHenry et al., 2014). Behaviourally high dose testosterone produced an anxiolytic effect in male mice due to neurosteroid metabolites of testosterone interacting with GABA_A receptors with a time course suggestive of rapid nongenomic mechanism of action (Aikey et al., 2002). In this study, spironolactone - which possesses MR antagonism and anti-androgenic properties - might interact through its upregulation of cortisol levels with testosterone levels (Terburg et al., 2009). In individuals with higher testosterone, spironolactone appears to synergise with testosterone, potentially enhancing resilience to traumatic memories. Conversely, in those with lower testosterone, spironolactone's anti-androgenic actions might further reduce testosterone activity, possibly increasing vulnerability to stress and facilitating intrusive memory formation. Although the mechanisms underlying this interaction remain unclear, and require further research, these findings suggest that individual hormonal profiles may moderate responses to MR-targeted interventions and that testosterone levels could inform personalized approaches to PTSD prevention.

4.5.1 Limitations

An acknowledged limitation in the current study is that as discussed in *Chapter 1, section 2*, sex is an important moderating variable in clinical neuroscientific research (Kornfield et al., 2018; Hiscox et al., 2023) given the sex differences in the epidemiology of stress-related disorders, glucocorticoid levels (Larsson et al., 2009; Wang et al., 2024) and HPA reactivity (Liu et al., 2017) with potential interaction between gonadal hormones and sex in the effects of corticosteroids on emotional memory expression. For example, the indirect link between sex and PTSD is contingent on impact of higher estradiol concentration on inflammatory cytokine levels after trauma being protective against probability of men to developing PTSD (Hiscox et al., 2023).

These results suggest that lower testosterone levels in men seem not just to negate the effects of spironolactone but rather increase the risk of the frequency of intrusive memories. Although an interaction between testosterone levels and spironolactone's effects on intrusion frequency is reported, this analysis was exploratory and not part of the pre-registered hypotheses. Future studies should include comprehensive hormonal assessments over time and larger, more diverse samples to validate these findings. Investigating this interaction in women and considering menstrual cycle phases and across the lifespan in both sexes could further elucidate sex-specific effects and hormonal influences on MR antagonism and memory consolidation.

The current results can only provide an incomplete picture regarding the interaction between sex hormones and effects of MR antagonism on trauma-like memories. The current study had originally planned (and had IRB approval) to recruit a balanced sample of men and women. In the previous study, (Hennessy et al., 2022) intrusion frequency is reported to be moderated by sex *and* gonadal hormone levels, confirming the importance of the biological variable sex in PTSD and experimental

medicine studies previously mentioned (Hsu et al., 2021; Hiscox et al., 2023). However, planning and approvals for the current study occurred prior to the COVID-19 pandemic. When the study eventually started (in July 2021), it was evident that recruitment would be much slower than previous studies. As such, to maximize statistical and experimental control and perform an adequately powered study within constraints of funding and time, pragmatically, it was decided to only recruit male participants (per our pre-registration), thereby removed sex and (menstrual cycle-related fluctuations in) gonadal hormone levels as a source of variability. Future studies are therefore needed that extend the current findings and should examine whether the observed effects are different in women and whether gonadal hormone levels interact differently in men and women receiving spironolactone v placebo.

4.6 Conclusion

In summary, this work helps elucidate the contribution of the MR to involuntary intrusive memories, with spironolactone appearing to demonstrate a selective effect upon the vividness and distress associated with intrusive memories, but not their frequency, or voluntary recall. Further investigation into the contribution of the MR to deleterious aspects of intrusive memories and its prevention of PTSD development is warranted.

Chapter 5

Discussion

5.1 Introduction

Currently, there are no reliable treatments offered to prevent development of PTSD and current psychological and pharmacological treatment options provide limited success in robustly treating the established aversive memories (Holmes et al., 2014; Fu, 2023) associated with this disorder. The sensory-perceptual memories associated with PTSD are generally robust, highly distressing and long held (Kessler, 2022) and often resistant to inhibition via extinction used in existing exposure based behavioural therapies, (Lewis et al., 2020) resulting in great individual suffering as well presenting a burden on worldwide healthcare resources particularly in regions of the world least able to meet these.

A novel behavioural manipulation of existing fear memory- reactivation prior to extinction, PRE – has been reported to provide robust protection against relapse following extinction-based (exposure) treatment (Else et al., 2018), a frequently occurring problem in current clinical settings (Joëls and Baram, 2009; Holmes et al., 2014). However, there are inconsistent findings of the benefits of PRE over extinction alone in both the animal (Luyten and Beckers, 2017) and human literature (Chalkia et al., 2020) and reviewed in Ecker (2021), with or without pharmacological adjuncts (Walsh et al., 2018). Animal studies (Bredy et al., 2007; Bredy and Barad, 2008) and current research literature suggests that the use of cognitive enhancing drugs (Whittle and Singewald, 2014; Singewald et al., 2023) as an adjunct to extinction based therapies may have the potential to improve current treatments. For example, the histone deacetylase inhibitor, sodium valproate (Kuriyama et al., 2011; Kuriyama et al., 2013) may act alone or synergistically with retrieval + extinction protocols to attenuate robust, long held intrusive aversive memories that are challenging to modify and this was experimentally explored in *Chapter 2*.

However, exposure to a traumatic event is not in itself sufficient to predict the development of a stress-disorder. While improving treatment outcomes for those with established PTSD would be highly beneficial it would be ideal to develop effective preventative strategies against the development of maladaptive persistent aversive mnemonic symptoms and such interventions and the potential underlying mechanisms involved was explored in *Chapter 3* and *Chapter 4*.

Preventing early hyper-consolidation of such memories during the brief (<6hr) protein synthesis dependent process of synaptic consolidation without altering the voluntary ability to recall the event may reduce risk of developing long term maladaptive, memory of experiencing trauma. As is the case for moderating established aversive memory, neuroendocrine and (epi)genetic variables associated with PTSD development have been shown to be amenable to experimental (pharmacological and/or behavioral) manipulation (Kamboj et al 2020) and potentially, to clinical intervention (Astill Wright et al., 2021; Kothgassner et al., 2021) with hydrocortisone. However, much of the clinical evidence for reduced PTSD incidence following hydrocortisone given soon after a traumatic event is from studies employing repeated dosing with hydrocortisone, in severely ill patients and in small sample sizes (Schelling et al., 1999; Schelling et al., 2001; Schelling et al., 2004; Weis et al., 2006; Delahanty et al., 2013). Therefore, despite small to large effect of hydrocortisone-dependent reduction of risk of PTSD in metaanalyses of the observational and small-scale clinical studies (Sijbrandij et al., 2015; Astill Wright et al., 2021), a placebo-controlled clinical trial, published subsequent to the study in Chapter 3 (Hennessy et al., 2022), administering IV hydrocortisone within the critical 6hr time window after a traumatic incident did not find the drug to be effective over placebo in preventing a diagnosis PTSD 13 months later (Carmi et al., 2023). Although interestingly the authors report that post-hoc analysis found that hydrocortisone

was effective if given when cortisol levels were low due to diurnal variation. Indeed, none of those given hydrocortisone at night developed PTSD (Carmi et al., 2023). However, the clinical literature provides limited opportunities to examine systematically underlying mechanisms of action or moderating factors (Sijbrandij et al., 2015; Astill Wright et al., 2019) and, as observed in Carmi et al., the relative contributions of the numerous cellular and molecular processes in inhibition or enhancement of maladaptive memory formation and consolidation are exquisitely sensitive to the levels of circulating glucocorticoids (Andreano and Cahill, 2006; Cohen et al., 2015) in HPA axis moderation of aversive memory.

Strategies for preventative efforts targeting moderation of early HPA responses and their interaction with sympathetic nervous system release of catecholamine during the initial stress response will likely be most effective if deployed within a critical window of opportunity (de Quervain et al., 2017). This will need to be clearly defined either to modify glucocorticoid and mineralocorticoid receptors response during the initial stress response or during physiological recovery period following the trauma event and before the fragile memory engram completes (synaptic) consolidation (Joëls and Baram, 2009; de Kloet and Joëls, 2023). The complexity of the mechanisms involved, while offering numerous potential targets for modulation, makes the provision of reliable, effective preventative and treatment options for all patients challenging.

The drugs examined in the chapters of this thesis are in regular use, in a wide demographic, including in emergency settings, making understanding their impact on post-trauma consequences particularly important. Additionally, there are clear differences in prevalence and morbidity between the sexes (Shansky, 2015; Ramikie and Ressler, 2018). Although social/cultural factors are likely to be critical in determining sex differences in vulnerability to post trauma and stress-related disorders, neurophysiological

differences prior and post a traumatic experience are also likely to be important determinants of the long term sequelae of such experiences (Kornfield et al., 2018). Therefore, interactions between brain systems and neuroendocrine mechanisms are potential candidates to influence and to explain differences in treatment outcomes in both sexes (Garcia et al., 2018; Ney et al., 2019; Hsu et al., 2021). The experimental studies contained within this current thesis aimed to make a significant contribution to the current body of research on the modification of human aversive memory in both sexes and to adequately explore the potential of

Novel psychopharmacological strategies to attenuate maladaptive aversive memories in men and women

Using three experimental double-blind placebo-controlled studies, the thesis specifically addresses the following research questions:

- 1) Can reconsolidation updating with or without psychopharmacological adjunct provide long lasting protection against return of consolidated experimentally conditioned fear memories?
- 2) Is the drug Sodium Valproate, a histone deacetylase inhibitor, viable to act alone to enhance extinction or as an adjunct to memory reconsolidation updating of experimentally conditioned fear memories?
- 3) Given the importance of glucocorticoid receptor activity in the consolidation of trauma memories and the modulating role of mineralocorticoid receptors in stimulus-response and habit learning that resembles the characteristics of stimulus driven-learning involved in sensory-perceptual memory formation, what might be the effects of (a) administering the mineralocorticoid antagonist spironolactone, immediately *prior* to a trauma-like event or (b) increasing the circulating

glucocorticoid (cortisol) levels via hydrocortisone administration immediately *post* the event, have on a transdiagnostic symptom of PTSD psychopathology (i.e. intrusive memory *frequency*)?

- 4) Does moderating mineralocorticoid and glucocorticoid receptor activity, either immediately prior- or post- a trauma-like event, alter the perceptual and emotional *quality* of post trauma sequelae and does voluntary memory of the event remain intact?
- 5) Does sex and/or gonadal hormone levels or their interaction, influence the efficacy of moderating aversive memories in experimental models of the onset and treatment of stress related disorders?

This final chapter will summarise the findings from this research and integrate these findings into the extant literature. While accounting for the limitations of the studies detailed here, potential implications and areas for future consideration will be addressed and some conclusions proposed.

5.2 Summary of findings

Sodium valproate (an histone deacetylase inhibitor, HDACi) as a pharmacological adjunct to reconsolidation updating of conditioned fear: Previous research suggests that retrieval and subsequent reconsolidation of a long held memory adaptively uses some overlapping and some distinct neurobiological processes to those that are engaged during memory consolidation (Silva and Gräff, 2023). Under certain circumstances this leads to the updating of the original memory (Monfils et al., 2009b; Monfils and Holmes, 2018). Extending the work of animal studies (Bredy et al., 2007; Bredy and Barad, 2008) of the potential of histone deacetylase inhibitors (HDACi) to

enhance extinction and reconsolidation-updating of fear memories, *Chapter 2* uses an associative fear conditioning three day protocol to explore the use of reconsolidation updating with or without pharmacological adjunct (sodium valproate (400mg) to protect against return of fear effects three days after acquisition of conditioned fear in men (N=40) and women (N=40). Sodium valproate facilitated new learning and enhanced extinction learning and re-extinction the following day compared to placebo possibly through HDACi 'permitted' increase in transcription and synaptic plasticity-related proteins. Contrary to previous research, however, valproate treatment failed to prevent return of fear response at test and had no additive effect with reconsolidation processes. Contrary to some but not other previous research no benefit of reconsolidation alone to prevent return of fear was found based on the pre-planned analysis. Exploratory, post hoc analysis suggests both drug and retrieval prior to extinction (PRE) were sex dependent with acute and lasting effects on the response to the safety signal (CS2). Speculatively, the effect of sodium valproate in attenuating fear responding described in the pre-planned analysis and an effect of behavioural manipulation of reconsolidation on fear memory significantly reduced differential fear responding at test primarily only in men given placebo.

Hydrocortisone given immediately after a trauma-like event as a secondary preventative measure to reduce PTSD-like symptomology (Chapter 3); Posttraumatic stress disorder (PTSD) is characterised by dysregulated hypothalamic-pituitary-adrenal axis activity and altered glucocorticoid receptor sensitivity (Lawrence and Scofield, 2024). Current research literature and recent meta-analyses suggest that early treatment to moderate the glucocorticoid response to a stressful event may be an effective preventative agent/'pharmacological vaccine' in PTSD (Astill Wright et al., 2021). In *Chapter 3*, the study described, replicated and extended previous work that explored the use of psychopharmacological agents (Das et al., 2016) to disrupt consolidation and/or

rehearsal, and with propranolol and hydrocortisone (cortisol) in particular (Kamboj et al., 2020). The double-blind randomised controlled experimental study conducted with 120 healthy men and women (N=60) given a 30mg dose of hydrocortisone (cortisol, N=60)), or matched placebo, immediately *following* the viewing of a highly distressing film. The results of the study supported the pre-registered hypothesis and previous findings showing a statistically significant Day $\times$ Drug interaction that is reflective of an accelerated reduction of intrusive, sensory-perceptual memories (the type that are a diagnostic symptom of PTSD) following hydrocortisone compared to placebo as reported by daily over the subsequent week. Also as the pre-registered hypothesis, voluntary recall of the events of the film was left unaffected (Hennessy et al., 2019). Further, in an extension to previous findings (Kamboj et al., 2020), results from exploratory analyses on sex and gonadal hormone interactions with drug group membership were reported. It was found that men receiving hydrocortisone, who had higher baseline estradiol levels experienced fewer intrusions, whereas women exhibited the opposite pattern. By contrast, progesterone levels were positively associated with intrusions only in men treated with hydrocortisone. These exploratory results tentatively suggest the need to account for individual neuroendocrine profiles as well as sex in the assessment of drug efficacy in both sexes.

Spirolactone given as an mineralocorticoid receptor antagonist prior to a trauma-like event to moderate encoding/early consolidation of aversive memory (Chapter 4); Post-trauma exogenous hydrocortisone administration purports to manipulate the glucocorticoid response via modulation of predominantly glucocorticoid receptors (GR) that modulate the ‘recovery’ phase after a traumatic event and the consolidation/rehearsal phase of fear memory development whereas the pre-registered study (osf.io/76yvk) described in *Chapter 4* was designed to target the initial stages of the stress

response/memory encoding by moderating mineralocorticoid receptor (MR) activity. Previous pharmacological studies suggest that MR antagonism may modulate neural processes contributing to memory formation (Karst et al., 2005; Groc et al., 2008; Karst et al., 2010; Vogel et al., 2016) and by extension, to maladaptive memory (Brewin, 2011). Nuclear mineralocorticoid receptors maintain and moderate tonic changes in basal levels of glucocorticoids but over acute stress response timescales membrane-bound MR mediate rapid, non-genomic effects on neuronal excitability (de Kloet et al., 2005; Groeneweg et al., 2012). MRs therefore play a role in rapidly shifting neural activity in the salience network driving the formation of basic, rigid stimulus-response representations of the traumatic episode (Schwabe and Wolf, 2012) while correspondingly downregulating cognitive resources by constraining hippocampal and prefrontal cortex neural activity (de Kloet et al., 2019) that mediates complex information processing involved in ‘contextualizing’ memories. Poorly contextualized and fragmented declarative (voluntary) memory and a bias towards simple stimulus-response processing is thought to underlie intrusive memories (Ehlers et al., 2004) contributing to impoverished contextual representations of the traumatic event and a tendency for the episode to be reexperienced as fragmented.

MR functioning was probed in humans using the MR antagonist, spironolactone, a potassium sparing diuretic (Schwabe et al., 2013; Vogel et al., 2017; Wingenfeld and Otte, 2019) using a similar methodology and trauma film as in *Chapter 3* but administering 400mg, or matched placebo, to 78 healthy men 90mins *prior* to the trauma-like event. This method ensured the drug reached peak plasma levels to exert MR antagonism during putative encoding/early consolidation of the trauma-like event (Figure 1.3.2). Consistent with the pre-registered hypotheses, participants receiving spironolactone reported less distressing and vivid intrusions throughout the subsequent

week relative to placebo, suggesting that during encoding/ early consolidation of traumatic stress-like events there is a critical role for MR-mediated information processing but no acceleration reduction. Contrary to effects of post-trauma glucocorticoid manipulation (Kamboj et al., 2020; Hennessy et al., 2022), the analysis failed to find a drug effect on total intrusions or an enhanced reduction in the rate of intrusions across the week compared to placebo. However, exploratory analysis found that, intrusion frequency was dependent on levels of the gonadal hormone testosterone depending on drug group. Specifically, men with *higher pre-drug* testosterone levels reported *fewer intrusions* with spironolactone and lower pre-drug levels were associated with a tendency to experience higher levels of intrusions. The opposite pattern was observed in the placebo group. The results suggest that reducing MR activity pharmacologically may promote a more resilient response to stressful events and that this effect is most pronounced when baseline testosterone levels are high. These exploratory results with spironolactone in *Chapter 4* and hydrocortisone in *Chapter 3* suggest that gonadal hormones are important moderators of memory functioning relevant to stress related disorders and of the importance of assessing gonadal hormone levels as predictors of response to preventative and treatment strategies to attenuate aversive memories in both sexes.

The studies described, provide supportive evidence that manipulating both mineralocorticoid and glucocorticoid receptor activity can moderate trauma memories during both initial and recovery phase of glucocorticoid activity while memories are in a fragile, pre-consolidated state, providing an opportunity to moderate synaptic consolidation leading to attenuated aversive memories and associated subjective distress. Additionally, extinction learning of established declarative memories following sodium valproate can be accelerated possibly via epigenetic manipulation. Exploratory analysis

from all three experimental studies suggests that sex and/or gonadal hormones may be important moderators and indications of potential novel treatment and preventative strategies for attenuation of aversive memories in both sexes.

5.3 Incorporating the current results into the existing literature

5.3.1 Epigenetic modification via histone deacetylase inhibition during fear learning/extinction

Exposure therapies that incorporate methods that enhance resistance to reinstatement and renewal effects would be an important advance in the treatment of anxiety and trauma disorders. An epigenetic driven alteration to the expression of stress-related genes following adverse events has been proposed as the mechanism to explain a pre-disposition to stress related disorders (Yehuda and LeDoux, 2007). Epigenetic alterations of the brain-derived neurotrophic factor, BDNF, gene known to be involved in extinction learning are seen following early life maltreatment in mice resulting in decreased expression of BDNF exon IV in the PFC in adulthood (Roth et al., 2009). Additionally, an accumulation of dysregulating epigenetic marks over a lifetime may drive some of the hippocampal and amygdala dysfunction seen in PTSD patients (Zovkic and Sweatt, 2013). This may explain the susceptibility to forming intense fear memories that are resistant to extinction.

Fear learning and maladaptive memory: Acutely, stress level glucocorticoid levels can act to enhance histone deacetylase activity (section [1.1.8](#) and glossary) to influence which elements of the event meet the threshold to be fully consolidated as lasting aversive

memories (Kwapis and Wood, 2014). HDAC activity has direct consequences on fear learning dependent gene expression and the de novo protein synthesis required to drive LTP and neural plasticity in both basolateral and central nucleus of the amygdala following trauma (Maren, 2011) as well as during consolidation of hippocampus-dependent memory (Roth and Sweatt, 2009). Inhibiting HDAC activity within the consolidation window (within hours of conditioning) disrupts long term fear memory processing and reduces conditional fear responses (Maren et al., 2003).

Extinction learning: Extinction learning recruits similar (but not identical) cellular and molecular mechanisms in similar neural circuitry, that is, engagement of hippocampus and amygdala and medial prefrontal cortex, as original consolidation (Orsini and Maren, 2012) although extinction memories are often not as robust as the original memory (Bouton, 2002). Inhibiting HDAC activity e.g. using an HDAC inhibitor such as sodium valproate (*Chapter 2*), during extinction learning has been reported to enhance extinction memory in humans (Kuriyama et al., 2011) and protect against return of fear effects.

Accelerated extinction learning: In *Chapter 2*, sodium valproate, was shown to accelerate within-session extinction learning (Day 2). Slower within-session extinction has been reported as a predictor of vulnerability to relapse (King et al., 2018b) and is an under reported effect of pharmacological adjuncts to extinction training (Cahill and Milton, 2019). Nonetheless, individual differences in faster within-session extinction learning have been shown to predict enhanced extinction learning retention with D-cycloserine, DCS, (King et al., 2018a). Enhanced extinction following VPA is also in line with the animal literature investigating epigenetic moderation of fear memory (Bredy et al., 2007; Lattal et al., 2007; Bredy and Barad, 2008) and in part with the limited human literature (Kuriyama et al., 2011; Kuriyama et al., 2013). Bredy et al. (2007) suggest that HDAC inhibition by VPA enhances the potentiation of specific patterns of histone

acetylation on BDNF gene promoters (i.e. P1 and P4) associated with successful extinction learning and HDACi enhances this effect to promote robust, long-term extinction learning even following partial fear extinction. This last effect is important for any potential use of VPA clinically as extinction learning deficits are a recognised problem in treating PTSD with extinction-based exposure therapies (Whittle and Singewald, 2014).

Failure of VPA to prevent return of explicit fear responding at test of reinstatement (Day 3); Following test of renewal (first trial of Day 3, 24 hours after extinction session) and after test of reinstatement (representation of UCS) in *Chapter 2*, a rapid re-extinction is reported with VPA compared to placebo. This is consistent with previous pre-clinical research proposing more robust fear extinction learning of consolidation memories due to VPA histone deacetylase inhibition (Silva and Gräff, 2023). However, the results did not support previous studies reporting a sufficiently robust efficacy of VPA to prevent fear responding at the reinstatement trial itself compared to placebo (Kuriyama et al., 2011; Kuriyama et al., 2013) although differences in analysis design make direct comparisons difficult.

Sodium Valproate effects on safety signal; A profound effect of VPA to reduce explicit fear ratings of the ‘safety signal’ (the stimuli never associated with a shock, CS2) an effect reported here across all post-drug sessions an effect which accentuated the explicit CS differential fear responding at test of VPA compared to placebo. Sodium Valproate is recognised as a GABAergic mood stabilizer and so, the accelerated reduction in fear responding described in *Chapter 4*, may suggest a more global, anxiolytic, GABAergic VPA effect (de los Ángeles Cintado et al., 2024) than any specific fear memory-related synaptic plasticity involvement. However, despite a general reduction in anxiety scores (STAI) from day of acquisition compared to subsequent sessions there were

no drug interactions with behavioural measures of anxiety, or positive and negative affect (PANAS) that would support a general anxiolytic effect and indeed SCR (a measure of arousal) were higher overall during extinction in VPA than in PBO.

The dissociable effects between the conditioned stimuli (CS1) and safety signal (CS2) may more likely be a function of VPA's HDAC inhibiting activity; a non-HDAC VPA derivative, Valpromide, has not been found to have the same effects on learning (Bredy et al., 2007). Other cognitive enhancing drugs, such as DCS, have been shown to cause generalisation of fear response to other fear associated CS (Ledgerwood et al., 2004). In this study, HDACi activity, by lowering the threshold to learning (Kwapis and Wood, 2014) may have enhanced learnt associations surrounding the safety signal (Gräff et al., 2014), especially as both CSs were from the same category, spiders, and this may have resulted in hyper attenuation of the learnt habituation to CS2 during extinction learning which was maintained over the session on Day 3.

Inhibiting HDAC activity as an adjunct to retrieval-extinction (PRE) protocol;

A key determinant of whether PRE + HDACi can robustly reduce fear memory (Kwapis and Wood, 2014; Singewald et al., 2015; Blouin et al., 2016) is the interaction of drug during destabilisation of the target memory at retrieval. Put another way, there is a threshold that needs to be met for memory destabilization to enable memory modification of consolidated memories or the memory will be returned to its inactive state without change (Bui and Milton, 2023). Destabilization engages reconsolidation mechanisms that are similar but dissociable from the inhibitory new learning mechanisms of extinction learning (Merlo et al., 2014; Cahill and Milton, 2019; Milton et al., 2023) and this includes activation of pathways that regulate translation and

transcription, so that neural synaptic modifications are promoted following reconsolidation (described in *Chapter 2*). HDACi has the potential to lower the threshold required for destabilisation of the original aversive memory even for older, extinction resistant memories (Costanzi et al., 2011).

However, contrary to what has been observed previously (Gräff et al., 2014) no interactive effect of PRE with a HDACi (sodium valproate, VPA) in preventing the return of fear for either implicit (SCR) or explicit (expectancy) measures in *Chapter 2*. The limited number of previous human studies (Kuriyama et al., 2011; Kuriyama et al., 2013) using VPA employed standard extinction protocols precluding direct comparison with the current findings, but these have found that VPA can broadly potentiate both the extinction and reconsolidation of memory, leading to either a weakening or strengthening of memory traces (Bredy and Barad, 2008). HDACi theoretically promote activity-dependent learning, regardless of its nature therefore what is learnt will be sensitive to current environmental conditions and VPA has been shown to attenuate (Lattal et al., 2007; Bredy and Barad, 2008; Stafford et al., 2012) but also to enhance fear learning at test (Bredy and Barad, 2008; Guan et al., 2009; Gupta et al., 2010) depending on the protocol employed. Bredy and Barad's (2008) series of experiments detail sensitivity of VPA to context, inter-trial training and reactivation protocol to determine either VPA enhancement or impairment of fear learning. Further research in humans is therefore required to clarify appropriate protocols if a consistent effect of HDACi as an adjunct to novel behavioural strategies to deliver long term protection against fear responding in stress-related disorders is to be achieved.

Additionally, in *Chapter 2*, physiological arousal of conditioned stimuli may not meet the threshold of required intensity as the memories were only 48hrs old and may have been too 'young'. Young and old memories are known to rely on different brain

regions for their maintenance (Frankland and Bontempi, 2005; Clem and Schiller, 2016) and so in this case epigenetic priming might have made no observable beneficial effect on these memories either alone or in combination with engagement of reconsolidation mechanisms (Gräff et al., 2014).

Retrieval prior to extinction (PRE) without adjunct; The pre-planned analysis here, found the PRE alone did not protect against return of fear, as result which is contrary to some previous studies (Schiller et al., 2009; Schiller et al., 2012) but consistent with others (Schroyens et al., 2017b; Chalkia et al., 2020), and in literature reviews (Kredlow et al., 2016; Ecker, 2021). The ineffectiveness of a brief retrieval trial prior to extinction learning was despite PRE effects altering respective responses to CS1 and CS2 compared to standard extinction learning (EXT) during extinction learning that was suggestive of some behavioural effect on conditioned memory. The rapid early reduction in expectancy of shock to CS1 immediately following the retrieval trial compared to EXT is similar to that reported in a retrieval plus in-vivo exposure therapy session that resulted in enhanced attenuation of fear >1mth later (Telch et al., 2017). However, rapid early reduction during extinction did not translate to significantly lower differential responding compared to placebo at test of renewal or reinstatement here. Indeed, the ‘occasion setting’ of expectancy ratings (explicit response) to the conditioned stimulus (CS1) at start of recovery testing on day 3 was significantly greater in those who had seen the retrieval trial (CS1-R) compared to those who had the standard extinction protocol.

Preventative strategies

Upregulation of glucocorticoid levels via hydrocortisone (HC) administration after a trauma like event; The results reported here, in *Chapter 3*, are broadly in line with evidence from previous experimental studies (Zohar et al., 2011; Kamboj et al., 2020) but not all (Graebener et al., 2017) that report intrusive memory frequency over the week following a trauma like event to be significantly accelerated by hydrocortisone-induced supraphysiological levels of glucocorticoids immediately *after* analogue trauma in healthy human participants although the faster decline in intrusions in *Chapter 3* more closely follows the pattern of nitrous oxide effects on intrusions in a similar study design (Das et al., 2016).

A highly complex relationship between exogenous cortisol levels and memory performance exists across different memory domains and memory phases resulting from a ‘neuro-symphony’ (Joëls and Baram, 2009) of stress memory mediating effects across molecular and cellular mechanisms to large scale brain network connectivity (Schwabe et al., 2022). These inter-relational effects are time (Het et al., 2005) and dose dependent (Schwabe and Wolf, 2014) with implications for how preventative strategies might specifically moderate the sensory perceptual representations that underlie aversive intrusive memories associated with stress related disorders. Supraphysiological levels of cortisol were achieved 60 min post hydrocortisone administration and trauma-like event in the study in *Chapter 3*. High cortisol levels at 60 min post stressor onset could represent the impairing right ‘arm’ of the u-shaped dose response curve to enhance GR mediated constraint of the endogenous initial stress response via negative feedback to the hypothalamus (de Kloet and Joëls, 2020) to induce the subsequent reduction in intrusions from day 2 after viewing the trauma film seen in *Chapter 3* while leaving the ability to voluntary recall the event unaffected. Selectively targeting and preventing intrusive

memory experiencing is therapeutically valuable and something that was not assessed in the previous clinical studies that reported a protective effect of post trauma hydrocortisone administration on PTSD symptomology more generally. However, whether the reduction in intrusion frequency across the week seen in this study is the result of raised glucocorticoid levels effects on consolidation sensory perceptual representations specifically was not determined here.

Hydrocortisone has been shown able to impair retrieval shortly after encoding (Kuhlmann and Wolf, 2005) and previous studies suggest that high cortisol levels as in *Chapter 3* could act on early spontaneous retrievals prior to the engram becoming hippocampal dependent and dispersed through neocortical networks and this could represent an indirect root to impaired ‘synaptic’ consolidation (Antony et al., 2017). However, the results here were not consistent with an effect on early retrieval processes as intrusion frequency assessed >1 hr after viewing the film found no differences between drug and placebo. The results here are more consistent with recent thinking that hydrocortisone’s impairing effects on retrievals can have longer lasting effects on consolidation, persisting up to 7 days later (Wolf, 2017). Additionally, the aforementioned upregulated GR mediated constraint of the stress response by hydrocortisone may have more generalised acute effects on cognitive processing though glucocorticoid enhancement of executive control via prefrontal cortex neural activation (Jentsch et al., 2019) to indirectly reduce intrusions by an extended duration of emotion regulation.

Although the results reported here are consistent with other observational and small-scale clinical studies and largely concur with the conclusions of recent meta-analyses (Astill Wright et al., 2021) they appear inconsistent with a clinical trial (Carmi et al., 2023) published after the completion of the study here (Hennessy et al., 2022). The pre-planned analysis of this placebo-controlled clinical trial reported no effect of

intravenous hydrocortisone over placebo when given in the emergency department > 6 hr after a traumatic event. However, whether supraphysiological cortisol levels post trauma impair intrusion frequency is likely dependent on individual differences in cortisol (and other neurosteroids) circulating at time of trauma and at time of intervention (Schwabe and Wolf, 2014). The authors of the clinical trial (Carmi et al., 2023) conducted post hoc analysis and report that hydrocortisone was effective (effect size .41) vs placebo in preventing PTSD symptom. In fact, no patients developed PTSD if hydrocortisone was given at night when the traumatic event occurred at night. (i.e. when cortisol levels were low due to diurnal variation). The study in *Chapter 3 (and Chapter 4)* was purposely scheduled so that testing occurred in the afternoon to control for diurnal and pulsatile variations in cortisol levels (Lightman and Conway-Campbell, 2010) and how these fluctuations, from high morning levels to low evening levels, might influence emotional learning and its attenuation (Pace-Schott et al., 2013). As such, the results reported in *Chapter 3* are consistent with the post-hoc analysis of (Carmi et al., 2023) that show hydrocortisone to have protective effect on subsequent PTSD symptomology under low circulating cortisol levels, itself a risk factor for PTSD (Lehrner et al., 2016).

Impact on aversive memory of MR antagonism during initial stress response to a trauma-like event (Chapter 4); Mineralocorticoid receptors (MRs) influence regulation of a glucocorticoid response to a threatening event at both genomic and non-genomic time scales via both nuclear and membrane-bound receptor variants with MRs abundantly expressed in key regions involved in fear learning; i.e. in the CA1, CA3 and dentate gyrus of the hippocampus (Karst et al., 2005), basolateral amygdala (Karst et al., 2010), and pre-frontal cortex (Joëls et al., 2024) where they are co-localised with GRs. Tentatively, results from *Chapter 4* indirectly support the importance of MR activity in acute stress responses (Schwabe et al., 2013) and memory encoding. Here MR antagonism (and by

implication, possible alteration of the relationship between MR/GR receptor activity) during the initial stages of an acute trauma-like event resulted in subsequent reduced distress and reported vividness associated with intrusive memories. Intrusive memories, as experienced by patients with PTSD, are typically associated with a heightened sense of ‘nowness’, as if events are being relived, as well as hypervigilance and avoidance of the environmental cues that trigger these highly distressing thoughts and memories (Kleim et al., 2013). The results of *Chapter 4* suggest that spironolactone down regulated -MR activity during encoding and/or early consolidation to reduce subsequent vividness and distress of trauma memories. Previous research suggests that non-genomic, membrane-bound, MR activity rapidly drives a shift in cognitive resources during the initial stress response towards the dorsal striatum to promote a rigid stimulus-response representation of the trauma (Kim, 2011; Schwabe et al., 2013) that heightens vigilance, attention and appraisal of the event prioritising retrieval of threat relevant memory at the expense of non-threatening resulting in poor contextualization of salient fear cues (de Kloet et al., 2019). The results in *Chapter 4* suggests MR antagonism prior to a stressor (analogue trauma) can constrain this shift towards the salience network allowing better connectivity within and between large scale networks (e.g. central executive control) that promote top down inhibitory control suggested to protect against psychopathology (Menon, 2011).

Acute orientation towards, and memory of, salient fear cues may be adaptive under threat but maladaptive cognitions under stress are a key factor in development of stress related disorders (Wirz et al., 2018). As discussed in *section 1.1.4*, stress-induced shift towards ‘habitual’ responding does so at the expense of hippocampal and PFC functioning on which mental flexibility and other higher order cognitive processing relies (Hermans et al., 2014b; Christina et al., 2017). Down-regulation of MR activity in humans, either due to loss of MR function genotype (Bogdan et al., 2010) or, pharmacological

inducement (Schwabe et al., 2013), as with MR antagonist spironolactone here, has been shown to reduce or prevent this shift (Vogel et al., 2015), although not always (Wingenfeld and Otte, 2019). Preventing this shift via pre stress MR antagonisms allows maintenance of higher order cognitive processing within brain regions important for safe-guarding neural plasticity and maintaining integrity of fear memory (Vogel et al., 2016). A reduction in reliance on amygdala processing therefore appears to generate more detailed, complex memories and results in less fragmented, better contextualized memory with reduced sensory-perceptual imagery. The reduced vividness ratings following spironolactone (MR downregulation) reported in *Chapter 4*, while supporting the previous literature suggesting this shift towards dorsal striatum habit, has not been directly tested here. Studies using neuroimaging data are still needed to clarify the neural basis of this spironolactone induced reduction in intrusive vividness and distress.

Dissociable memory effects

Preventative strategies: In line with the theory of dual representation of memory in PTSD (Brewin, 2001; Brewin and Holmes, 2003; Brewin and Burgess, 2014) (discussed in section 1.1.6), the dissociable effects of both stress and intervention between sensory perceptual and context-rich voluntary memory are reported throughout this thesis. In *Chapter 4*, although distress and vividness associated with intrusive memories was reduced, contrary to our pre-registered hypothesis and previous work with nitrous oxide (Das et al., 2016) and with hydrocortisone and propranolol administered post-trauma (Kamboj et al., 2020), downregulating MR activity during initial memory encoding did not decrease the frequency of sensory-perceptual intrusive memories and, as predicted, voluntary recall was unaffected. Similarly, post-trauma GR modification did not alter voluntary recall of the film events compared to placebo, despite the reported accelerated reduction of sensory-perceptual aversive memories (*Chapter 3*).

In the literature, glucocorticoid memory research generally reports an enhancing effect of glucocorticoids on consolidation and an impairing effect on both working memory and memory retrieval which may make reconciling some of the results reported in *Chapter 3* and *Chapter 4* somewhat counter intuitive. However, glucocorticoid effects on memory are dependent on levels of emotional arousal induced by noradrenergic transmission (Dominique et al., 2009) to influence hypothalamic-pituitary-adrenal/synaptic nervous system (HPA-SNS) responding and stress response effects on subsequent memory. Therefore, memory outcomes and their interaction with glucocorticoid moderation will be affected by the intensity and emotional value of the stimuli as well as the temporal relationship between HPA-SNS response and subsequent exogenous rise in glucocorticoids (Schwabe et al., 2022). In addition, effects of glucocorticoids are different depending on the memory domain assessed as evidenced by differences between episodic-verbal, voluntary, memory (the focus on much of the previous literature) compared to spontaneous, highly emotional, intrusive memory the frequency the focus of this thesis. However, an effect of drug over time, i.e., a day-dependent effect on voluntary memory (in the absence of a main effect) was not assessed in this study so it is possible that if it were and matched to aspects of involuntary memory there may indeed be an effect (Lau-Zhu et al., 2021). It could also be possible that the measure used was not sensitive to pick up a small effect in this sample.

Epigenetic modification of fear extinction learning and reinstatement; Chapter 2 reports that declarative memory was affected by HDAC inhibition (but did not have similar effects on implicit memory as measured by physiological data i.e. skin conductance). Contrary to much of the human literature on modulation of long term fear memory (Schroyens et al., 2017a) drug modulation of extinction learning reported here relates to accelerated (re)-extinction of *explicit* fear responses compared to placebo. In

contrast, much of the previous literature in this area has found a dissociation between implicit and explicit pharmacological fear memory modulation in the opposite direction (Soeter and Kindt, 2010b). It should be noted that the physiological response measured here was SCR which may not have been as sensitive to the changes in relatively low levels of arousal on Day 3, whereas fear potential startle (FPS) is a regularly used alternative measure and is considered a more sensitive measure of emotional valence.

5.3 Gonadal hormone and sex dependent effects on pharmacological and behavioural strategies for moderation of aversive memories

A recurring theme in the studies of this thesis is the influence of sex as a biological variable in response to the discussed novel psychopharmacological and behavioural strategies to moderate aversive memory. Speculatively, sex is suggested as a factor in predicting explicit but also some implicit responses to both behavioural and pharmacological manipulation of conditioned associative fear (*Chapter 2*). However, exploratory results from the preventative strategies reported in *Chapters 3 and 4* suggest that it may rather be sex as an interaction with baseline gonadal hormone levels rather than sex alone that moderates the effects of the novel preventative strategies in aversive memory modification seen here.

Testosterone: Exploratory results reported in *Chapter 4* show the interaction between MR antagonism with spironolactone and pre-stress/pre-drug testosterone levels in men to influence subsequent intrusion frequency whereas without the moderation of testosterone no effect of drug was observed on intrusion frequency. Despite a few inconsistent reports (Kenny et al., 2004) the majority of clinical and preclinical studies suggest that testosterone yields protective benefits against anxiety and depression in both sexes (although women were not included in *Chapter 4*) and administration of testosterone to men with low testosterone levels has been shown to improve affect (Kanayama et al., 2007). Additionally, higher testosterone to cortisol diurnal ratio has been associated with improved extinction learning (Pace-Schott et al., 2013). Understanding the role of gonadal neurosteroids in aversive memory modification is complicated by their interconnected biosynthesis pathways (see Figure 1.1.4). For example, testosterone is converted from progesterone and then itself converted via

aromatization, to estradiol (Lalonde et al., 2021) meaning levels of testosterone could be indirectly influencing memory processing through variable bioavailability of other neurosteroids (Toufexis et al., 2014; Sze and Brunton, 2020). However, neither pre-stress progesterone nor estradiol levels were found to be significant moderators of intrusion frequency in either the spironolactone or placebo group in the men in this study. Research on the role of MR in fear memory formation is relatively nascent, and while it is difficult from these exploratory results to identify the specific role testosterone plays in MR-dependent processes directly, they do suggest that accounting for hypothalamic-pituitary-gonadal (HPG) involvement may illuminate the role of MR in encoding and early consolidation more effectively.

Progesterone: In *Chapter 3* higher intrusive memory frequency was positively correlated to pre-analogue trauma progesterone levels in men but only in those administered hydrocortisone and who therefore experienced exogenously generated supraphysiological levels of cortisol post analogue trauma. This suggests an interaction between progesterone and cortisol in potentiating rather than attenuating intrusive aversive memory under stress conditions in men. There is extremely limited data in the experimental research literature on the effects of sex hormones on aversive fear memory in men, but particularly surrounding the role of progesterone, either in naturalistic or conditioning studies (Garcia et al., 2018) making it difficult to make comparisons. No effect of progesterone on intrusion frequency was seen in women in this study but the results in men are in line with some results seen in previous research in women (Garcia et al., 2018; Ney et al., 2019; Hsu et al., 2021).

Estradiol: Additionally, sex dependent effects on the influence of pre-stress estradiol levels on intrusion frequency was seen within the hydrocortisone group but, as with progesterone, not the placebo group in both sexes although in opposite directions

(*Chapter 3*). The results in women are in line with considerable evidence for estradiol in moderating the HPG/HPA interaction in fear and aversive memory under stress (Garcia et al., 2018; Ney et al., 2019; Hsu et al., 2021) due to the estrogen receptors found throughout brain regions important for fear extinction processes (Bartholomew et al., 2022). In healthy female populations higher estradiol levels (or menstrual phase associated with higher estradiol (i.e. luteal phase compared to follicular) have been associated with higher intrusions, (Ferree et al., 2011; Cheung et al., 2013) and early luteal > late luteal and mid follicular (Soni et al., 2013). However, as discussed in section 1.2, lower estradiol in women has also been associated with higher intrusions (Wegerer et al., 2014). In general, the relationship between sex hormone levels and frequency of intrusions in this study in women is in line with previous similar research showing potentiating effects of estradiol intrusive memories in experimental settings but with more complicated relationships and conflicting results found in clinical settings (Hsu et al., 2021). As with progesterone, evidence for a role of estradiol under stress on aversive memory is limited in men (Garcia et al., 2018). However, the results here are consistent with data from clinical populations that suggest that in men there is an indirect link between sex hormones and PTSD through interaction between higher estradiol concentration and inflammatory cytokine levels markers after trauma and subsequent PTSD development (Lalonde et al., 2021).

Hormone contraceptives: It should be noted that women taking part in the hydrocortisone preventative study (*Chapter 3*) were taking hormone contraceptives > 1 month in line with previous research (Kamboj et al., 2020). Hormone contraceptive use maintains consistent, artificially low endogenous estradiol and progesterone levels. They maintain progesterone and estradiol levels at levels comparable to in men (Bartholomew et al., 2022), and this is as seen in *Chapter 3* where no significant difference between the sexes is reported, although in both sexes a degree of individual variation in these

baseline levels is maintained. Hormone-based contraceptives contain synthetic estradiol and it is unclear if the relationships with exogenous moderation of the stress response after a trauma-like event and gonadal hormone levels on aversive intrusive memory as reported in *Chapter 3* would be maintained in naturally cycling (NC) women. Previous fear studies, report men and women taking hormone contraceptives can respond differently compared to naturally cycling women (Nielsen et al., 2014; Raeder et al., 2019; Spalek et al., 2019). Additionally, there is a sensitivity to synthetic vs endogenous hormones with a differential response to hormone contraceptive use if given post trauma according to the particular combination of synthetic estradiol/progesterone administered when given as emergency contraceptive (EC) post sexual assault as compared to synthetic progesterone EC alone or no EC (Ferree et al., 2012). However, results were not found to vary between hormone contraceptive type or length of use in the study here.

Categorical sex: the sex dependent effects described of both drug and behavioural memory manipulation in *Chapter 2*. In men drug effects were much as described for the pre-planned analysis but were largely absent in women. The sex dependent effects reported and discussed in *Chapter 2* are exploratory and not from randomised samples and as such must be treated with circumspection. However, due to the sex and/or sex hormone interaction with pharmacological strategies presented in *Chapters 3* and *Chapter 4*, and the larger effect sizes from the within sex analysis compared to unstratified (although see Ioannidis et al., 2001) a brief discussion of the possible explanations for the sex dependent effects described in *Chapter 2* seems warranted.

In men, in the absence of drug, retrieval plus extinction (PRE) induced a reduction in CS differential in expectancy of shock ratings at test (i.e. fear generalisation) but had no significant effect on women in this study. This is to some extent consistent with Meir

Drexler et al. who reports sex dependent effects on measures of SCR, with reinstatement effects only evident in men (Meir Drexler et al., 2015) and not women (Meir Drexler et al., 2016) in a similar retrieval+extinction protocol with pharmacological adjunct (cortisol) administered prior to retrieval trial of conditioned fear. However, a lack of an effect in women is largely contrary to other studies that report reconsolidation updating in both men and women in clinical trials (Gray et al., 2019; Gray et al., 2021). In experimental reconsolidation studies, contrary to *Chapter 2*, reconsolidation updating is widely reported in samples including (sometimes >50%) women (Ecker, 2021) with or without pharmacological adjunct (Walsh et al., 2018) although not always consistently so (Schroyens et al., 2017a; Stemmerding et al., 2022).

Memory at retrieval of conditioned stimulus; Sex dependent differences in response at the retrieval trial in both expectancy ratings and skin conductance response (SCR) are reported in *Chapter 2*. Whether a memory is destabilised (Forcato et al., 2009) at reactivation and the fate of that memory is exquisitely sensitive to both external and neurophysiological conditions (Cahill and Milton, 2019; Bui and Milton, 2023), as previously described in Chapter 1, Box 1.2. Therefore, speculatively, it is possible that one or more of the conditions required for the fear memory to reach the threshold for destabilization (Bui and Milton, 2023) may have affected the subsequent fear memories differently in PRE women to PRE men.

Fear generalisation and safety signals; Spatial or context-bound cues surrounding reactivation will likely influence the specificity of the reconsolidation-induced updating of sensory memories or whether generalization to non-reactivated elements of the memory occurs (Debiec et al., 2013). Sex differences in hippocampal-amygdala-pre-frontal cortex (PFC) responding during retrieval may contribute to individual differences in fear generalization (Keiser et al., 2017). In *Chapter 2* fear is reported as generalising to the

safety signal (CS2), the cue never paired with a shock, particularly in men. Compared to fear extinction learning (Garcia et al., 2018) less research has been conducted on sex differences in fear discrimination in both human and animal models but research so far in humans suggests a possible role for sex in moderating the ability of men and women to discriminate or generalise between cues that predict threat (e.g. response to CS1) or safety (to CS2) although there is inconsistency in direction of sex effects (Day and Stevenson, 2020). Day and Stevenson (2020) suggest that sex is not a strong or consistent moderator of fear discrimination/generalisation if hormones are not considered. Gonadal hormone levels were not assessed in the study here, so it has not been possible to determine the sex-dependent effects observed were influenced by sex alone or due to or in interaction with gonadal-hormone levels as seen in the studies exploring preventative strategies (*Chapter 3* and *Chapter 4*). This emphasises the limitations surrounding the reliance of using categorical sex alone as an explanatory factor. Additionally, it is important to emphasise that the effect of an intervention on aversive memory was not moderated by an effect of sex per se, but rather by interactions between sex and either the pharmacological or behavioural manipulations employed.

Drug x sex-dependent effects of HDAC inhibition on retrieval and extinction of conditioned fear; the study in *Chapter 2* reports that the accelerated extinction and re-extinction of shock expectancy following sodium valproate (VPA) was sex dependent with a significant effect predominantly only seen in men with additional Sex x Drug effects at the retrieval trial on skin conductance responses. Results from the limited previous research exploring VPA as a pharmacological adjunct to fear extinction learning in humans, while including both sexes, did not report separate analysis by sex with which to directly compare these results (Kuriyama et al., 2011; Kuriyama et al., 2013). Additionally, much of the animal models on which this research is based was conducted

in male rodents with implications for validity in generalising the results to women (Cahill, 2012; Shansky and Murphy, 2021). However, epigenetic mechanisms that influence patterns of memory related gene expression have been previously reported to differ between men and women (Keiser and Wood, 2019) influenced by gonadal steroid hormones in concert with coordinated HAT or HDAC activity that is moderated by sodium valproate in a range of fear and memory related brain mechanisms (Bonomi et al., 2022). However, these are exploratory results and no gonadal hormone or neuroimaging data e.g. from positron emission tomography (PET) using radio tracers with HDAC targeting imaging agents (Bonomi et al., 2022), was employed. Therefore, any gonadal hormone x HDACi enhancement of gene expression (e.g. BDNF) or HDACi mediated sex differentiation in relevant brain regions (e.g. moderation of estrogen receptor activity in bed nucleus of the stria terminalis (Murray et al., 2009) or hippocampus (Fortress et al., 2014) could not be assessed directly in the study here. However, behaviourally because HDAC inhibitor effects are neural activity dependent, any sex differences in recruitment of brain regional neural activity due to sex dependent learning strategies under stress (Beck and Luine, 2010) could differentially affect neural priming prior to or during extinction learning (Gräff et al., 2014). Such differences may speculatively hint at an explanation for sex dependent fear memory response effects of HDACi on fear memory responses seen in this study. However, there is limited previous research reporting sex differences in the use of HDACi as adjuncts to moderation of aversive memory in humans with which to compare the exploratory sex effects found in *Chapter 2*.

5.4 Challenges and limitations of using experimental models of PTSD and anxiety to identify potential novel preventative and treatment interventions

Challenges for retrieval extinction as a novel behavioural strategy with or without pharmacological adjunct; Attempts to engage reconsolidation mechanisms to attenuate aversive memory, especially explicit memory, have been mixed (Lee et al., 2017; Elsey et al., 2018). As described in *Chapter 1*, temporal dynamics are particularly important in aversive memory (re)consolidation (De Quervain, Silva and Graff). To establish efficacy of novel strategies to moderate aversive memory it will be essential to accurately and consistently engage the targeted mechanisms of a specific memory phase whether using behavioural and/or through pharmacological means; a requirement if any such strategy is to be used effectively, safely and within the appropriate time frame in any potential clinical setting.

Mixed results within the reconsolidation literature reflects inconsistent success in moderating aversive memories to prevent/or not renewal of fear effects. This variability stems from a complex interplay of factors related to both the memories being studied, the study protocol and reporting methodologies employed (Elsey et al., 2018). This methodological heterogeneity may account for some of the discrepancies observed in reconsolidation findings and may explain the inconsistencies with results reported here and previous findings. Interestingly, the most consistent results in human fear reconsolidation research have emerged from a single laboratory using propranolol, where

procedural variations are likely minimized (Walsh, 2019). This observation underscores the importance of methodological consistency in this field of research.

Important for translating the promise of reconsolidation into beneficial clinical therapies will be to determine how the rewriting or blocking of an aversive memory following reactivation can be made reliable under varied conditions and across wide demographics. Reconsolidation exists as a memory updating process ensuring memory can be efficiently updated and remain relevant in the face of changing environmental demands and to guide future behaviours (Lee et al., 2009). It therefore makes sense that these processes are sensitive to the aforementioned ‘boundary conditions’ (Box 1.2) to prevent unnecessary and indiscriminate reconsolidation (Zuccolo and Hunziker, 2019). A challenge particularly relevant to *Chapter 2* is that different parameters are required depending on the pharmacology of the adjunct used (Bos et al., 2014; Whittle and Singewald, 2014) and less success has been reported in defining such boundary parameters for remote memories or for novel adjuncts, such as HDACi (Singewald et al., 2015; Silva and Gräff, 2023) although see (Elsey and Kindt, 2017). The inconsistencies and failures of post-retrieval memory updating in the literature have been largely attributed to the difficulty, in achieving quantifiable and reliable retrieval procedures between different memory domains and paradigms with different pharmacological adjuncts (Whittle and Singewald, 2014) to determine level of prediction error at retrieval that correlates with subsequent extinction recall at test, something not found in *Chapter 2* in either memory domain. In an animal model, memory lability or the ‘limbo state’ has been associated with specific biomarkers, (Jarome and Helmstetter, 2013) but in humans other than the behavioural index of PE proposed (Sevenster et al., 2013; Sevenster et al., 2014) only retrospective self-reported violations of expectancy have been identified as a proxy for such a biomarker of successful destabilization (Das et al., 2018b). However, this

is a challenge that needs to be met to attain translatable, reliable reconsolidation attenuation of trauma memories in stress and anxiety disorders (Elsey and Kindt, 2017; Bui and Milton, 2023; Milton et al., 2023).

Consistency in reporting between studies is important (Elsey et al., 2018). In *Chapter 2* an effect of PRE in men to afford protection against fear renewal can be said to have occurred if CS differential between conditioned and unconditioned stimuli is non-significant as was reported here. A reduced CS differential fear responding response to pre-acquisition/chance-level at test can be interpreted as successful ‘overwriting’ of the memory of the original fear contingencies. This would be indicative of achieving memory destabilization and memory updating prior to reconsolidation. However, if the stimuli are analysed independently the reduced CS differential is revealed as a potentiated response to the safety signal rather than to a reduction in absolute fear responding to conditioned stimuli (as reported here) and could be considered over generalization of fear responding rather than successful updating. Some of the inconsistencies in results between studies may therefore reflect different approaches to analysis and reporting (Elsey et al., 2018).

Ecological validity of models of PTSD: Given the complexity of real-life trauma memories compared to specific CS/US pairings constructed in fear conditioning studies (Gazarini et al., 2023) and known analogue trauma films there will be unknown potential associations encoded concurrently in time and space during the trauma event that could impede successful reconsolidation such as potential generalisation to the safety signals reported in *Chapter 2*. Although optimistically this may also provide additional potential associations that could be targeted, it is still crucial that reliable conditions to weaken them are determined (Maddox et al., 2019). This is a particular challenge for novel strategies to address long held, robust trauma memories in that, in addition to their complexity, these memories have a potency understandably not generated in experimental

settings (as discussed in *Chapter 1.3*). Laboratory generated memories tend to fade naturally over a week following trauma-like events as reported in *Chapter 3* and *Chapter 4* using the trauma film paradigm (Holmes and Bourne, 2008). Associative conditioned memories are deemed fully consolidate ‘old’ memories after 24 hours (as in *Chapter 2*), but as results in the preventative studies show (*Chapters 3 and Chapter 4*) memory consolidation/retrieval effects can be ongoing up to 7 days later (Miller et al., 2015; Wolf, 2017).

Similarly, experimental models of PTSD elicit adaptive aversive memories (the type that, for most people, fade naturally over time). However, it is not adaptive aversive memories but rather maladaptive that are often resistant to extinction. Indeed, a diagnosis of PTSD requires intrusive memories to have been occurring for longer than one month. This is a challenge for both associative fear conditioning and also the more purported ‘naturalistic’ studies as in trauma-film paradigm (*Chapter 1.3*). As discussed, following the analogue trauma in *Chapter 3*, salivary cortisol levels rose fairly modestly in response to the analogue trauma (Mean nmol/L = 16.65) and may not reflect the response to real life trauma (e.g., major surgery levels can rise as high as 1452 nmol/L (Prete et al., 2018). However, an additional challenge in modelling trauma and phobia is that experimentally generated healthy responses to trauma even if ecologically sound, may not reflect stress responses of those most likely to develop PTSD i.e. those who have experienced trauma previously or have pre-existing mental health disorders (Kessler et al., 2017). For clear ethical reasons those vulnerable to harm from experimental trauma are excluded from such studies (Stirling et al., 2023), as they were here. However, experimental models of aversive learning do need to engage with the mechanisms involved in PTSD to be valid. Clinical PTSD patients are known to have dysregulated stress responses compared to healthy and non-PTSD trauma controls (Yehuda, 2009) with differences in stress

responding exist between PTSD patients depending on multiple factors e.g. trauma type (Kessler et al., 2017).

Dosage of novel pharmacological interventions; A limitation shared by all studies is that the required dosages of the pharmacological agents used has not been previously identified. The dosage administered (*Chapter 2*) were based on limited previous research in humans for sodium valproate (VPA) as an HDACi (Kuriyama et al., 2011). The authors noted that 400mg single dose based on the tolerability and safety profile at this intake rather than knowledge of any specific dose-dependent epigenetic requirements. A limitation here is that no plasma levels of VPA were measured so it is not possible to comment on any dose-dependent relationships between VPA and behavioural outcomes or interactions with sex effects. Similarly, hydrocortisone dosages are based on previous use in humans and not specifically the level of plasma concentration of cortisol that this will achieve (Schelling et al., 2001; Weis et al., 2006). The 400mg spironolactone and 30mg hydrocortisone are moderately high doses (Howlett, 1997) compared to chronic medical use and this was chosen to promote supra-physiological cortisol response, which was observed as expected to be more pronounced with hydrocortisone in *Chapter 3* than seen following mineralocorticoid blockade in *Chapter 4* with spironolactone.

5.5 Future directions for novel strategies for the prevention or treatment of aversive memory modification

Neuroimaging of neural network activity and ‘hotspots’ during trauma film;

Neuroimaging methods could be incorporated into future studies using the trauma film paradigm. Hotspots at encoding (e.g. while viewing the trauma film) have been shown to produce greater neural activity in the salience network (Box 1.1) during subsequent fMRI imaging (Menon, 2011). The diary entries, such as in *Chapters 3* and *4*, can show how ‘hotspots’ within distressing films develop into subsequent intrusive memories (Visser et al., 2022) and if they are associated with voluntary memory recollections (Lau-Zhu et al., 2021). Elucidating neural mechanisms underlying potential maladaptive aversive memories in this way may help explain how two people who experience the same trauma can either develop adaptive or maladaptive memories and pinpoint more accurately how and why moderation by novel pharmacological and or behavioural strategies can succeed or fail to inhibit expression of aversive memories.

Future direction of Spironolactone as probe of inhibitory control during initial encoding and early consolidation of emotional memory. MR involvement in a stress induced shift in cognitive processing away from PFC executive control networks towards amygdala sensory-perceptual, salient network processing (Henckens et al., 2011; Hermans et al., 2014b) is tentatively supported by results in *Chapter 4* and with its potential for therapeutic value of down-regulation of ‘nowness’ of aversive memories, future experimental trials are warranted. Future studies using similar MR blockade would benefit from the addition of EEG and inhibitory cognitive tasks such a Think-No think (Anderson, 2003) to model the mechanisms underlying memory suppression and retrieval

control. Additionally, administering spironolactone at different time points relative to a trauma-like event might clarify better the temporal dynamics of MR activity and corresponding neural network activity and corresponding aversive memory performance.

Maladaptive vs adaptive aversive memory; In *Chapter 3* and *Chapter 4*, there was a wide variation in response to trauma with some participants not recording an intrusion across the study (N=10(8%) in *Chapter 3*, N=6 (8%) in *Chapter 4*) whereas others had greater than double the average number of intrusions across the week (N = 13(11%) in *Chapter 3*; N=13 (15%) in *Chapter 4*). Stratifying participants according to either minimal or extreme responding to a pre-stress test might capture minimal vs extreme human responders similar to a protocol employed in animal models (Cohen and Zohar, 2004; Kozlovsky et al., 2009). Such a design might better identify markers of those vulnerable to developing a stress-related disorder within necessary ethical constraints (Franke et al., 2022; Ney et al., 2022). In addition, it may better capture outcomes of novel strategies in those whose response to analogue trauma more closely resembles maladaptive aversive memory development (Kozlovsky, 2009, Cohen 2004, 2006).

Time to treat: The results in *Chapter 3*, and previous similar experimental studies (Das et al., 2016; Kamboj et al., 2020) that administer psychopharmacological interventions immediately following a trauma-like event, suggests that mechanistically there is viability in delivering a pharmacological preventative strategy within the theoretical constraints of the consolidation ‘window’ of >6hrs. Previous small scale clinical trials have reported a preventative effect against aversive memory development event if given outside of this time window (Astill Wright et al., 2019) and it may be that the first night’s sleep is a determining factor (see below). Future studies are needed to empirically test this. Carmi et al. (2023) suggests that the role of diurnal variation in endogenous cortisol levels (high cortisol in the morning, low at night) is also implicated

in response to preventative treatment with hydrocortisone. The moderating effect baseline gonadal hormones x cortisol levels play in the exploratory results from the *Chapter 3 and 4* are consistent with this. Future studies might sample and/or pharmacologically manipulate baseline steroid hormone (and catecholamine levels) at various time points before and after a trauma-like event to understand the temporal interaction between them, at what levels, and in what ratio to each other (Ali and Pruessner, 2012) on subsequent aversive memory. Understanding how and when an individual's baseline neuroendocrine status works synergistically with timely exogenous administration of high dose glucocorticoids post (analogue) trauma is important to ensure attenuation (rather than upregulation) of subsequent decontextualization and re-experiencing of aversive memory to increase resilience to development of PTSD.

Delayed HC effects on intrusive memory, the role of sleep: In *Chapter 3*, time dependent (delayed) effects of hydrocortisone post a trauma-like event (Kamboj et al., 2020) and similar studies (Das et al., 2016) manifest over the following days. As discussed in *Chapter 3*, the time delayed effects of post trauma GR administration on memory reactivations (i.e. retrieval), could possibly occur during or be influenced by slow wave sleep (Antony et al., 2017; Antony et al., 2019), with REM sleep, both before and after trauma, shown to influence subsequent intrusive memory development (Porcheret et al., 2020; Alkalame, 2022) although also see (Cohen et al., 2012). In *Chapter 4*, MR blockade altered the affective quality of aversive memories over the same 7 day period. Additionally, sodium valproate (*Chapter 2*) has been found to facilitate sleep-dependent learning of both extinction and habituation of conditioned fear in humans (Kuriyama et al., 2013). Sleep scores were not found to be predictive of outcomes in the studies of this thesis, the impact of sleep in both preventative studies here was assessed via self-report (Buysse et al., 1989) not by direct EEG or neural imaging measurement and no sleep

measures were recorded in the reconsolidation study something future studies might explore as the role of sleep in the moderation of aversive memory with glucocorticoids might be particularly relevant for first responders who may have irregular sleep patterns, a potential risk factor for PTSD development (Alkalame, 2022).

Histone deacetylase inhibitors to enhance extinction of aversive memories: The mixed results reported here suggests considerably more pre-clinical work is needed to further assess the role of epigenetic modifications in response to stress to treat remote, established fear memories and to better identify protocols that reliably attenuate rather than enhance fear responses. Future studies need to identify precise timing of HDAC inhibitor administration e.g. possibly administered after successful fear reduction or after rather than before retrieval. However, the accelerated extinction/re-extinction of explicit responding reported in *Chapter 2* produced by sodium valproate (VPA) alone is of interest as dropout rates from exposure-based therapies can be associated with patients being unable to tolerate or unwilling to engage in prolonged or repeated exposure to the aversive memory (Kessler et al., 2022).

5.6 The challenges and limitations of addressing differences in response to moderation of aversive memories between men and women; the role of gonadal hormones

Despite the measures taken by the NIH (1993,2014) to move towards better understanding of the role of sex as a biological variable (SABV) has on psychopathology (Beltz et al 2019), understanding the role of sex and gonadal hormones in PTSD, particularly as moderators of response to treatments for the condition still remains a challenge for experimental research (Lonsdorf and Merz, 2017; Shansky, 2019; Shansky and Murphy, 2021). Tentative steps are taken in *Chapter 2, Chapter 3 and Chapter 4* to explore sex and gonadal hormone as moderators of the novel strategies studied in this thesis.

Considering sex as opposed to gender: Biological sex has been the focus of the exploratory analysis in this thesis. However, it is recognised that men and women are likely to respond differently to trauma, both experimentally driven and in real life, due to socio-cultural influences that affect cognitive appraisal, and emotional and behavioural responses to trauma (Kornfield et al., 2018). Post trauma coping styles contribute to response to trauma and later PTSD development (Hiscox et al., 2023). Women's reliance on emotional coping styles are associated with increased later PTSD severity than the more typical men's problem-focused coping style. Coupled with differences in mental health outcomes depending on trauma type and exposure, these factors could be characterized as gender rather than sex differences as they sit within an individual's socio-cultural and historical context and do contribute to differences between men and women for PTSD risk. However, these factors do not account for a substantial amount of the

difference observed in response to PTSD treatment (Kornfield, 2018, Hiscox, 2023) and no behavioural x sex x treatment effects were reported in the studies here. It is therefore useful to consider sex as a biological variable in explaining differences in response to potential new interventions.

There are inconsistencies between results from both the animal and human experimental model and how men and women exhibit PTSD-like symptoms and response to treatments in clinical settings (Garcia et al., 2018; Hiscox et al., 2023) and this will need to be addressed in future research. To do this effectively will require changing attitudes as well as the protocols and methods that have been relied on for decades; this is a major challenge, particularly for pre-clinical research (Shansky and Murphy, 2021) but one that has consequences for human models built on this research. Gonadal hormone variations are not just a ‘female problem’ (Shansky, 2019) as demonstrated in both preventative studies here with influence of hormones in men on subsequent memory formation not just via testosterone levels and mineralocorticoid receptor activity (*Chapter 4*) but also in the hormones more often considered ‘female’ hormones, that is, progesterone and estradiol (Taxier et al., 2020) and seen in their moderation of intrusive memory in response to post trauma exogenous hydrocortisone (*Chapter 3*).

As seen in *Chapter 3*, although average baseline estradiol and progesterone levels were not significantly different between men and women taking a hormone contraceptive, however individual variations in these hormones were found to moderate the effects of hydrocortisone given after a trauma-like event on intrusion frequency in a sex dependent manner. Therefore, ideally future studies would consider measuring gonadal hormone levels in both sexes and include sex hormone levels, as well as sex, as potential moderators of aversive memory and response to treatment.

Studies exploring sex differences (Garcia et al., 2018) in intrusive memory moderation in humans regularly considers sex as a dichotomise factor (an acknowledged limitation of study in *Chapter 2*). In most cases when ‘hormones’ are considered they reflect either self-reported menstrual phase as a proxy for ‘high’ vs ‘low’ progesterone (Garcia et al., 2018) or when estradiol hormone levels are measured, contrary to much of the animal literature, it is mostly in women (Hsu et al., 2021, although see Andreano and Cahill, 2006; Cheung et al., 2013) possibly making comparisons between the two models problematic (Shansky and Murphy, 2021).

Analysis of sex differences: There is a concern when both men and women are included in studies but analysis is done only at the group level that this might not generate meaningful information about sub-groups (i.e. men and women) (Lonsdorf and Merz, 2017). The ‘one size fits all ‘ approach can lead to Type II errors if a sub-group responds well to an intervention but another responds in the opposite direction (Cahill, 2012). In our study we saw both behavioural (e.g. PRE) effects potentially masked by the lack of effect in women (*Chapter 2*). Additionally, in both preventative studies, drug group analysis alone would have missed the higher attenuation of intrusive men by hydrocortisone on men who have higher estradiol levels and of spironolactone administration prior to trauma-like event to moderate intrusive frequency depending on testosterone levels. Just as importantly it would not have identified that low estradiol in women and high progesterone in men are potential contra-indications for the use of hydrocortisone to moderate aversive memory given after trauma.

Limitations: The sex/gonadal-hormone differences observed following the preventative strategies were from exploratory analysis and not pre-registered. Additionally, analysis in moderating consolidated memories (*Chapter 2*) was under powered for within-sex analysis and non-randomised for sex which has been suggested to

result in misleading results and as such needs to be interpreted with caution. Detailed proposals for *a priori design*, outcome measures and analysis plans to take account of potential sex differences have been proposed elsewhere (Lonsdorf and Merz, 2017; Elsey et al., 2018; Beltz et al., 2019; Garcia-Sifuentes and Maney, 2021) and will not be detailed here but while a challenge, if adopted, in both animal and human research, it provides an opportunity for future fear memory research that should assist in untangling the importance of the complicated relationship between sex, gonadal hormones and modification of maladaptive aversive memory.

5.7 Future directions for HPA/HPG research

In Chapter 3, the preventative potential of glucocorticoid moderation to ameliorate sensory-perceptual memory symptomology was associated with baseline gonadal hormone levels in both women taking hormone contraceptives and men (Hennessy et al., 2022). In light of this, and comments above, not exploring the influence of MR antagonism on sensory-perceptual memory in *Chapter 4* in women taking hormone contraceptives (as the pre-registered <https://osf.io/698q7/>), is a limitation that needs urgent addressing in a replication of the study.

Use of hormone-based contraception in Chapter 3 and Chapter 4; Hormone-based contraceptive use has substantial effect on fear memory extinction learning (Spalek et al., 2019; Graham, 2022) and there is a high prevalence of use, approx. 26% of reproductive aged women (Graham, 2022), and attempts to attenuate sensory-perceptual hyper consolidated memory of aversive event is moderated by contraceptive status with differential outcomes between women on hormone contraceptives compared to free cycling women both clinically (Raeder et al., 2019) and in experimental studies (Hsu et al., 2021). This suggests the need to extend the research detailed in *Chapter 3* and *4* to both free cycling women as well as those taking hormone contraception.

The results here suggest considering sex hormone status in both sexes in future studies is important not just to assess treatment effectiveness but also to assess potential risks within the drug groups. This is consistent with pharmacological treatments for other mental health disorders that are influenced by hormone levels e.g. estrogen influence on response to antidepressants (LeGates et al., 2019). Indeed, future studies may wish to explore co-administering estradiol antagonist in conjunction with hydrocortisone in men to moderate sex hormone effects on hydrocortisone efficacy to reduce intrusive memory

frequency (*Chapter 3*). Future studies are warranted to further explore these findings especially as hydrocortisone is a regularly used medication in emergency post-trauma clinical settings and may be influencing subsequent mental health recovery post-trauma depending on acute gonadal hormone status.

5.8 Ethical considerations for using strategies to modify aversive memories in humans

There are ethical considerations involved with using both pharmacological and drug treatment of mental (or indeed physical) health disorders. It is a challenge to balance the risks associated with the intervention involved against the risk of developing or living with a difficult to treat long term health condition (Kessler et al., 2022). It is the case that only a proportion of those who experience a trauma develop long term PTSD symptoms (Kessler et al., 2017). Therefore, a case needs to be made for interfering in the health of people who, despite having experienced trauma, might not go on to experience harm. In the case of pre-trauma preventative potential interventions, no trauma would have yet been experience (e.g., prior to major surgery or for first responders to high risk events). The proposed pharmacological preventative pharmacological measures are not dissimilar in purpose to other preventative measures in use for physical health conditions, such as vaccines for infectious diseases. As with existing primary and secondary preventative measures, there must be enough information available for health professionals and patients to make informed decisions. This is a compelling case for conducting robust clinical trials building on reliable and valid experimental models so that advice and decisions are based on empirically sound evidence for risk factors for developing long term PTSD symptomology, for efficacy of the drug and for individual risks vs benefits. As described, future research should aim to develop reliable biomarkers predictive of who would, or would not, benefit from a novel pharmacological strategy to attenuate aversive memories prior to development of PTSD and develop relevant screening protocols e.g. rapid neurosteroid hormone profiling or using self- reported menstrual phase. Although, as with any intervention it is unlikely to be possible to eliminate all risk.

Post-trauma behavioural interventions have been found to be effective, for example, visual interference (Holmes et al., 2009; Astill Wright et al., 2021), and it raises the question, would a behavioural intervention perhaps be more ethical and pose fewer risks than a pharmacological one? Evidence for early debriefing in the immediate aftermath of a trauma has not been shown to have significantly positive effects to reduce subsequent PTSD (Rose et al., 2002; Stileman and Jones, 2023). Some trauma victims may not be in a position to be able or wish to undertake a behavioural task (playing the game Tetris™ or similar visual task) and current behavioural treatments for longer-held aversive memories such as prolonged exposure or cognitive processing therapy, especially trauma focused, are prone to high dropout rates with 17% in one study citing requirement to engage in ‘imaginal exposure’ as the reason (Najavits, 2015; Fu, 2023). Ethical questions therefore need to be carefully considered with the use of either behavioural or pharmacological aversive memory modification strategies reported in this thesis.

In both preventative and treatment studies here, the drugs used are drugs that are widely prescribed (van Staa et al., 2000; Vizzardi et al., 2014) and have good safety records (NICE). No participant in our studies withdrew due to side effects of the drugs and participants were no better than chance at guessing whether they received drug or placebo. The drugs administered were relatively cheap and did not require special training to deliver. The reconsolidation behavioural protocol is quick and would not need more than one (or possibly two) sessions to be effective. These considerations mean that the novel strategies described could be universally available removing the ethical dilemma of only being available to the wealthy or adding a financial/ resource burden to under resourced healthcare services particularly important in regions of conflict where PTSD rates are highest (Watson, 2019a).

However, more generally, it is worth considering the implications of memory modification by either means. The promise of ‘erasing’ maladaptive memories (Maren, 2011) would to some suggest the possibility of science fiction type effects that could philosophically alter an individual’s sense of identity or more pragmatically prevent a victim of crime or conflict be able to testify to their experience. However, results reported here emphasise the intervention effects on aversive memory, is dissociable and that effects of both pharmacological and reconsolidation behavioural interventions differentiate between explicit (e.g. expectancy ratings) implicit (e.g. physiological arousal levels) and autobiographical memories (voluntary recall of what occurred). As importantly, and possibly more clinically relevant, is that the emotional, sensory perceptual elements can be selectively targeted, the distress, the ‘nowness’, as with spironolactone, the anxiety of anticipated harm, e.g. phobia with sodium valproate. However, targeting of the intervention needs to be accurate. Reactivation of a memory with or without a pharmacological adjunct aims to update the original memory. This raises valid ethical concerns because there is the potential, as we have seen here (*Chapter 2*) and elsewhere in both fear and appetitive updating (Lee et al., 2009) for the intervention to update the memory counter to the desired direction. In a clinical setting this would present the potential for harm. As such, while new pharmacological and behavioural intervention strategies to both prevent and treat the type of extinction resistant memories associated with the distress of PTSD (Silva and Gräff, 2023) more pre-clinical research is needed to ensure appropriate, evidence-based and safe protocols for their beneficial use in patients.

5.9 Concluding remarks

In summary, the studies in this thesis show controlled mechanistic manipulation by novel preventative and treatment strategies to moderate aversive memory under certain conditions. The results observed in this pre-clinical, experimental research can guide future studies and large-scale clinical trials. Such future research will need to further clarify the conditions under which such strategies do and do not work in order that precision empirically based psychiatric medicine that moderate aversive memories towards better contextualisation and reduced re-experiencing and deliver them safely and efficiently in both sexes. In summary, this thesis provides the following contributions to research in this field:

1. Sodium Valproate, an HDACi, may have use as a clinical enhancer of exposure-based clinical therapy for declarative maladaptive aversive memory but was not found to be a useful adjunct of reactivation prior to extinction behavioural manipulation.
2. Replication and extension of previous research confirmed that administration of high dose hydrocortisone immediately after a trauma-like event can significantly accelerate the forgetting of intrusive, spontaneous memories in men and women suggesting such treatment may be an effective preventative clinical strategy for disrupting maladaptive aversive memories and warrants large-scale clinical trials.
3. Use of a mineralo-corticoid antagonist (spironolactone) prior to a trauma-like event can subsequently reduce the distress and vividness associated with sensory-perceptual aversive memories and is a useful mechanistic probe of MR/GR influence on subsequent aversive memory consolidation.

4. Exploratory analysis found that both pharmacological and behavioural manipulation of aversive memory to be sex and/or gonadal hormone dependent. Including sex as a biological variable in future studies of sensory-perceptual maladaptive aversive memory modification appears warranted.

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APPENDIX INFORMATION

Appendix item 2.1 Participant criteria Chapter 2

Inclusion criteria

- (i) no current or pre-existing medical conditions,
- (ii) no contraindications to use of a single 400 mg dose of Sodium Valproate (i.e. pregnancy or breast feeding, psychosis, migraines or seizure disorders, a mood disorder or psychosis, cardiovascular or respiratory disease, kidney or liver disorders or known allergy to sodium valproate)
- (iii) fluency in English,
- (iv) normal or corrected to normal colour vision and
- (v) normal body mass index.

Exclusion criterion

- i) blood pressure was <90/60 and heart rate < 60bpm on testing day prior to drug administration.

The study was approved by the UCL Research Ethics Committee (Project ID Number): 3901/002 and was conducted in accordance with the World Health Association Declaration of Helsinki.

Appendix item 3.1 for Chapter 3

1. Criteria for Participation Chapter 3

The following criteria needed to be met for a participant to be included in the study:

Inclusion criteria

1. Healthy women and men
2. Women participants must be taking oral hormone-based contraception (for >1 month prior to participation)
3. Age between 18 and 35 years
4. Fluency in English
5. Normal or corrected to normal colour vision and hearing
6. Able to swallow capsules with water
7. Able and willing to complete daily diaries on the first three days and on at least five out of the seven days

In addition, potential participants were excluded from participating if they meet any of the following criteria:

Exclusion criteria

1. Pregnancy or breastfeeding
2. Historical or current diagnosis of a psychiatric disorder that was/is sufficiently severe as to require treatment
3. Use (including non-prescribed) of hydrocortisone or other steroid hormone, /glucocorticoid or noradrenergic-modulating medications.
4. Beck Depression Inventory-II (BDI-II; Beck et al, 1996) score > 20 or reporting significant suicidal ideation
5. History of blood/injury/ phobia
6. Experience (including witnessing) of an event (especially a violent event) that is judged at screening to be sufficiently stressful as to have led to risk of developing post-traumatic stress disorder (PTSD)
7. Likely to be highly distressed by film scenes of a graphic nature involving interpersonal (including sexual) violence
8. History of fainting

9. Have glaucoma or ocular herpes simplex
10. History of steroid myopathy
11. Self-reported memory problems
12. Have a diagnosis of asthma requiring steroidal treatment
13. Have a diagnosis of diabetes
14. Have breathing problems e.g. Chronic Obstructive Pulmonary Disease (COPD)
15. Have a cardiac pacemaker implant
16. Have a cardiovascular condition or hypertension
17. Have a history of epilepsy or neurosurgery or tuberculosis
18. Have impaired liver or kidney function or hypothyroidism
19. Have a history of severe anaphylactic reaction
20. Have a known hypersensitivity to hydrocortisone
21. Have diagnosis of osteoporosis or peptic ulcer
22. Have untreated infections
23. Currently taking cardiovascular or psychiatric medication
24. Regular (>twice a month) illicit recreational drug use
25. Regularly consuming over 14 units of alcohol per week
26. Daily smoker
27. Lactose intolerance

Appendix 3.2 Verbal instructions given to participants in Chapters 3 and 4

Film instructions for the recording of intrusive, spontaneous thoughts about the film

“We are now going to ask you to watch the film. The film is a total of approximately 15 min long and consists of two separate scenes. We are going to ask you to watch them with your head resting in the chin rest and we will calibrate your eyes again just before we start (if they asked why, we explained that it made sure everyone saw the film in the same way)

As we mentioned on the telephone this is a challenging watch for some people and there may be times you want to look away but we ask that you do your best to keep your eyes focused on the screen at all times. We won't sit here watching you but will be sitting back here doing some work so please let us know when the film has finished. However, if you find yourself getting too distressed and want to stop and end the experiment let us know and we will of course switch off the film.

Immediately, at the end of the film we will ask you to take two tablets which will be either hydrocortisone (cortisol) or placebo with a glass of water, we don't know which they will be so I'm sorry we can't tell you. We will then ask you to give us another saliva sample.

Do you have any questions about either the film or taking the tablets?

If you are ready to start, we will close the curtains and set up the eye tracker.

Just before they press the key to begin ask them

We would like you to blink 5 times like you did before

At the end of the film say

Thank you very much for watching that”.

Recording memories

Intrusive memories in lab on Day 1

After viewing the film and reading a description of the nature of intrusive memories (see (Kamboj et al., 2020); also osf.io/76yvk/), participants recorded momentary intrusions for 60 min on an E4 device (Empatica, Boston, MA, USA), starting after the 1 hr drug absorption period (i.e. t_{+60} to t_{+120}) during a second filler (music) task period (see Chapter 2, Figure 2). Thus, while listening to a standard set of classical music pieces and periodically rating these for pleasantness, they were instructed to press the response button on the E4 once for every occurrence of an involuntary memory relating to the film. This acute period of memory monitoring was intended to capture post-encoding memory retrieval which may contribute to early consolidation differently in the two drug groups. Any early difference in groups would be expected to reflect non-genomic effects of hydrocortisone/cortisol (Joëls, 2008).

Instructions for Music and acute intrusion task

“Now you’ll be listening to some music clips and at the end of each track rating them for how much you like them using this pleasantness scale. Between the music clips there will be silence. Other than rating the pleasantness of the music, you don’t have to do anything special - you can just relax, but we’d like you to notice if any involuntary thoughts or images about the video occur while you’re just sitting and resting or when listening to the music. What we mean by involuntary thoughts and images is that they occur without you intending them to occur; in other words, they happen spontaneously. If you do have any spontaneous thoughts or images about the video, *briefly* press the button on the watch once for every thought or image you have. If you don’t have any thoughts about the video, please don’t press the button. Otherwise press the button as many times as you have an involuntary thought about the video, regardless of whether you have the same thought or image repeatedly or if you have a number of different thoughts or images. Does that make sense?”

Intrusive memory recording over the following week (Day 1 -7)

Before leaving the laboratory, participants were given detailed instructions on how to record intrusive memories over the following week (see below and (Das et al., 2016; Kamboj et al., 2020)). The definition of intrusive memories provided to participants emphasized their involuntary nature and distinguished between sensory and verbal mental events. Based on the descriptions provided in the memory diaries, all recorded intrusions conformed to the definition of sensory-perceptual memories.

Starting at the end of day 1, and on each subsequent day at 9 p.m., participants received an email reminder to complete the memory diary as close to bedtime as possible. On day 1, the interval between the end of the film and completion of the memory diary (which is assumed to reflect the initial, pre-sleep rehearsal-consolidation period) was similar in the placebo (8.8 ± 2.7 hr) and hydrocortisone groups (9.3 ± 3.4 hr; $t(117)=0.86$, $p=0.393$). For the diary entries, participants were instructed to record a brief description of each thematically distinct intrusive memory (allowing subsequent verification of film-relatedness of intrusions) and the number of times they occurred. Additionally, participants recorded distress and vividness of each distinct intrusive memory (1 = ‘not at all’; 5 = ‘extremely’).

Instructions for completion of the daily intrusion diary, days 1 – 7

At the end of the testing session we explained how to record intrusive memories via online platform Qualtrics Qualtrics (Provo, UT) as follows:

“The aim of our research is to look at spontaneous, involuntary memories because they seem to be important in many psychological disorders, especially Post-Traumatic Stress Disorder.

By ‘spontaneous’, we mean memories of the film that suddenly pop into your mind automatically. We do not mean times when you deliberately think about it or mull it over. The spontaneous memories may pop into your mind when you are doing or thinking about something completely unrelated. Or you may be reminded of the film by things that happen in your environment. The main thing is that you didn't mean to think about the film, but recall something about it, 'out of the blue.'

Spontaneous memories may take the form of 'mental pictures' of the film you saw. They can be visual, but they can also be in any other sensory modality, so you might have memories of the sounds you heard. They can also take a verbal form – that is, words or sentences, statements or questions. Or they could be a mixture of sensory and verbal forms. The key thing is that they should be related to the film and they should occur out of the blue, without you trying to think about them.

Holding this in mind, have you experienced any intrusive memories of the film today? Yes/No

The purpose of the diary is to record any unwanted involuntary memories you may have had during the day. This is a very important part of the research and we appreciate your time in filling the diary out. In column 1, record anything you recalled involuntarily about the film. Record different involuntary memories on different rows. Write a brief description of what came to your mind, but be as specific as you can. You may have had exactly the same memory about the same thing several times in the day, in which case, please record the number of times you had the memory in column 2 in the same row as your description of that memory. If you recalled a memory only once, please also indicate this (i.e. by putting '1' in the box). In column 3 and column 4, indicate respectively how distressing and how vivid the memory was by selecting the most appropriate number on a scale of 1-5 (1=not at all, 5=extremely). If the same memory was experienced more than once, indicate the maximum level of distress the memory caused. If you had no intrusions, please put 0 in columns 1 and 2 for each row. If you had fewer intrusions than the amount listed, please put 0 in columns 1 and 2 for any row which does not apply.”

Voluntary (recall) memory on Day 8

Voluntary memory was assessed on one occasion only (day 8). For free recall, participants were asked to recall “as much information and detail as possible including information about where things happen, when they happen, who they happen to, what the people and scenes look like, etc.,” typing this directly into a text box with no time limit. Performance was determined by counting the number of recalled ‘idea units.’ After the free recall task, participants provided open text responses to 19 questions about events in the two clips (cued recall). These questions related to contextual aspects of the narrative (i.e., where events take place; what happens to the actors; who does what to whom, and when).

There was no time limit for either recall task. Every participant's free and cued recall task performance was independently scored either 0 (inaccurate), 0.5 (partially accurate), or 1 (accurate) by two treatment-blind researchers. High interrater agreement was achieved (>90%) in scoring both tasks with disagreements resolved through discussion. Positive and negative affect (PANAS, (Watson et al., 1988)) ratings were taken before and after the memory recall tasks.

Instructions for Voluntary memory assessment

Free recall, participants were asked to recall "as much information and detail as possible including information about where things happen, when they happen, who they happen to, what the people and scenes look like, etc.," typing this directly into a text box with no time limit. Performance was determined by counting the number of recalled 'idea units'. Performance was assessed based on percentage correct of total possible units as determined and previously agreed by three independent experimenters. For

Cued recall task; participants provided responses to 19 questions about events in the two clips. Free and cued recall were independently scored either 0 (inaccurate), 0.5 (partially accurate), or 1 (highly accurate) by two treatment-blind researchers. High interrater agreement was achieved (>90%) across recall metrics, with disagreements resolved through discussion.

Protocol and instructions given for Saliva Collection by Passive Drool

At the start of the testing session, it was confirmed that the participant had not had anything to eat, drink or smoke for two hours prior to taking the sample and not to have undertaken any excessive exercise that morning. We also confirmed that they were not working shifts or jet lagged. The timing of saliva collection was the same as for the state measures, namely at T1 (pre-film), T2 (immediately post-film, as a measure of peri trauma effects) and T3 (post-drug), except for an additional sample at 15 min post film. Participants were asked to gently wash their mouth out with 10 ml of distilled water. At each collection point participants were asked to swallow all the saliva in their mouth and then to sit in a relaxed position with their head tilted slightly forward. They were asked to allow saliva to collect inside their mouth without swallowing while the saliva collected in their mouth and asked to refrain from making active facial or jaw movements for this time. Once some saliva had collected, they were asked to allow the saliva to gently dribble into cryovials via a small funnel. Samples were frozen immediately and stored at 80°C

To encourage saliva production verbal imagery was given e.g. 'image you are sucking on something bitter or sour such as a lemon'.

Between 0.5 – 1ml of saliva was collected.

Appendix item 3.3 Ethical approval UCL Ethics Project ID 5583/002

Chapter 3 Probing the role of the stress system in consolidation of involuntary and declarative emotional memory using a single dose of cortisol in healthy humans

Appendix item 3. 4 Information provided to participants in study for Chapter 3

Participant Information Sheet for Adults in Memory Consolidation Studies Using Hydrocortisone

YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET

Title of Project: Probing the role of the stress system in consolidation of involuntary and declarative emotional memory using a single dose of cortisol in healthy humans

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 5583/002

You are being invited to take part in a research study which is part of a graduate research degree at the Department of Clinical, Educational and Health Psychology at UCL. Before you decide to take part, it is important for you to understand why the research is being done and what participation will involve. Please take the time to read this information carefully and discuss it with others if you wish. Take time to decide whether, or not, you wish to take part and ask us if there is anything that is unclear or if you would like more information. You should only participate in this study if you want to; choosing not to take part will not disadvantage you in any way.

Details of Study:

What is the project's purpose?

Emotional events have a privileged status in our daily lives. However, intensely emotional events or chronic exposure to stressful experiences can create unwanted memories which are distressing. Therefore, it is important to learn about the brain mechanisms involved in the formation of unpleasant emotional memories. Some drugs might be helpful in this regard, particularly drugs that affect the 'stress response' – such as the steroid drug 'hydrocortisone' – which can affect the strength of memories when they are initially laid down. Participants in this study will therefore receive hydrocortisone or a placebo to see how this affects their subsequent memories of unpleasant events. By taking part in this study you will contribute to the scientific knowledge of the effect of this drug on memory consolidation, which may inform future treatments for psychological disorders such as posttraumatic stress disorder (PTSD).

Why have you been chosen to take part?

We are intending to invite 120 women and men aged between 18 and 35 to take part in this study. You will need to be fluent in English, in good physical health, with normal or corrected to normal colour vision. **Women must also be taking oral contraception in order to take part.** Unfortunately, you cannot take part if you have any of the following: a historical or current diagnosis of a mental health issue that required/requires treatment, a history of post-traumatic stress disorder (PTSD) or have experience of violence, such as witnessing a violent injury or death, that might have led to PTSD or phobia to sight of blood, have glaucoma, ocular herpes simplex, known memory problems, asthma, diabetes, breathing problems like Chronic Obstructive Pulmonary Disease (COPD), a cardiac pacemaker implant, other cardiovascular conditions, history of tuberculosis, peptic ulcer, osteoporosis, steroid myopathy, epilepsy or neurosurgery, impaired thyroid, liver or kidney function, or a history of peptic ulcer or severe anaphylactic reaction. This study involves receiving one active medication or placebo. As a result, you will also not be able to take part if you are hypersensitive to hydrocortisone, or if you are pregnant/likely to become pregnant during the study or breastfeeding, have untreated infection or currently taking cardiovascular or psychiatric medication, or taking regular (>twice a month) recreational drugs other than alcohol, nicotine and caffeine. Lastly, you should not be consuming alcohol excessively (i.e. not regularly drinking >14 units of alcohol per week), and not be a daily smoker.

Do I have to take part?

Your participation in the study is entirely voluntary and you are free to withdraw from the study at any time without giving a reason, even if you have previously given your written consent and without any loss to any benefits to which you are entitled. If you do agree to take part, you will be asked to sign a consent form and will be given this information sheet to keep. If you do withdraw from the study, you will be asked what you wish to happen to the data you have provided. When we collect and analyze your data it will be pseudo-anonymized after which all the data will be anonymized and at that stage, we will no longer be able to identify an individual's data. At this point we would no longer be able to remove your data.

What will I have to do?

If you agree to participate in this study, you should contact the experimenter by email and provide a telephone number and a convenient time to call. You will then receive a call from us, and we will ask

you some questions to check your eligibility for the study. Please note that we have strict criteria for participation, and based on your answers to these questions, you may not be eligible to take part in the study.

If you fulfill our study criteria, we will arrange for you to attend an appointment at UCL. Before this session we will ask you to complete some questionnaires online for which you will need access to a computer with internet connection. Your answers to these questions may mean that we need to contact you to inform you that you are ineligible to participate in this study. Otherwise, we will ask you to attend the appointment at UCL. During this session, which takes approximately 3 hours, you will be asked to complete some questionnaires about your past, current mood and usual cognitive and emotional state. We will ask you to complete some computer tasks, and we will collect some samples of your saliva to measure hormone levels. You will be asked to place some sticky probes on your body to measure heart rate and skin conductance as well as take regular blood pressure readings. We will also be measuring 'brainwaves' via an EEG headset. These are completely safe and do not involve any discomfort. Following this, you will watch a short film (~15 minutes). We will be monitoring your physiological and biological response before, during and after the film. **You should be aware that the film contains highly graphic scenes of sexual assault, interpersonal violence, injury and death which are designed to be distressing.** *Please do not take part if you are likely to become very distressed by such scenes.* You will then be given some capsules (containing hydrocortisone or placebo) to swallow with water and remain in the Department for about 2 hours so we can monitor your bodily reactions. While you wait, you will listen to some music and before leaving you will complete a final computer task.

Following the session at UCL you will be required to fill in a simple online diary of any spontaneous thoughts/memories about the film for the next 7 days. You will be reminded to do this by daily email. **The daily information provided between sessions is crucial for our experiment and to the collection of good data. Drawing conclusions from research with missing or insufficient data can result in the potential for harmful outcomes.** If you are unable or unwilling to complete the brief daily diaries on the first three days and on at least five out of the seven days, we will not be able to ask you to complete the second online session. You may contact the researchers at any time during or after the study if you experience any difficulties with this requirement.

The final assessment takes place online one week later. You will first complete mood measures via questionnaires, and subsequently do two memory tasks which will take approximately 30 min. At the end of this online session, you will be debriefed and reimbursed £35 for your participation in the study via bank transfer through UCL finance. Please note, we cannot pay any addition travel expenses.

What are the possible disadvantages or risks of taking part?

Depending on which group you are randomly allocated to, you will receive hydrocortisone or placebo. Cortisol (hydrocortisone) is an important natural stress hormone in humans and is used to reduce inflammation in conditions, such as rheumatoid arthritis. Once you have received hydrocortisone or placebo, you will be monitored by a researcher in the Department of Clinical, Educational and Health Psychology at UCL. The placebo contains lactose powder.

Note that hydrocortisone can have side effects (e.g. fatigue, sleep difficulties, nausea, drowsiness/weakness, exacerbation of existing breathing problems). Therefore, there are strict criteria for inclusion in the study. You will be asked to remain in the department and be monitored until drug effects have completely abated. Your mood will also be carefully monitored using different measures.

You will watch scenes from a film as part of the study. You should be aware that the film contains graphic scenes of sexual assault, interpersonal violence, injury and death which are designed to be distressing. After the film, some people may have spontaneous thoughts and images from the film. These will be short-lasting. In previous similar research with hundreds of participants, no one

experienced longstanding intrusive thoughts or emotional problems in response to the film. Any clips you see are in the public domain. However, it is not possible to guarantee zero risk to you. You should therefore not take part if you have personally experienced psychological trauma, have concerns about your mental health, or think that you may be strongly psychologically affected by the film.

What are the possible benefits of taking part?

Whilst there are no immediate benefits for people taking part in the study, it is hoped that your participation will help add to the scientific knowledge of the effect of hydrocortisone formation of emotional memories. This may eventually help inform the future development of treatments for psychological disorders such as posttraumatic stress disorder (PTSD).

What if something were to go wrong?

If you have any complaints about your treatment by the researchers while taking part in this study you should contact the principal supervisor whose details are at the top of this document. However, should you feel your complaint has not been handled to your satisfaction after speaking to Dr Kamboj you can contact the Chair of the UCL Research Ethics Committee –

Will my taking part in this study be kept confidential?

All information which is collected about you during your participation in the research will be kept strictly confidential and will be securely stored electronically, using a numbered code so that you cannot be directly identified. Only researchers directly involved in the study will have access to the data. Any biological samples we collect from you will be anonymized before being analysed by a third party at another university in the UK. These samples will be destroyed once they are analysed. All data will be stored in accordance with the Data Protection Act 2018. The results of the research will be disseminated in peer-reviewed scientific journals, but you will in no way be identifiable from such publications. You may request feedback when the study is completed.

Limits to confidentiality

- Please note that assurances on confidentiality will be strictly adhered to unless evidence of wrongdoing or potential harm is uncovered. In such cases the University may be obliged to contact relevant statutory bodies/agencies.
- Please note that confidentiality will be maintained as far as it is possible, unless during our conversation I hear anything which makes me worried that someone might be in danger of harm, I might have to inform relevant agencies of this.
- Confidentiality will be respected subject to legal constraints and professional guidelines.
- Confidentiality will be respected unless there are compelling and legitimate reasons for this to be breached. If this was the case, we would inform you of any decisions that might limit your confidentiality.

Will I be recorded and how will the recorded media be used?

No, there will be no visual or audio recordings made of your participation in this study. Our research sometimes generates interest from the media which helps our sponsor, a registered charity, generate awareness of the needs of those who experience real life trauma. If you are interested in being asked for your feedback about taking part in this study for this purpose there will be an opportunity for

you to declare this on the consent form otherwise your participation in this study will be kept anonymous.

What will happen to the results of the research project?

The results from this study are likely to be published in a peer reviewed journal (such as the Journal of Psychopharmacology) some months after the end of collecting the data from participants. If you wish to be sent a copy of the published results you should consent to this on the consent form. It will not be possible to tell you which arm of the project you were involved in until the results are published and the data collected during the course of the project might be used anonymously for additional or subsequent research. Please be assured that you will not be able to be identified in any report or publication.

1. Local Data Protection Privacy Notice

Notice:

The controller for this project will be University College London (UCL). The UCL Data Protection Officer provides oversight of UCL activities involving the processing of personal data.

This 'local' privacy notice sets out the information that applies to this particular study. Further information on how UCL uses participant information can be found in our 'general' privacy notice

The information that is required to be provided to participants under data protection legislation (GDPR and DPA 2018) is provided across both the 'local' and 'general' privacy notices.

The categories of personal data used will be as follows:

Name, address, electronic contact details and special category personal data (such as health and biometric data)

The lawful basis that would be used to process your *personal data* will be performance of a task in the public interest

The lawful basis used to process *special category personal data* will be for scientific and historical research or statistical purposes.

Your personal data will be processed so long as it is required for the research project. If we are able to anonymise or pseudonymise the personal data you provide we will undertake this, and will endeavour to minimise the processing of personal data wherever possible.

If you are concerned about how your personal data is being processed, or if you would like to contact us about your rights, please contact UCL in the first instance.

15. Who is organising and funding the research?

The research is organised by the Clinical, Education and Health Psychology Department, UCL and funded by The Bobby Charlton Foundation's charity, Find A Better Way, UK Registered Charity No. 1140911

Contact for further information

Thank you for reading this information sheet and for considering to take part in this research study. Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

Note – You will be given a copy of this information sheet and consent form to keep. If you have any further questions regarding this study please do not hesitate to contact any of the researchers.

This study has been approved by the UCL ethics committee

Study Registration Details:

All data will be collected and stored in accordance with the Data Protection Act 2018. UCL data protection registration number for this research is Z6364106/2016/10/28

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 5583/002

It is up to you to decide whether, or not, to take part. If you choose not to participate, it will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

Thank you for reading this information sheet and for considering take part in this research.

Appendix item 3.5 supplementary results

Intrusive memories across days

Intrusion counts

The summary regression Table from the ZIP model equation below is outlined in Table S1.

$$\text{Intrusions} \sim \text{Drug} + \text{Day} + \text{Drug} : \text{Day} + (1 | fID)$$

Table S1: regression coefficient estimates from the ZIP count model run using the glmmTBM command in R.

	Estimate	SE	Z value	P(> z)
(Intercept)	1.039243	0.180256	5.765	$8.15 \cdot e^{-09}$
Placebo	0.003923	0.231487	0.017	0.98648
Day 2	-0.806622	0.129493	-6.229	$4.69 \cdot e^{-10}$
Day 3	-1.5388	0.195329	-7.878	$3.33 \cdot e^{-15}$
Day 4	-1.95509	0.216973	-9.011	$<2.00 \cdot e^{-16}$
Day 5	-2.76308	0.322534	-8.567	$<2.00 \cdot e^{-16}$
Day 6	-3.20159	0.379329	-8.44	$<2.00 \cdot e^{-16}$
Day 7	-3.31531	0.404417	-8.198	$2.45 \cdot e^{-16}$
Placebo: Day2	0.426168	0.180698	2.358	0.01835
Placebo: Day3	0.585453	0.248312	2.358	0.01839
Placebo: Day4	0.754535	0.294237	2.564	0.01034
Placebo: Day5	0.904374	0.400667	2.257	0.024
Placebo: Day6	1.484992	0.442473	3.356	0.00079
Placebo: Day7	1.252689	0.485635	2.579	0.00989

Estimated marginal means for placebo and hydrocortisone are displayed in Table S2.

Table S2: EMMs for placebo and hydrocortisone Note the EMMs are in log rather than response units.

<i>Placebo</i>				
Day	Est marginal mean	SE	95% CI (lower)	95% CI (upper)
1	1.0432	0.165	0.72	1.3665
2	0.6627	0.176	0.317	1.0083
3	0.0898	0.2	-0.302	0.4816
4	-0.1574	0.238	-0.624	0.3089
5	-0.8155	0.274	-1.354	-0.2768
6	-0.6734	0.265	-1.193	-0.1537
7	-1.0195	0.303	-1.615	-0.4242

<i>Hydrocortisone</i>				
Day	Est marginal mean	SE	95% CI (lower)	95% CI (upper)
1	1.0392	0.18	0.685	1.3931
2	0.2326	0.194	-0.148	0.6133
3	-0.4996	0.241	-0.972	-0.0267
4	-0.9159	0.259	-1.424	-0.4075
5	-1.7238	0.353	-2.418	-1.0301
6	-2.1623	0.405	-2.958	-1.3665
7	-2.2761	0.43	-3.119	-1.4327

Response units can be recovered from the logarithmic values using the equation:

$$\text{Intrusion count}_{i,j} = \text{Exp}(\text{EMM}) \cdot (1-\pi)$$

Where π = a constant representing the probability of a false zero (here π =0.2845).

As such Day 1 count values in the placebo and hydrocortisone groups were:

$$\text{Intrusions count}_{\text{Day1,Placebo}} = \text{Exp}(1.0432) \cdot (1-0.2845)=2.0308$$

$$\text{Intrusions count}_{\text{Day1,Hydrocortisone}} = \text{Exp}(1.0392) \cdot (1-0.2845)=2.02226$$

Day 2 counts were

$$\text{Intrusions count}_{\text{Day2, Placebo}} = \text{Exp}(0.6627) \cdot (1-0.2845)=1.38809$$

$$\text{Intrusions count}_{\text{Day1, Hydrocortisone}} = \text{Exp}(0.2326) \cdot (1-0.2845)=0.90287$$

These values correspond to the predicted intrusions on days 1 and 2 in Figure 2 in the main paper.

Appendix item 4.1 Supplementary Protocol Information and exclusion criteria for Chapter 4

1. Criteria for Participation

The following criteria needed to be met for a participant to be included in the study:

Inclusion criteria

1. Healthy men
2. Age between 18 and 35 years
3. Fluency in English
4. Normal or corrected to normal colour vision and hearing
5. Able to swallow tablets with water
6. Able and willing to provide four saliva samples.
7. Able and willing to complete daily diaries for all seven days after the laboratory-based session

Exclusion criteria

1. Historical or current diagnosis of a psychiatric disorder that was/is sufficiently severe to require treatment
2. Beck Depression Inventory-II (BDI-II; Beck et al, 1996) score > 20 or reporting significant suicidal ideation at screening
3. History of blood/injury phobia
4. Experience (including witnessing) of an event (especially a violent event) that is judged at screening to be sufficiently stressful as to have led to risk of developing post-traumatic stress disorder (PTSD)
5. Likely to be highly distressed by film scenes of a graphic nature involving interpersonal (including sexual) violence
6. Systolic blood pressure < 90 mmHg
7. History of fainting
8. Current hypotension
9. Adrenal insufficiency e.g. Hypocortisolism/Addison's Disease
10. Heart/liver disease
11. Renal impairment/anuria or any kidney disease
12. Acute porphyria
13. Hyperkalaemia

14. Untreated infections
15. Currently taking any cardiovascular or psychiatric medication or medication for inflammatory conditions (e.g. steroid-based medications)
16. Taking regular (> twice monthly) recreational drugs, other than alcohol, nicotine and caffeine
17. Self-reported memory problems
18. Have a cardiac pacemaker implant
19. Have a cardiovascular condition or hypertension
20. Have a history of epilepsy or neurosurgery or tuberculosis
21. Have a history of severe anaphylactic reaction
22. Regularly consuming over 14 units of alcohol per week
23. Daily smoker
24. Use of medication that may result in drug interaction with Spironolactone, e.g. Ibuprofen, Diclofenac, Ketorolac or Naproxen

Appendix item 4.2 Ethics approval for Chapter 4 Project ID 3419/003 The effects of a single dose of spironolactone on involuntary and voluntary emotional memory in healthy adult volunteers. Approved by UCL Research Ethics Committee 25th April 2020

Appendix item 4.3 Information for participants Chapter 4

You are invited to take part in a study at the Clinical Psychopharmacology Unit at UCL. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what participation will involve. Please take time to read this information carefully and discuss it with others if you wish. Take time to decide whether or not you wish to take part. Choosing not to take part will not disadvantage you in any way. Thank you for reading this information sheet.

What is the purpose of the project?

Stressful experiences can sometimes lead to the formation of unwanted memories which are distressing. In some cases, very stressful experiences can lead to psychological problems, like posttraumatic stress disorder (PTSD) – in which people find it difficult to forget their negative experiences. Therefore, it is important to learn about the brain mechanisms involved in the formation of unpleasant emotional memories. Some drugs might be helpful in this regard, particularly drugs that affect the ‘stress response’ – such as the drug *spironolactone*. While *spironolactone* is normally used to treat high blood pressure and other heart problems it also seems to temporarily affect memory formation. To examine whether spironolactone affects the formation of negative emotional memories, Participants in this study will be asked to take tablets containing either spironolactone (400 mg total) or a placebo (vitamin C) to see how these affect subsequent memories for unpleasant events. By taking part in this study you will therefore contribute to the scientific knowledge of the effect of this drug on emotional memory formation which may inform future treatments for psychological disorders such as posttraumatic stress disorder (PTSD).

Why have you been chosen to take part?

We are going to test 78 healthy men aged between 18 and 35 to take part in this study. To take part, you will need to be fluent in English, in good physical health, with normal or corrected to normal vision. Unfortunately, you cannot take part if you have any of the following: any neurological problems, a current mental health problem that requires treatment, including phobia to sight of blood, a previous diagnosis or suspected diagnosis of post-traumatic stress disorder (PTSD). As this study involves giving half of the participants a medication (spironolactone), you will also not be eligible to take part if you suffer from hypotension, heart/liver disease, renal impairment/anuria or any type of kidney disease, acute porphyria, hyperkalaemia (abnormally high potassium levels) or Addison’s disease. You should also not take part if you have untreated infection or are currently taking cardiovascular or psychiatric medication, or taking regular (>twice a month) recreational drugs other than alcohol, nicotine and caffeine. To take part, you should not be consuming alcohol excessively (i.e. not regularly drinking >14 units of alcohol per week), and not be a daily smoker.

If you are currently taking any type of medication or supplement on a regular basis, you must provide details to the experimenter prior to participating in order to avoid drug interactions. You should also inform the researcher about any other studies you have participated in recently, or are currently enrolled in.

Note: this is not a full list of inclusion/exclusion criteria and a full assessment will be carried out to determine if you are eligible to take part in this study.

Do I have to take part?

Your participation in the study is entirely voluntary and you are free to withdraw from the study at any time without giving a reason, even if you have previously given your written consent and without any loss to any benefits to which

you are entitled. If you do agree to take part, you will be asked to provide an e-signature on a consent form and will be given this information sheet to keep. If you do withdraw from the study, you will be asked what you wish to happen with the data you have provided. When we collect your data, it will be pseudo-anonymized and at the analysis stage, all data will be anonymized. Once your data have been anonymised, we will no longer be able to link it to you. At this point we would no longer be able to remove your data.

What will I have to do?

If you agree to participate in this study, you should either contact the experimenter by email or scan the QR code provided on the advertisements. This will give you access to a brief online screening questionnaire. You should also provide a telephone number and a convenient time for us to call. If the online screening survey indicates that you may be eligible, you will then receive a call from us. At this stage of the screening process, we will ask you some further questions to check your eligibility for the study. *Please note that we have strict criteria for participation and based on your answers to the online and telephone screening questions, you may not be eligible to take part in the study.*

If you fulfill our study criteria, we will arrange for you to attend a lab session at UCL. During this session, which takes approximately 5 hours, you will be asked to complete some questionnaires about your past and current mood, your usual cognitive and emotional state and any history of childhood adversity. We will ask you to complete some computer-based tasks. You will be asked to place some sticky probes on your body to measure heart rate and skin conductance. We will also take regular blood pressure readings during the session. We will be recording brain signals via an Electroencephalography ('EEG') headset. EEG involves placement of electrodes on the scalp. To ensure good contact between the electrodes and the scalp, we need to slightly abrade the skin and apply some gel. This usually does not cause discomfort and is considered completely safe. Please note that due to Covid restrictions, no washing facilities will be available to clean your hair after the experiment. You may therefore wish to bring a head covering and take leave sufficient time to return home and wash your hair before attending other engagements.

During the session, you will watch a short film (~15 minutes) while we monitor your physiological and biological response before, during and after the film. **You should be aware that the film contains highly graphic scenes of sexual assault, interpersonal violence, injury and death which are designed to be distressing.** *Please do not take part if you are likely to become very distressed by such scenes.* During the session, you will be given capsules containing spironolactone or placebo to swallow with water. The placebo pills contain Vitamin C.

After the lab session at UCL you will be required to fill in a simple online diary of any spontaneous thoughts/memories about the film **for the next 7 days**. You will be reminded to do this daily via email. **The daily diaries provided after the lab session are crucial for our experiment. Please only participate if you are able and willing to complete all the brief daily online diaries.** If you cannot do this, we will have to withdraw you from the study. You may contact the researchers at any time during or after the study if you experience any difficulties with this requirement.

A final longer online assessment takes place 8 days after the first lab session. You will be asked to complete some additional measures and tasks during this session. After this session, you will be debriefed and reimbursed £60 for your participation in the study via bank transfer. Please note that additional travel expenses cannot be reimbursed.

What is the drug 'spironolactone'?

Spironolactone is a so-called potassium-sparing diuretic (or 'water pill') that clears fluid and salt from your body whilst keeping your potassium levels from getting too low. Spironolactone is used to treat heart failure, high blood pressure (hypertension), or hypokalaemia (low potassium levels in the blood).

Spironolactone is considered among the safest drugs in medicine (it is on the WHO's list of essential medicines), and significant side effects typically only occur due to chronic use of the drug. As part of this experiment, those who are randomised to the placebo group will receive tablets containing vitamin C. Those randomised to receive spironolactone will receive only **one dose** of 400 mg spironolactone in tablets which are taken with water. With a one-off dose, most people do not experience side effects. However, it is important to note that, although this happens rarely, a single dose of spironolactone may cause hypotension (low blood pressure) in some individuals.

Who is organising and funding the research?

The research is organised by the Clinical, Education and Health Psychology Department, UCL and funded by The Sir Bobby Charlton Foundation.

Further information

Please discuss the information above with others if you wish, and please contact the researchers if there is anything that is not clear or if you would like more information. You will be given a copy of this information sheet and a consent form to keep.

Registration Details

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 3419/003

UCL Data Protection Registration Number: Z6364106/2019/12/50

**CONSENT FORM FOR HEALTHY ADULT VOLUNTEERS TAKING PART IN RESEARCH
STUDIES CONCERNING ADDITIONAL COVID-19 PROCEDURES**

Approved 18/8/2020 by VPRO Ethics



Name and Contact Details of the Researcher(s):

Please complete this form after you have read the Information Sheet for procedures in place for reducing COVID-19 transmission risk in experiments involving human participants.

If you are happy to take part in the study, we will need you to give your consent. To do this, please read the statements below and tick the appropriate boxes.

		Tick Box
1.	I confirm that I understand that by ticking each box below I am consenting to this element of the study. I understand that it will be assumed that unticked boxes mean that I DO NOT consent to that part of the study. I understand that by not giving consent for any one element that I may be deemed ineligible for the study.	
2.	I confirm that I have read and understood the Information Sheet for procedures in place for reducing COVID-19 transmission risks. I have had an opportunity to consider the information and what will be expected of me. I have also had the opportunity to ask questions which have been answered to my satisfaction.	
3.	<u>Personal Information</u> I understand that: - For purposes of Government-led contact tracing, I will need to supply my mobile phone number, address and email address. These data will be stored in accordance with General Data Protection Regulation (2018) that protects the privacy of personal information. - No one other than the researchers (and authorized government officials, if necessary) will have access to this data. - This data will be securely deleted 3 weeks after the participation date as shown on this form.	
4.	<u>Right to withdraw:</u>	

	I understand that my participation in this study is voluntary and that I am free to withdraw from the study without giving a reason.	
5.	<u>COVID-19 transmission risk:</u> I understand that whilst every effort is made to reduce COVID-19 transmission risks down to manageable levels, the risk cannot be abolished completely.	
6.	<u>My responsibility:</u> • I confirm that I will adhere to the instructions detailed in the information sheet including those that relate to face covering, hand sanitizing and social distancing. • I understand that my contact details will be shared with Government officials in case contact tracing is necessary. • I will inform the experimenter, using the contact details provided to me, in the event that I test positive for COVID-19 within 1 week of today's date.	
7.	I am aware of who I should contact if I wish to lodge a complaint.	
8.	I voluntarily agree to take part in this study.	

Participant details for contact tracing:

Name:

Email:

Address:

Mobile phone number:

****Note to researcher: to adhere to GDPR guidelines, this information should be stored safely under lock and key for a period of 3 weeks, and disposed of safely thereafter.***