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Investigating the Behavioural and Electrophysiological Consequences of Early Life Stress

Investigating the Behavioural and Electrophysiological Consequences of Early Life Stress

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Abstract

Early life stress (ELS) is one of the biggest known risk factors for the development of a range of psychiatric disorders including depression. Events exceeding a child's ability to cope lead to elevated glucocorticoid concentrations which cause abnormal brain development with regions involved in reward circuitry such as the hippocampus, amygdala and prefrontal cortex being most impacted. Impairments of reward learning (RL) have been reported to act as an intermediate phenotype in the aetiology of depression. This thesis therefore investigates the hypothesis that ELS predisposes to depression through reprogramming of brain regions associated with reward which causes reward processing deficits that in turn lead to the development of depression.

Reward learning was initially attempted to be assessed in a mouse model of ELS using a modified version of the affective bias test. ELS mice showed no differences in RL compared to controls, however there was evidence for a lack of an affective phenotype in the mouse model. A translational reward learning assay, the probabilistic reversal learning task (PRLT), was therefore validated in a cohort of rats for use in future ELS studies. This task was initially sensitive to manipulations of serotonin but showed non-specific impairments following a range of other manipulations including ketamine and amphetamine administration. Task sensitivity also decreased over time suggesting a window of opportunity for successful use. Next the electrophysiological consequences of ELS were assessed in the hippocampus of rats. Maternally separated rats showed increased NMDA but not AMPA receptor function but no changes in basal transmission. Finally reward learning was assessed in a cohort of humans with high levels of ELS. ELS was associated with decreased positive feedback sensitivity and reduced initial learning in the PRLT. The results from this thesis suggest that the proposed hypothesis is plausible, however further work is needed to both confirm this and translate findings into patient benefit.

Covid-19 Statement

Work in this thesis was substantially impacted by the Covid-19 global pandemic. Much of the electrophysiology work in Chapter 4 is underpowered due to the premature termination of experiments due to lockdown. A final cohort of 30 animals had been bred and put through an early life stress protocol to complete long term potentiation experiments but were required to be terminated. Validation experiments were sufficiently powered while AMPAR/NMDAR and miniEPSC experiments, although lower than liked, had a large enough sample size to allow conclusions to be drawn. However, long term potentiation data is heavily underpowered meaning that any conclusions need to be drawn tentatively from this unfinished data. Pilot data from plateau potential experiments that were going to be completed in early life stress animals has also been included to illustrate the direction of this project had work not been interrupted. It was not possible to mitigate against these circumstances due to restrictions on animal experimentation due to Covid-19.

A key aim of this thesis was to assess reward learning deficits in early life stress using the probabilistic reversal learning task validated in Chapter 2. As this was not going to be possible due to Covid-19 an alternative approach of testing humans using an online platform was therefore carried out in Chapter 5. Although these rodent experiments still merit being done in the future this best enabled the meeting of the research objectives in the given time remaining.

Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed: ..... Date: 15th January 2021

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It is also important to thank both the Mellor and Robinson labs for keeping me sane when experiments did not always go the way they were intended. I would like to extend specific thanks to Megan Jackson, Daryl Purawijaya, Chloe Slaney, Haris Organtzidis, Tanuj Sircar, Christian Wood, Justyna Hinchcliffe, Sarah Stuart and Claire Hales for their great support, friendship and advice over the course of this PhD. From the Mellor lab I would like to thank Matt Udakis and Travis Bacon whose endless enthusiasm and support when inevitably the rig broke made any of the electrophysiology possible.

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List of Abbreviations

5-HT	5–hydroxy tryptamine
ABT	Affective bias test
ACd	Dorsal agranular cingulate cortex
ACE	Adverse childhood experience
aCSF	Artificial cerebrospinal fluid
ACTH	Adrenocorticotropic hormone
ADHD	Attention deficit hyperactive disorder
AF	Alexa-fluor
AHP	Afterhyperpolarisation
AIC	Akaike information criterion
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
ap	Action potential
AUC	Area under the curve
BD	Bipolar disorder
BDI-II	Beck's depression inventory II
BDNF	Brain derived neurotrophic factor
BIC	Bayesian Information Criterion
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
BrdU	5-bromo-2'-deoxyuridine
CA1	cornu Ammonis region 1
CA2	cornu Ammonis region 2

CA3	cornu Ammonis region 3
CeA	Central nucleus of the amygdala
CI	Confidence interval
CNS	Central nervous system
CORT	Corticosterone
CrEL	Cremophor EL
CRH	Corticotropin-releasing hormone
CSA	Childhood sexual abuse
CSF	Cerebrospinal fluid
DA	Dopamine
DAPI	4′,6-diamidino-2-phenylindole
DAT	Dopamine transporter
DCX	Doublecortin
DG	Dentate gyrus
DH	Dorsal hippocampus
DMSO	Dimethyl sulfoxide
DRN	Dorsal raphe nucleus
EC	Entorhinal cortex
ELA	Early life adversity
ELS	Early life stress
ELSQ	Early life stress questionnaire
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
FFT	Fast Fourier transform
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid

GLMM	Generalised linear mixed model
GR	Glucocorticoid receptor
GWAS	Genome wide association study
HCl	Hydrochloric acid
HFS	High frequency stimulation
HPA	Hypothalamic pituitary adrenal
I_{clamp}	Current clamp
IFNα	Interferon alpha
IL	Infralimbic cortex
i.p.	Intraperitoneal
KS-test	Kolmogorov Smirnov test
LFP	Local field potential
LG	Licking grooming
LNBM	Limited nesting and bedding material
LPS	Lipopolysaccharide
LTD	Long term depression
LTP	Long term potentiation
MA	Monoamine
MD	Maternal deprivation
MDD	Major depressive disorder
miniEPSC	miniature excitatory postsynaptic potential
MR	Mineralocorticoid receptor
MS	Maternal separation
MS180	Maternal separation (180m per day)
NA	Noradrenaline
NAc	Nucleus accumbens

NFS	Negative feedback sensitivity
NMDA	N-methyl-D-aspartate
NRI	Noradrenergic reuptake inhibitor
NSFT	Novelty supressed feeding test
PaS	Para-subiculum
PB	Phosphate buffer
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline with 0.2% tween20
PC1	Principal component 1
PCA	Principal component analysis
PFA	Paraformaldehyde
PFC	Prefrontal cortex
PFS	Positive feedback sensitivity
PL	Prelimbic cortex
PND	Postnatal day
PPR	Paired pulse ratio
PRLT	Probabilistic reversal learning task
PrS	Pre-subiculum
PSST	Probabilistic stimulus selection task
PTSD	Post-traumatic stress syndrome
PTX	Picrotoxin
PV	Parvalbumin
PVN	Paraventricular nucleus of the hypothalamus
RAAD	Rapid acting antidepressant
RBPRT	Response bias probabilistic reward task
R_{in}	Input resistance

RL	Reward learning
RLA	2vs1 reward learning assay
RM-ANOVA	Repeated measures analysis of variance
R_{ser}	Series resistance
s.c.	Subcutaneous
SC	Schaffer collateral
SERT	Serotonin transporter
SGZ	Sub-granular zone of the dentate gyrus
SHAPS	Snaith-Hamilton pleasure scale
SHAPS-C	Snaith-Hamilton pleasure scale continuously scored
slm	Stratum lacunosum moleculare
SNRI	Serotonergic noradrenergic reuptake inhibitor
SNP	Single nucleotide polymorphism
SPT	Sucrose preference test
SSRI	Selective serotonergic reuptake inhibitor
SST	Somatostatin
sr	Stratum radiatum
TA	Temporoammonic
TBS	Theta burst stimulation
TBZ	Tetrabenazine
TCA	Tricyclic antidepressant
TTX	Tetrodotoxin
V_{clamp}	Voltage clamp
V_m	Membrane voltage
VH	Ventral hippocampus
VTA	Ventral tegmental area

Chapter 1

General Introduction

1.1 Major depressive disorder

1.1.1 Mental health disorders

Psychiatric disorders are a major burden upon society and can be defined as a behavioural or psychological syndrome or pattern that occurs in an individual causing clinically significant distress or disability which is not an expectable response to common stressors (Stein et al., 2010). It has been estimated that mental health disorders account for 32% of all years lived with disability and 13% of disability adjusted life years globally (Vigo et al., 2016). This translates into 1 in 6 adults in the UK suffering from a common mental disorder in any one week with this increasing over time (McManus et al., 2014). Mental health does not just have a prominent effect upon the individual but also impacts society more widely. It is estimated that up to 12.4% of all sick days in the UK can be attributed to mental health conditions (Office for National Statistics, 2019b) with only 26% of people who have suffered from a mental health problem for over a year being in work (Trades Union Congress, 2008). This culminates in an estimated wider cost to the UK economy of around £80 – 115 billion per year (adjusted for inflation, Davies, 2013), equal to around 4.5% of GDP.

1.1.2 Prevalence and symptoms

Of all psychiatric diseases depression has the largest impact on society, causing the largest number of years lived with disability compared to all other psychiatric disorders (see Figure 1.1, Global Burden of Disease Study 2013 Collaborators, 2015). Around 3.3 in 100 people suffer from depression at any one time in the UK which rises to 7.8 in 100 if mixed anxiety and depression is included (McManus et al., 2014). In its most common machination, major depressive disorder (MDD), patients suffer persistent low mood, diminished interest or enjoyment in normally pleasurable activities (known as anhedonia), fatigue, feelings of worthlessness and significant changes in bodyweight (American Psychiatric Association, 2013). *In-extremis*, feelings of worthlessness and anhedonia can be so strong this can lead individuals to consider taking their own life. MDD patients are 21 times more likely to commit suicide than the general population (Harris and Barraclough, 1998) and suicide is now the leading cause of mortality in both men and women between the ages of 5 and 34 in the UK (Office for National Statistics, 2019a).

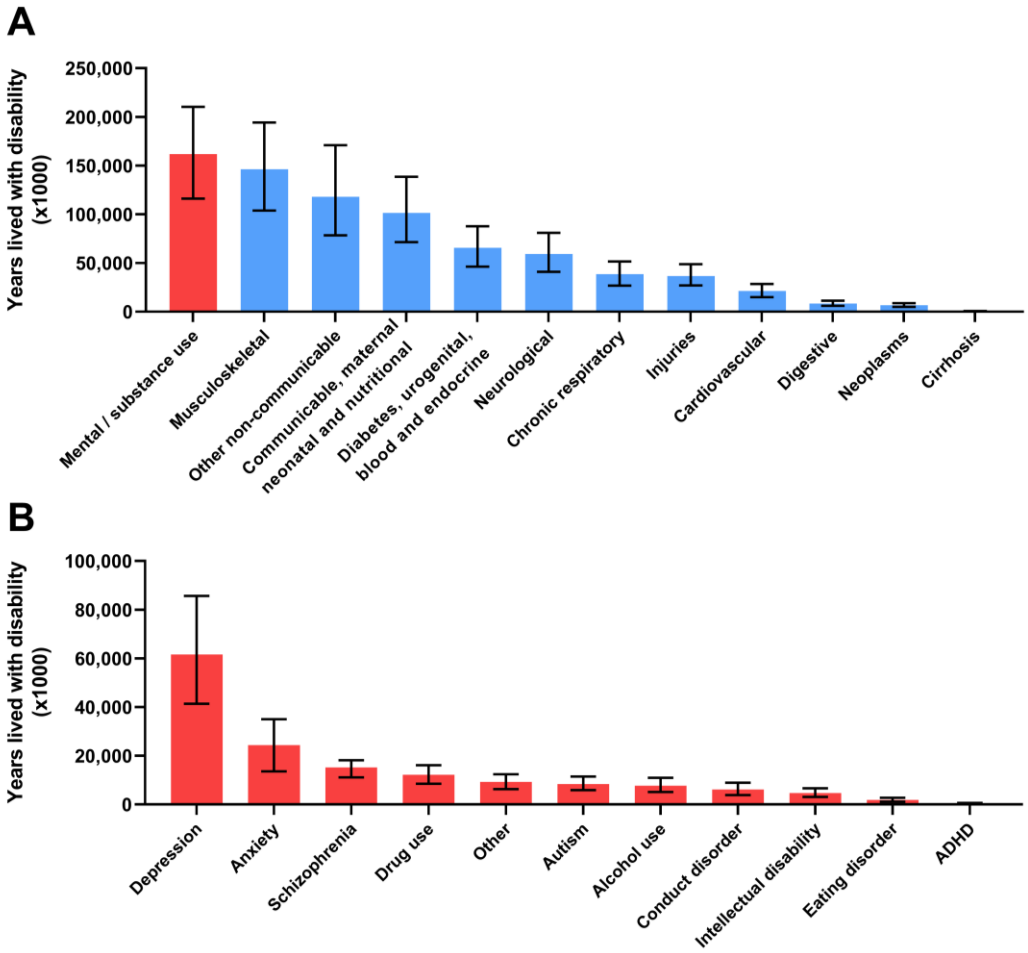


Figure 1.1 The global health burden of psychiatric disease. (A) Global years lived with disability split into top level disease domains from the 2013 global burden of disease study (Global Burden of Disease Study 2013 Collaborators, 2015). **(B)** Data from the top level mental and substance use disorders category split into its respective subcomponents. All data is shown as mean ± 95% confidence interval.

1.1.3 Aetiology

The aetiology of depression is complex and involves interaction between vulnerability factors such as genetic background or stress in early life and lifetime events including pharmacological exposure, stress and infection (see Figure 1.2 for overview). This is coupled with a varied pathophysiology involving changes in neurotrophic signalling, monoaminergic neurotransmission, neurogenesis and inflammation. Pathopsychological processes are also at the core of disease development and progression with profound changes in both reward learning and cognitive affective biases being associated with depression aetiology.

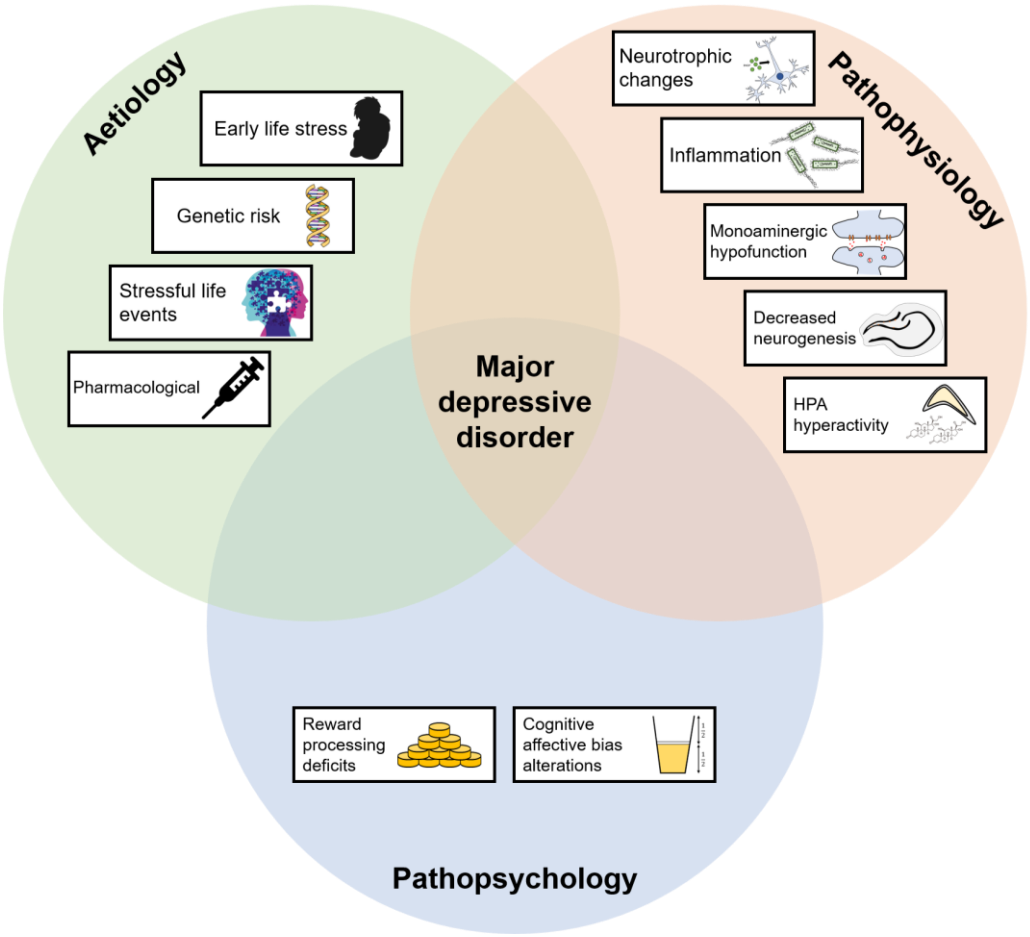


Figure 1.2 Summary of the interplay between disease aetiology, pathophysiology and pathopsychology in major depressive disorder.

1.1.3.1 Genetic risk

As with many diseases the risk of developing depression has a large genetic component with a heritability of between 31-42% found in monozygotic twin studies (Sullivan et al., 2000). This seems to be mainly driven by the presence of numerous low impact genetic loci having an additive effect as opposed to single loci having a large effect (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013). These combinations of risk loci can be described using a polygenic risk score with this being able to account for 2% of the variance between control and depressed patients (Mcintosh et al., 2019). However, as the effect size of the polygenic risk score is small this means that having a score in the top 10% only confers a 2.5x higher lifetime risk of developing depression compared to persons in the bottom 10% of polygenic risk (Wray et al., 2018). Interestingly there are strong correlations between genetic risk for depression and other psychiatric disorders such as schizophrenia, bipolar disorder and ADHD alongside weaker

correlations with autism and anorexia (Mcintosh et al., 2019). Previous studies have suggested a range of candidate genes containing polymorphisms linked with depression risk including SLC6A4, SLC6A3, HTR2A, TPH2, APOE and BDNF amongst others (Gatt et al., 2015; López-León et al., 2008; Shadrina et al., 2018; Smoller, 2016). However, recent evidence has suggested that these links are not replicated in large population based case-control samples (Border et al., 2018). Large genome wide association studies (GWAS) have also been carried out with risk loci identified including CACNA1C, SIRT1 and LHPP although it should be noted that multiple studies reported no genome-wide significant associations (Cai et al., 2015; Green et al., 2010; Smoller, 2016). This complexity of linking individual genes to depression has made it difficult to utilise genetics as a tool to understand the mechanisms leading to depression development.

Much interest has also been directed towards putative interactions between genetic risk and environmental exposure. Caspi et al., 2003 reported that life stress and a functional polymorphism in the 5-hydroxy tryptamine (5-HT) transporter gene (5-HTT) interacted to increase the risk of developing depression. However, recent studies utilising much larger sample sizes have failed to replicate this discovery (Culverhouse et al., 2018; Fergusson et al., 2011). Two other polymorphisms have also been described to interact with stress, specifically stress in early life (ELS, discussed in detail later). Polymorphisms in FKBP5, a protein able to moderate glucocorticoid receptor (GR) sensitivity, have been found to interact with ELS to cause increased risk of developing both MDD and post-traumatic stress disorder (PTSD, Wang and Shelton, 2017). CRHR1, the gene coding for the corticotrophin releasing hormone (CRH) receptor 1, has also been implicated in increasing the risk of depression following ELS (Nugent et al., 2011).

1.1.3.2 Stress

The genetic component for depression vulnerability is significant although it is difficult to ascribe mechanistic links between individual polymorphisms and vulnerability. However, stress in life has emerged as a much clearer factor. Stress has been implicated in both the aetiology and pathology of depression with environmental exposure being associated with around 63% of the variance in developing depression (Mullins and Lewis, 2017; Sullivan et al., 2000). Stress in early life is also a key predisposing factor for the development of depression and is discussed in detail later.

Across multiple studies exposure to stressful life events has been associated with subsequent depressive episodes (Kessler, 1997) with this acting in a cumulative fashion such that having more stressful events further increases the risk of MDD development. Around 80% of major depressive episodes are preceded by major life events (Hammen, 2005). Within the general population increased morning cortisol levels are also associated with a higher risk of developing MDD. (Goodyer et al., 1991; Harris et al., 2000). Additionally, animal models utilising either chronic mild stress (Willner, 2017), chronic social defeat stress (Slattery and Cryan, 2017) or chronic corticosterone administration (Gourley and Taylor, 2009) are widely used to probe depression like behaviours and appear to offer face validity with animals showing anhedonia like traits through reduced sucrose consumption in the sucrose preference test. Finally, introduction of exogenous glucocorticoids through treatment with drugs such as dexamethasone is associated with a greatly increased risk of both suicide and the development of MDD in humans (Fardet et al., 2012; Otte et al., 2016).

1.1.4 Pathophysiology

1.1.4.1 Hypothalamic pituitary adrenal axis

Life stress leads to elevated cortisol levels (Miller et al., 2007). This has been suggested to lead to loss of the glucocorticoid receptor (GR) containing cells within the hippocampus that mediate negative feedback of the hypothalamic pituitary adrenal (HPA) axis (see Figure 1.3, Barden, 2004; Sapolsky et al., 1984, 1990). There is strong evidence that depressed patients have both alterations of HPA axis function and its regulation (Otte et al., 2016). Depressed patients show increased basal corticosterone levels in addition to impaired negative feedback control of cortisol release following administration of a synthetic glucocorticoid such as dexamethasone (Stetler and Miller, 2011). Depressed patients also have elevated adrenocorticotropic hormone (ACTH) levels while no reliable differences in corticotropin-releasing hormone (CRH) have been found between control and depressed patients (Stetler and Miller, 2011). Interestingly although the HPA axis plays an important role in disease aetiology it does not appear to be associated with disease progression (Verduijn et al., 2015). This is supported by the evidence that antidepressant treatment reverses elevated cortisol levels in only 44% of patients (McKay and Zakzanis, 2010) and that much effort has been expended studying the use of CRF antagonists for antidepressant efficacy but with limited clinical success (Spierling and Zorrilla, 2017).

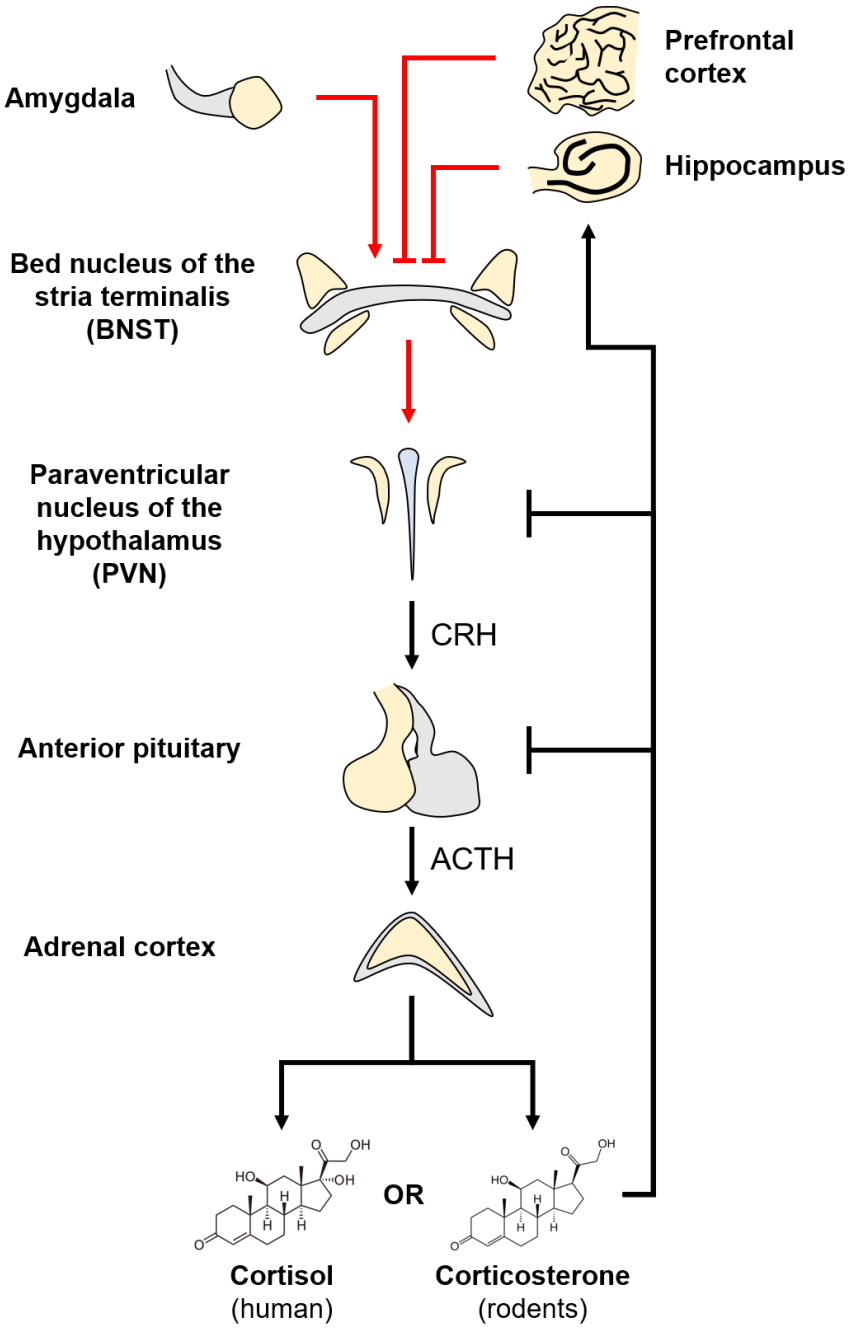


Figure 1.3 The hypothalamic pituitary adrenal axis and its regulation by the limbic system. Black lines represent the action of hormones through the blood while red lines indicate direct neural signalling. GABAergic neurosteroids can also act to directly inhibit CRH release from the PVN (see Gunn et al., 2015). Arrows show excitatory or stimulatory connections while blunt ended lines indicate inhibitory connections. Abbreviations: CRH, corticotrophin releasing hormone; ACTH, adrenocorticotropic hormone. Diagram constructed using information from (Herman et al., 2016; Smith and Vale, 2006)

1.1.4.2 Monoaminergic neurotransmission deficits

Deficits in monoaminergic neurotransmission were one of the first theorised causative factors in depression (Schildkraut, 1965). All current conventional antidepressants act by increasing synaptic concentrations of the monoaminergic neurotransmitters 5-HT (also known as serotonin) and noradrenaline (NA) through inhibition of their reuptake transporters (Heninger et al., 1996). In addition, depletion of central nervous system (CNS) 5-HT through administration of large tryptophan free amino acid loads leads to low mood in some participants (Young, 2013).

However, there are numerous issues with monoaminergic signalling deficits as a complete theory of depression pathophysiology. Firstly, conventional antidepressant treatments acutely raise synaptic monoamine concentrations, while taking weeks for patients to report subjective improvements in mood (Oswald et al., 1972). Additionally, around 30% of MDD patients do not respond to conventional antidepressant treatment, suggesting solely increasing monoamine levels is not sufficient to treat the symptoms of depression (Knochel et al., 2015). Finally, drugs of abuse such as cocaine or amphetamine, which both increase synaptic monoamine concentrations, have no efficacy as antidepressant treatments (Stuart et al., 2013). Efforts have been made to explain these discrepancies by suggesting that acute increases in monoamine concentrations lead to neurotrophic changes alongside changes in 5-HT receptor feedback regulation that themselves are responsible for antidepressant efficacy (Frazer and Benmansour, 2002; Sharp et al., 2007).

1.1.4.3 Neurotrophic changes in MDD

Neurotrophins are growth factors, expressed within the adult brain, which regulate the survival, development, function and plasticity of neurones (Huang and Reichardt, 2001; Krishnan and Nestler, 2008). This class of molecules has been suggested to provide a link between stress, brain structural changes observed in depression and the delayed onset of conventional antidepressant efficacy (Krishnan and Nestler, 2008). Pre-clinical studies have observed that stress exposure reduces brain derived neurotrophic factor (BDNF) expression in the hippocampus and can cause neuronal atrophy (Duman and Monteggia, 2006). Indeed, decreased hippocampal volume has been reported in depressed patients

with this returning to normal following antidepressant treatment (Duman and Monteggia, 2006). Post-mortem hippocampal samples from depressed patients have also been found to have lower BDNF expression (Karege et al., 2005). These changes appear to be ameliorated by chronic antidepressant administration increasing BDNF, vascular endothelial growth factor (VEGF) and VGF expression within animal studies while also increasing neurogenesis within the sub-granular zone (SGZ) of the dentate gyrus (Krishnan and Nestler, 2008). Blocking this neurogenesis has been reported to inhibit the efficacy of antidepressant administration in rodent models (Sahay and Hen, 2007). Finally, direct administration of BDNF into the rodent hippocampus in the forced swim test led to decreased immobility, a change that is commonly interpreted as showing antidepressant effects (Shirayama et al., 2002).

Interestingly there are also direct links between hippocampal neurogenesis and stress resilience. Enhancement of adult neurogenesis in the ventral hippocampus has been found to enhance resilience to chronic social defeat stress while inhibition of adult born granule cells leads to stress sensitisation (Anacker et al., 2018). These effects also appear to persist after the cessation of stress with increased adult neurogenesis following social defeat stress leading to the amelioration of some negative outcomes including basal corticosterone concentrations (Culig et al., 2017).

However, as with other observations about the pathophysiology of depression, there are caveats to the hypothesised causal role of BDNF and neurogenesis in depression pathogenesis. Firstly, work from rodent studies has shown that conditional knockout of the BDNF gene does not lead to depression behaviour and indeed administration of BDNF to the ventral tegmental area (VTA) or nucleus accumbens (NAc) has been found to have pro-depressant effects (Krishnan and Nestler, 2008). A single nucleotide polymorphism (SNP) has also been described in the BDNF gene in humans (G196A) which leads to impaired activity dependent release of BDNF (Egan et al., 2003) and decreased hippocampal volume (Szeszko et al., 2005) but does not show an association with MDD risk (Gratacòs et al., 2007). Finally, while stress has been found to reduce SGZ cell proliferation, decreased neurogenesis does not lead to depression (Krishnan and Nestler, 2008). There has also been recent debate as to the relevance of neurogenesis to humans with conflicting data as to whether neurogenesis occurs at an appreciable rate in adult humans (Boldrini et al., 2018; Kriegstein et al., 2018).

1.1.4.4 Inflammation

Recent data has thrown light on the ability of inflammation and the microbiome to interact with brain neurocircuitry to contribute to the pathophysiology of depression (Foster et al., 2017; Miller and Raison, 2016). It is hypothesised that stress leads to increased intestinal permeability, allowing bacterial translocation and immune activation involving mediators such as interleukin-6 and interferon gamma (Foster et al., 2017). MDD patients show increased immune activation with increased pro-inflammatory cytokine, cytokine receptor and chemokine concentrations reported in both cerebrospinal fluid (CSF) and blood (Miller et al., 2009). Clinical studies following patients being treated with interferon-alpha (IFNα), a proinflammatory cytokine used for treating leukaemia and hepatitis, have found that up to 50% of patients develop the symptoms of MDD and have increased risk of suicidality which reverses following cessation of treatment (Raison et al., 2005, 2006). Anti-inflammatory drugs have also been investigated for both antidepressant efficacy and as an adjunct to conventional antidepressants with mixed success (Adzic et al., 2017; Kopschina Feltes et al., 2017). Pro-inflammatory interventions are also used within pre-clinical studies to generate depression-like disease models with both lipopolysaccharide (LPS) and IFNα being widely used in this regard (Krishnan and Nestler, 2011).

A final factor that has been found to be associated with depression vulnerability is vitamin D deficiency (Anglin et al., 2013) with MDD patients being found to have lower concentrations than controls. Vitamin D has been described as a powerful neuroimmunomodulatory molecule and additionally is hypothesised to be a risk-modifying factor for development of the neuroinflammatory disease multiple sclerosis (Fernandes de Abreu et al., 2009).

1.1.5 Current therapeutic strategies

The first true generation of antidepressant compounds, the tricyclic antidepressants (TCAs), were developed from the antipsychotic chlorpromazine. However, this early generation of drugs had poor tolerability and a narrow therapeutic window due to interacting with a poorly targeted range of receptors and transporters. Early investigations into the mechanism of action of these antidepressants observed that increased monoaminergic neurotransmission (Robinson, 2018) was a feature of these

drugs. This led to efforts to develop a second generation of compounds which were more selective to monoamine reuptake transporters. Introduction of the selective serotonergic reuptake inhibitors (SSRIs) were the result of this effort amongst other family members with slightly different transporter specificities. The so called "conventional" antidepressants have a greatly improved tolerability and safety profile compared to older generations of compounds and are widely prescribed (Cipriani et al., 2018). However, as previously discussed, a major drawback of these compounds is the therapeutic lag, lasting weeks to months, between the time a patient starts taking the drug and when they report subjective improvements in mood (Machado-Vieira et al., 2010). Great interest has been generated by the relatively recent discovery of compounds with rapid-onset antidepressant efficacy such as ketamine (Zarate et al., 2006) where effects can be observed in as little as hours. This has led to the S-ketamine enantiomer receiving marketing approval for treatment resistant depression when used in combination with a conventional antidepressant (Jauhar and Morrison, 2019). Other compounds such as scopolamine have also been identified as having rapid-acting antidepressant efficacy (Jaffe et al., 2013). Of the rapid onset antidepressants ketamine has been the most extensively investigated, however the mechanism of action is still largely unclear. One of the key findings that has been reported is that ketamine blocks burst firing in lateral habenula glutamatergic neurones to rapidly improve mood (Yang et al., 2018). Another mechanistic discovery was that ketamine preferentially inhibits NMDA receptors on GABAergic interneurons as opposed to excitatory glutamatergic neurones (Widman and McMahon, 2018; Zanos and Gould, 2018) with this often referred to as the disinhibition hypothesis. A neuropsychological framework has also been proposed whereby ketamine, unlike conventional antidepressants, attenuates previously learnt negative biases (Stuart et al., 2015). Finally, it is also worth acknowledging that cognitive behavioural therapy is another widely used therapeutic strategy with efficacy approaching that of antidepressant psychotherapy (Baer, 2003; Butler et al., 2006).

However, although there are now a rich variety of compounds with both delayed and rapid-acting antidepressant efficacy there is still a need for much better therapeutic strategies. Firstly, around 30-40% of patients do not show at least a 50% reduction in symptoms following treatment and only around a half of all patients achieve a complete remission of disease (Nestler et al., 2002; Zhou et al., 2014). Current antidepressants also still have a significant side effect burden with SSRIs commonly causing gastrointestinal disruption, sexual dysfunction and emotional blunting in addition to many rapid-acting antidepressants having psychotropic effects and a potential for abuse

(Robinson, 2018). Finally, current strategies fail to take into account the highly heterogenous nature of MDD patients (Goldberg, 2011). This compares poorly to other disease areas such as cancer where personalised medicine is now part of standard treatment pathways (Krzyszczyk et al., 2018).

1.2 Reward learning

While the previously described structural and molecular alterations in MDD patients are the current best understanding of the aetiology of depression there is still a missing link between these phenomena and the subjective psychological experience that MDD patients suffer. Alongside alterations in cognitive affective biases (reviewed in Hales et al., 2014), reward learning deficits (Pizzagalli, 2014) have been proposed as a key intermediate phenotype of MDD.

1.2.1 Reward: an overview

Reward processing is critical for the survival and wellbeing of any organism and is defined as the ability to modulate future choices and behaviours as a function of reward feedback from the environment (Hélie et al., 2017). Reward processing can be subdivided into separate domains which encompass experience of reward, motivation for reward, reward learning and decision making (Slaney et al., 2018). Patients with MDD show wide ranging deficits in reward experiencing, motivation and decision making (Nestler and Carlezon, 2006; Slaney et al., 2018). It could however be argued that reward learning is the integration of these modalities. Reward learning has been described as "a process by which individuals experience, learn and repeat goal-directed actions that maximise the probability of receiving future rewards" (Der-Avakian et al., 2016) with this also applying to the avoidance of non-rewarding actions. Successful reward learning therefore requires motivation to engage with a potentially rewarding situation, good decision making to best optimise outcomes and accurate experiencing of reward to guide future behaviour.

Studies regarding the neural basis of reward learning have implicated multiple brain regions and circuits including the basolateral amygdala (BLA), prelimbic cortex, insular cortex and orbitofrontal cortex (Cardinal et al., 2002; Der-Avakian et al., 2016) in addition to the hippocampus (Delgado and Dickerson, 2012, discussed in detail later). These circuits however are suggested to converge upon the mesolimbic dopaminergic neurones of the basal ganglia. Cell bodies for these dopaminergic neurones are located in the VTA and substantia nigra but project axons to the striatum, NAc and prefrontal cortex (PFC) amongst other brain regions including the hippocampus (Mcnamara and

Dupret, 2017; Schultz, 2002). Dopaminergic neurones preferentially respond to reward through modulation of activity but over the course of conditioned learning they switch from responding to the reward to the conditioned stimulus (Schultz, 2002, 2016). Through modulation of dopaminergic activity by both unexpected reward and unanticipated lack of reward this allows these neurones to code reward prediction errors, how much the anticipated reward differs from that received (Schultz et al., 1997).

1.2.2 Assessment of reward learning

Tasks that assess reward learning have been widely used in both humans and animals (Der-Avakian et al., 2016) in order to probe the links between reward learning impairments and a wide range of diseases and manipulations. A valuable element of many of these tasks is that they are directly translatable between animals and man. One widely used task in both man (Pizzagalli et al., 2005) and to a lesser degree in animals (Der-Avakian et al., 2013) is the response bias probabilistic reward task (RBPRT). In this task participants have to identify whether the mouth of a face rapidly presented to them is long or short (ambiguous stimuli) while being, unknown to the participants, probabilistically rewarded such that one stimulus is rewarded three times more often than the other upon correct identification. Healthy participants develop a bias towards the more highly rewarded stimulus, indicating a reward induced learning bias (Der-Avakian et al., 2016).

Another example of a probabilistic reward task is the probabilistic stimulus selection task (PSST) which has been utilised to probe learning associated with positive and negative reinforcement in man (Frank, 2004) and to an extremely limited extent in animals (Trecker et al., 2012). Participants are required to learn three pairs of reward contingencies presented to them (80:20, 70:30, 60:40 chance of reward : chance of no reward). Once contingencies are learnt, participants are then presented with the stimulus paired with an 80% chance of reward alongside the other lower rewarded stimuli. Additionally, participants are presented with the stimuli paired with a 20% of reward alongside the other higher rewarded stimuli. Greater discrimination ability on stimuli pairs containing the 80% probability of reward are thought to reflect greater learning from positive feedback while greater performance on pairs including the 20%

reward contingency are conversely associated with greater learning from negative feedback (Der-Avakian et al., 2016). Healthy participants learn at an equal rate from positive and negative feedback (Frank, 2004; Frank et al., 2007).

Probabilistic learning tasks containing contingency reversal as an extra level of cognitive difficulty have also been widely used in both animals (Bari et al., 2010; Ineichen et al., 2012; Slaney et al., 2018; Wilkinson et al., 2020) and man (Cools et al., 2002; Murphy et al., 2003). In the probabilistic reversal learning task (PRLT) participants are presented with two stimuli rewarded probabilistically such that there is a "rich" stimulus (80% chance of reward) and a "lean" stimulus (20% chance of reward). After a variable period of time either based upon task performance in animals (Bari et al., 2010) or a set number of trials (Cools et al., 2002) the contingencies reverse such that the "rich" stimulus becomes "lean" and vice versa. Subjects then have to learn the new probabilistic rule to maximise reward.

1.2.3 Reward learning in depression

Reward processing deficits appear to be a key feature of MDD with patients less able than controls to bias their responding due to reward in the RBPRT (Pizzagalli et al., 2008). Additionally it was observed that the degree of response bias impairment correlated with disease persistence after 8 weeks (Vrieze et al., 2013). These impairments also appear to persist following the remission of symptoms (Pechtel and Pizzagalli, 2013). A recent meta-analysis also concluded that depressed participants show wide ranging reward processing deficits compared to controls (Halahakoon et al., 2020)

Within the PRLT patients suffering from MDD were impaired in their ability to maintain responding in the face of negative feedback that was misleading (i.e. when not rewarded at a "rich" stimulus) while had no impairment in acquiring and negotiating the reversal element of the task (Murphy et al., 2003). Another study focusing on youths with bipolar disorder (BD) or MDD found a strong association between errors following reversal in BD patients but only a trend in MDD patients (Dickstein et al., 2010). Linked to the monoamine theory of depression, animal experiments have shown that 5-HT depletion impairs reversal ability and decreases positive feedback sensitivity while chronic

treatment with the selective serotoninergic reuptake inhibitor (SSRI) citalopram improves positive feedback sensitivity (Bari et al., 2010). However, a recent study in humans found that following tryptophan depletion in healthy subjects there was no change in any measure within the PRLT (Kanen et al., 2020). This implies that reward processing deficits in depression are independent of short term 5-HT synaptic availability and require pathology over a longer timescale to take effect.

Deficits in reward processing are also suggested to be present prior to the emergence of symptoms and are able to predict the risk of disease development. Healthy patients with high trait levels of anhedonia also show reduced reward induced biases in reward learning tasks (Pizzagalli et al., 2005). This is in addition to lower reward seeking and blunted feedback sensitivity being able to predict the risk of developing depression in a cohort of at risk adolescents and adolescent girls respectively (Bress et al., 2013; Rawal et al., 2013).

1.2.4 Impaired reward learning and stress

Impaired reward learning may also be a key link between the precipitating effects of stress and the development of MDD symptomology. Participants performing the RBPRT under the threat of shock had a lower reward induced response bias compared to those performing under standard conditions (Bogdan et al., 2010, 2011; Bogdan and Pizzagalli, 2006). Interestingly stress combined independently with SNPs in the CRH receptor 1 and mineralocorticoid receptor (MR) genes to increase the reward learning impairment observed compared to just stress alone (Bogdan et al., 2010, 2011). However, using the PSST it was only found that participants who were high responders with regards to cortisol and self-report measures had impairments in reward learning while at a whole population level there was no effect of stress (Berghorst et al., 2013).

Stress has also been found to have a profound effect upon the meso-limbic reward circuitry with chronic unavoidable stressors acutely reducing spontaneous DAergic neuronal activity in the VTA while also reducing DA release in the NAc for up to 14 days following the cessation of stress (Cabib and Puglisi-allegra, 2012; Pizzagalli, 2014). Chronic stress has also been found to potentiate mesocortical DA release in response to novel stressors up to 14 days following the cessation of chronic stress (Pizzagalli, 2014).

Through the inhibitory action of mesolimbic DA neurones upon NAc DA terminals (King et al., 1997) this stress-induced sensitisation may be a mechanism linking stress to longer-term reward learning impairments and the aetiology of depression.

These data therefore suggest that impaired reward learning may be a key intermediate phenotype in the development of MDD whereby stress and other factors (such as the to be discussed ELS) impair reward learning which then leads to the development of later depressive episodes.

1.3 Early life stress

As previously discussed, there are numerous factors involved in the aetiology of depression and it is hypothesised that reward learning is a key intermediary in the process by which a person goes from being at risk to developing symptomology. Early life stress (ELS), also known as early life adversity (ELA) has been shown to be the most important factor determining the risk of a person developing MDD (Green et al., 2010) while also having powerful effects upon reward learning (Novick et al., 2018).

1.3.1 Definition and prevalence

Early life stress can be defined as any exposure of a child to negative stressful events that exceed their ability to cope (Pechtel and Pizzagalli, 2011). Examples of these events include loss of a parent, abuse, neglect and poverty alongside war, adoption and parental divorce (Cohen et al., 2006). In the UK it is estimated that up to 1 in 5 adults have experienced some form of child abuse (emotional, physical, sexual or witnessing domestic violence), 1 in 100 adults have experienced physical neglect and 3.1 million adults were the victim of sexual abuse before the age of 16 years old (Office for National Statistics, 2020a). Utilising the early life stress questionnaire (ELSQ), a survey of adverse childhood experiences (ACEs), it was found that only around 28% of respondents suffered no form of ELS in childhood (see Figure 1.4 for overview, Cohen et al., 2006). It should also be noted that this study was carried out exclusively in European, North American, and Australian participants; globally the situation is likely more severe.

1.3.2 Links between ELS and psychiatric disease

Early life stress is strongly associated with an increased risk of developing psychiatric disease in later life. In a nationwide survey of comorbidities within the USA it was found that stress in childhood explains up to 30% of adult onset and 44% of childhood onset psychiatric disorder diagnoses (Green et al., 2010). Exposure to ELS is also able to account for around 67% of the population attributable risk of attempting suicide (Dube et al., 2001).

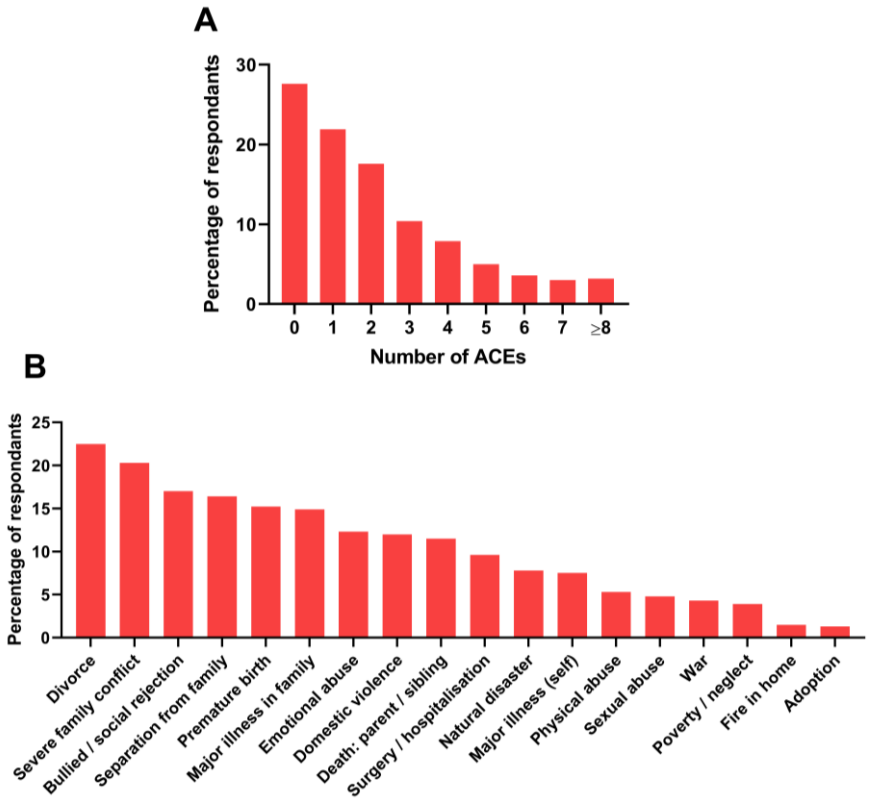


Figure 1.4 The prevalence of early life stress within western populations. (A) Data from Cohen et al., 2006 showing the number of adverse childhood experiences (ACEs) experienced across the population. **(B)** Data from the same study broken down into the separate ACEs that constitute stress in early life within the ELSQ.

Adversities in early life even have a powerful effect upon overall mortality risk with persons who suffered 6 or more ACEs dying on average nearly 20 years younger than those with no exposure to ELS (Anda et al., 2006; Brown et al., 2009). However, one caveat with these findings are that people with high levels of ELS are much more likely to be socioeconomically disadvantaged with this being also associated with increased mortality (Office for National Statistics, 2015) although not to such a large degree.

There are now well established links between ELS and an increased risk of developing depression (Agid et al., 1999; Lemoult et al., 2019; McCauley et al., 1997; Sadowski et al., 1999), anxiety (McCauley et al., 1997; Phillips et al., 2005), schizophrenia (Agid et al., 1999), lower adulthood cognition (Hedges and Woon, 2011; Richards and Wadsworth, 2004), substance abuse disorders (Andersen et al., 2008; McCauley et al.,

1997), ADHD (Harold et al., 2014), personality disorders (Ball and Links, 2009), epilepsy (van Campen et al., 2014) and visceral pain (Chaloner and Greenwood-Van Meerveld, 2013) in addition to metabolic disorders such as obesity, diabetes and cardiovascular disease (Joung et al., 2014). The risk conferred by ELS exposure has also been found to act in a cumulative fashion such that higher numbers of ACEs have been associated with a higher risk of developing MDD (Sadowski et al., 1999). Interestingly ELS has also been associated with accelerated telomere shortening leading to exaggerated cellular aging (Coimbra et al., 2017; Price et al., 2013). It should also be noted that prenatal stress, in addition to maternal depression, has also been found to lead to neurodevelopmental abnormalities in children which present in a similar fashion to ELS (Fatima et al., 2017; Kinsella and Monk, 2009; Maxwell et al., 2018).

People who have experienced ELS are also more sensitive to the effects of stress in life. These people require lower levels of stress in adulthood to increase the risk of developing depression compared to people with a normal childhood (Hammen et al., 2000). It is also the case that the higher the amount of adversity that a person suffers correlates with their stress sensitisation such that people with a history of higher adversity are more sensitive than those with a lower history (Mclaughlin et al., 2010). People who have experienced ELS are also more likely to be exposed to abuse or assaulted in adulthood, creating the stressful conditions necessary to confer a high risk of developing depression (Schaaf and McCanne, 1998).

While stress in adulthood potentiates the risk of developing MDD following ELS, other factors have been found to be associated with mitigating this risk. High educational attainment has been found to be associated with a reduced risk of developing MDD following ELS while robust social relationships have also been found to be associated with protection (Friedman et al., 2015). Rodent studies have also observed that high environmental enrichment is able to reverse the phenotype seen following application of an ELS model (discussed in detail later, Francis et al., 2002).

The link between ELS and psychiatric disease has also been found to be modified by genetic risk. As previously briefly discussed, interaction between FKBP5 genotype and ELS can increase the risk of developing MDD (Wang et al., 2017). FKBP5 is a chaperone protein who's expression is induced by GR activation and enables GR binding to

glucocorticoid response elements in the cell nucleus (Wang et al., 2017). One meta-analysis found an association between three SNPs in FKBP5 and increased risk of developing both PTSD and depression following ELS (Wang et al., 2017). These have been found to act in a summative fashion and be independent of stressful events in midlife (Lahti et al., 2016).

1.3.3 Animal models of ELS

Animal models of early life stress have been crucial to both understand the phenotypic consequences of ELS and to provide mechanistic insights into how ELS reprograms the brain in order to predispose to psychiatric disease (Schmidt et al., 2011). Animal models of ELS offer numerous advantages over studying human populations including greater experimental controllability and consistency in addition to allowing probing of brain neurocircuitry that is simply not possible in humans. In common with all mammals, neonatal rodents are cared for by their mother during their early development whereby a strong attachment between infant and caregiver is formed (Rincón-Cortés and Sullivan, 2014). Most forms of early adversity disrupt this attachment and additionally with humans it has been found that the earlier an adversity the more severe its consequences for mental health outcomes (Kaplow and Widom, 2007). As such the two most common models of ELS (see Figure 1.5), maternal separation (MS) and maternal deprivation (MD), seek to replicate this disrupted caregiver attachment in an early post-natal period to maximise the effects of the intervention. Both models revolve around removal of pups soon after birth from the mother for extended periods of time (Murthy and Gould, 2018).

1.3.3.1 Common ELS models and the resulting phenotype

Within the maternal separation paradigm animals are typically removed from the mother for 180 minutes per day from postnatal days (PND) 1 to 14, however between laboratories there is a wide degree of variation in both separation times and days of exposure (Tractenberg et al., 2016). Maternal deprivation takes separation a step further and pups are separated from their mother for at least 24 hours before being returned, although this normally only occurs once (Tractenberg et al., 2016). Recent efforts have been invested into developing more naturalistic ELS models with a commonly used one being the limited nesting and bedding material (LNBM) model.

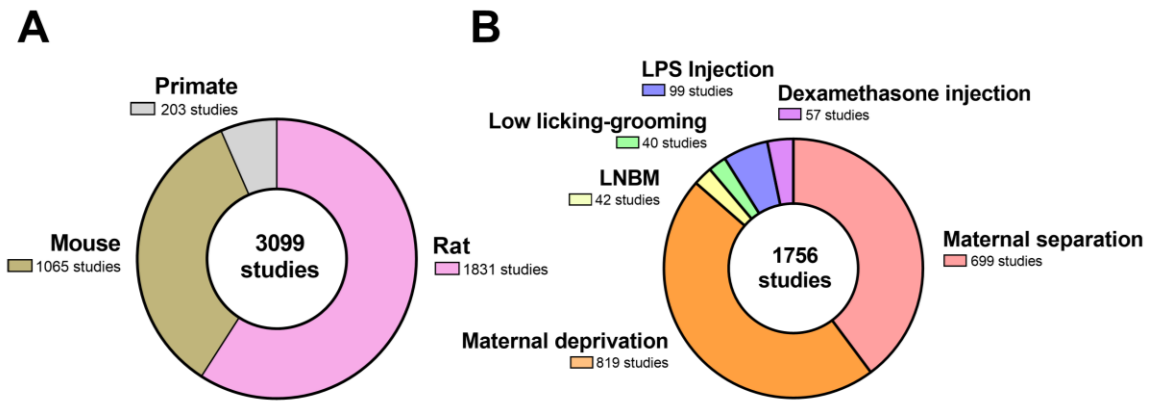


Figure 1.5 Modelling of ELS in animals by species and induction model. Number of primary publications as of 16th April 2020 on SCOPUS containing ELS split by **(A)** species and by **(B)** ELS model used. Search terms used: ELS: (early AND life AND stress) OR (early AND life AND adversity), mouse: (mouse OR mice), rat (rat OR rats), maternal deprivation: (maternal and deprivation), maternal separation (maternal AND separation), LPS injection : (LPS OR lipopolysaccharide), LNBM (limited AND nesting) and low licking grooming (licking AND grooming), dexamethasone injection (dexamethasone). Abbreviations: LPS: lipopolysaccharide, LNBM: limited nesting and bedding material.

Within this model animals are restricted to a sparse cage environment containing limited bedding between PND 1 and 9 and this has been successfully applied to both mice and rats (Rice et al., 2008; Walker et al., 2017). Another widely used naturalistic paradigm employs natural variation in maternal care (Champagne et al., 2001). By classifying rat mothers as either high or low licking-grooming (high LG or low LG) it is possible to assess the effect of maternal care upon offspring neurodevelopmental outcomes.

Finally, pharmacological models have been employed either utilising synthetic glucocorticoids or lipopolysaccharide injections. By injection of lipopolysaccharide (LPS) into pups postnatally, studies have attempted to study inflammation in early life as a model of ELS (Ong et al., 2017; Saavedra et al., 2018). Synthetic glucocorticoids such as dexamethasone are used to model the elevated HPA activity caused by stressors, however these may display divergent pharmacodynamic properties compared to endogenous CORT (Schmidt et al., 2011).

There are broad differences in phenotypic outcomes of offspring bred in ELS models. This is both between different models and within the same model due to methodological variation. However, at an overall level, a recent meta-analysis has found that ELS leads to increased anxiety in tests such as the elevated plus maze, light dark box, novelty supressed feeding and open field tests (Bonapersona et al., 2019; Wang et al., 2020). Improved memory under stressful conditions such as in fear conditioning was also observed while impaired learning under low stress conditions in tasks such as the object recognition and object in location tasks was an additional key feature (Bonapersona et al., 2019). Finally impairments in social behaviour are a key phenotypic observation in tests such as the social interaction test (Bonapersona et al., 2019). The phenotypic impact of ELS models is also potentiated by additional negative experiences such as stress manipulations or using a stress sensitive animal strain. However, most effects of ELS within animal models appear to be limited to males only with the exception of non-stressful learning following ELS and after additional negative experiences (Bonapersona et al., 2019). This may be due to male pups naturally receiving higher levels of maternal care than females which is then disrupted by the ELS model (Bath, 2020; Richmond and Sachs, 1984). When looking at methodological differences between models the largest effect sizes are found in rats as opposed to mice and with low licking grooming animals as opposed to other models (Bonapersona et al., 2019).

1.3.3.2 Caveats to animal models of ELS

It is however important to be aware that humans and model species such as rodents have different neurodevelopmental trajectories (Workman et al., 2013). While hippocampal development in a PND5-7 rodent is similar to a full-term human neonate, the amygdala of a PND10 rat is closer to that in a 6-9 month old human baby (Walker et al., 2017). Many elements of a rodent's brain development in the early postnatal period are more similar to a prenatal period in humans. For example, while the establishment of the blood brain barrier occurs in PND1-3 in rodents this has already happened by around 23-32wks gestation in humans (Semple et al., 2013). Peak gliogenesis, growth and axonal/dendritic density also occur around PND7-10 in rodents but after 36-40wks of gestation in humans (Semple et al., 2013). These neurodevelopmental differences may explain the discrepancy between rodents and man in the effect of sex upon outcome following ELS. In rodent models, male animals are more vulnerable to ELS (Bonapersona et al., 2019) as opposed to females in human cases (Herbison et al., 2017). However,

male humans are more susceptible to the effects of prenatal stress (Herbison et al., 2017) suggesting that rodent models are potentially having important effects upon brain circuits that in humans are still developing prenatally.

There are also important considerations relating to how the modality of stressor differs between humans and animal models of ELS. Animal models most commonly focus on stress in the early neonatal period which disrupts the interaction between dam and pups. This is however a period and modality more equivalent to attachment disorders in humans, disorders related to aberrant interactions between the offspring and caregiver (Newman and Mares, 2007). Nevertheless, as previously discussed, stress throughout the entire neurodevelopmental period in humans can lead to altered cognition in adulthood. Animal models have been widely used to investigate this and, although the timing and modality is different, lead to similar increases in CORT that are able to reprogram developing neurones.

These observations should be taken as a whole to suggest that while the current animal models do not perfectly map onto the stress modalities and neurodevelopmental time periods to that seen in humans, they do appear to recapitulate many of the phenotypic observations seen in humans who have experienced ELS.

1.3.4 ELS and the HPA axis

Corticosteroids (CORT: corticosterone in rodents and cortisol in humans) have a powerful impact on neurodevelopment through modulation of gene expression and synaptic plasticity (Kamin and Kertes, 2016). In order to minimise exposure, shortly after birth (≈ 6-12 months in humans and ≈ PND 3–14 in rodents) the CORT response to stressors is markedly diminished in a period known as the stress hypo-responsive period (SHRP, Dashkalakis et al., 2013). Strong enough stressors such as those encountered in ELS, can overpower the SHRP enabling elevated circulating levels of CORT. In addition to this, extended elevated CORT concentrations occurring outside of this period are also able to cause the same effects (Daskalakis et al., 2013). Elevated CORT binds to glucocorticoid (GR) and mineralocorticoid (MR) receptors in the brain with the hippocampus and amygdala being most significantly affected due to their high GR expression. The pre-frontal cortex is also detrimentally effected by CORT due to its slow development

(Tottenham, 2009). Due to a roughly tenfold lower affinity for CORT, GRs as opposed to MRs are the main mediators of the effects of ELS. This is because MRs are easily saturated by basal CORT and the onset of stress as opposed to GRs which require higher CORT levels to activate (Kamin and Kertes, 2016). It is also worth stating that CORT has been found to have rapid acting effects through non-genomic mechanisms. Multiple receptors are hypothesised to be responsible for these effects including GRs, MRs and yet to be identified receptors (Groeneweg et al., 2011).

In addition to the HPA axis being the main mediator of developmental reprogramming of the brain there is evidence that the HPA axis itself exhibits aberrant behaviour in adults with a history of ELS. Although it was long believed that ELS manifested itself with exaggerated HPA responses to stress in adulthood, recent evidence has suggested a more nuanced situation. A recent meta-analysis found that ELS leads to a hyporesponsive stress response following social stress with the largest effect sizes found in adults as opposed to children and maltreatment compared to other forms of adversity (Bunea et al., 2017). However, another similar meta-analysis found no overall effects of ELS on CORT awakening response, basal levels or upon exposure to stress (Fogelman and Canli, 2018). If just participants who experienced emotional, sexual or physical abuse were analysed, a heightened cortisol awakening response was observed. The age at which adversity occurred has also been found to have important effects upon the consequences for the HPA axis. In 16 year olds with a history of ELS it was found that if the adversity occurred in late childhood (6-11 years) this led to high basal CORT levels as opposed to adversities in early to middle adolescence (12-15 years) which caused CORT hyposecretion (Bosch et al., 2012). Adversities in this study before the age of 5 were not associated with any changes in HPA activity. It should however be noted that in adolescents the brain has still not fully developed therefore it is possible that effects of ELS might not have had time to yet manifest. Additional mediators of ELS-HPA axis outcome heterogeneity include genetic background with one study finding that the Val158Met polymorphism in catechol-o-methyl transferase, the enzyme responsible for DA and NA metabolism, interacted with the level of ELS to cause blunted HPA reactivity (Lovallo et al., 2017).

Regardless of the effects of ELS upon the HPA axis it is well established as previously discussed that those with experience of adversity in childhood are sensitised to the effects of stress in adulthood. This may be due to increased expression of the

glucocorticoid receptor which has been reported in animal models (Turecki and Meaney, 2016). Data from both humans and animal models have also reported that epigenetic methylation of the GR gene is a marker for ELS (Turecki and Meaney, 2016). Interestingly it appears that mineralocorticoid (MR) receptors may have a protective role with MR overexpression in a mouse model of ELS rescuing observed deficits in neurogenesis and contextual memory formation (Kanatsou et al., 2017).

It should also be noted that while the effects of ELS upon human HPA reactivity are complex a consistent observation in maternal separation (MS) rodent models of ELS has been HPA axis hyperactivity (Aisa et al., 2007; Daskalakis et al., 2013; Molet et al., 2014; Stuart et al., 2019). Interestingly animals bred from mothers exhibiting low maternal care exhibited hypoactive CORT responses to stress (Liu and Meaney, 1997). Elevated basal CORT concentrations have also been reported following animals bred in impoverished environments (Chen and Baram, 2016). Altered regulation of the HPA axis has also been reported in animal models of ELS with LNBM mice showing increased glutamatergic neurotransmission and insensitivity to GABAergic neurosteroids in CRF expressing neurones in the hypothalamus (Gunn et al., 2013). These neurosteroids normally act to supress CRF release therefore providing one potential mechanism for increased HPA axis activity in animal models of ELS (Gunn et al., 2015).

1.3.5 Reward learning impairments in ELS

Similarly to both people suffering from MDD and those at risk of developing the disease, there is a wealth of evidence to suggest that individuals with a history of ELS also have deficits covering the whole spectrum of reward processing (reviewed in Novick et al., 2018; Pechtel and Pizzagalli, 2011). As a component of this, reward learning deficits have been reported in both human and animal model studies.

Utilising the PSST, Pechtel and Pizzagalli, 2013 reported that women with a history of childhood sexual abuse showed lower reinforcement learning ability than controls. Another study conducted using a probabilistic learning task found that adolescents who experienced high levels of adversity in childhood exhibited lower associative learning compared to controls (Hanson et al., 2017).

Pre-clinical literature has also provided valuable insights into the effects of ELS upon reward learning. Marmosets bred under a postnatal social isolation paradigm show impairments in reversal in a simple visual discrimination paradigm (Pryce et al., 2004). Evidence from the affective bias task (ABT), a test of reward learning and how this is modified by affective state, suggests that rats bred under a maternal separation paradigm show markedly impaired reward learning when required to learn an association between reward magnitude and digging substrate (Stuart et al., 2019).

Alterations in the core reward neurocircuitry have also been observed following ELS. Decreased reward anticipatory activity in the left pallidus and putamen, key basal ganglia components involved in the processing of reward predicting cues, has been reported in abused individuals compared to controls (Pechtel and Pizzagalli, 2011). Alterations in dopaminergic neurotransmission have also been found using preclinical ELS models with a recent meta-analysis finding decreased striatal dopamine precursor and increased metabolite concentrations after ELS (Bonapersona et al., 2018). Functionally, both increased and decreased mesolimbic dopaminergic signalling has been reported following ELS (Novick et al., 2018). ELS animals have been reported to show increased NAc DA release in response to stress or amphetamine (Novick et al., 2018) while other studies have reported decreased sensitivity to the effects of amphetamine upon approach motivation behaviours (Matthews and Robbins, 2003).

1.3.6 Aberrant brain development as a result of ELS

The developing brain is a dynamic environment which is extremely sensitive to outside perturbations; disturbances can then cause changes in developmental trajectory leading to abnormal information processing in adulthood. As previously discussed, the most impacted brain regions are the prefrontal cortex, amygdala and hippocampus (reviewed in detail later) however there is evidence that a much wider array of brain regions are also affected. It should also be noted that ELS has important effects upon the epigenome (see Burns et al., 2018 for review) and immune system (see Brenhouse et al., 2019 and Fagundes et al., 2013 for reviews) but in the interests of brevity these will not be discussed in detail.

1.3.6.1 Prefrontal cortex

The prefrontal cortex (PFC) is one of the more evolutionary recent brain regions to develop and can be described as being responsible for carrying out goal directed actions (Fuster, 2015; Passingham and Wise, 2012). Decreased PFC volume has been reported in humans with a history of ELS (Cohodes et al., 2020; Gold et al., 2016). Although it should be noted that comparisons between human and rodent PFC are difficult, especially with regard to specific areas, interesting findings worth discussion have been found in animal models of ELS (Roberts and Clarke, 2019; Seamans et al., 2008). Within animal models there is now consistent evidence of altered cellular morphology and synaptic plasticity following ELS in the PFC. Reduced dendritic length and decreased spine density has been found on layer II/III pyramidal neurones in the mPFC (Chocyk et al., 2013; Rincel et al., 2018) which is coupled to impaired LTP and decreased glutamate receptor levels. Alterations in dendritic complexity have also been observed in the prelimbic (PL), infralimbic (IL) and dorsal agranular cingulate (ACd) cortices with the degree of ACd layer V apical shrinkage being able to predict cognitive deficits in stressed mice. (Farrell et al., 2016; Yang et al., 2015). ELS does not only appear to affect neuronal populations in the PFC; there are also reports of altered astrocyte and microglia populations (Abbink et al., 2019; Majcher-Maślanka et al., 2019). Reduced myelination has also been observed with this hypothesised to be due to dysfunction of oligodendrocytes (Rojas-Carvajal et al., 2019).

Alterations in PFC activity have also been observed with one study finding that maternally separated rats exhibited lower mPFC firing rates. These rates were differentially modulated by manipulations of γ-aminobutyric acid (GABA), suggesting that ELS influences inhibitory neurotransmission in the PFC (Ali et al., 2011; Stevenson et al., 2008). ELS has also been found to influence a 5-HT mediated inhibitory current whereby ELS amplifies this current in layer II/III pyramidal neurones in the mPFC at postnatal weeks 3 and 4 but in adulthood this current is attenuated in ELS but not control rats (Goodfellow et al., 2009) .

Within humans there is mixed evidence for the effects of ELS upon functional activation of the PFC. In response to emotionally valent words, people who suffered childhood emotional maltreatment were reported to show reduced activation of the mPFC (Van Harmelen et al., 2013). Conversely adolescents with a history of maltreatment were

reported to show enhanced activation of vmPFC and anterior cingulate cortex while conducting an emotional discrimination task (Hart et al., 2018).

1.3.6.2 Amygdala

Another key component of the limbic system is the amygdala. This region has key roles in emotional processing including appraisal of stimuli valence, motivation, reward learning and fear (Janak and Tye, 2015). The amygdala experiences protracted development from birth until late childhood in humans although interestingly development has been found to be complete by age 4 in females but not until 18 in males (Tottenham, 2009). Studies have suggested that permanent alteration of amygdala volume are a consequence of exposure to ELS. However, consensus has not been achieved; one study reported a larger volume from institutionalised children which did not normalise following placement into foster families of high socioeconomic status (Mehta et al., 2009; Tottenham et al., 2010). Other studies have however reported smaller amygdala volumes (Cohodes et al., 2020; Tottenham, 2009) with a correlation between severity of childhood sexual abuse and smaller amygdala volume. This is in addition to stress exposure accounting for around 25% of amygdala size variance during childhood (Cohodes et al., 2020; Pechtel et al., 2014; Veer et al., 2015). Functionally, people with a history of ELS show heightened activity in the amygdala in response to emotional cues compared to controls (Murthy and Gould, 2020). These findings are consistent with those from animal models where ELS increases cFos expression, a common marker of neuronal activity, in the basolateral amygdala (BLA) in response to stress throughout both development and adulthood.

Furthermore animal models have also suggested that accelerated amygdala development due to ELS is responsible for increased anxiety and aggression in adulthood (Kikusui and Mori, 2009). Early myelination and increased CRH containing neurone populations have also been reported with elevations in CRH mRNA being reported to last until adulthood (Ono et al., 2008; Tottenham, 2009). Interestingly this appears to be coupled with a decrease in GR mRNA in the amygdala which, when reversed using viral introduction of mRNA to the central nucleus to the amygdala (CeA), restored anxiety, fear and sociability deficits in maternally separated mice (Arnett et al., 2015).

1.3.6.3 Other brain regions

In addition to the key brain regions already identified, ELS has been found to have influence on a wide variety of other brain regions and structures to varying degrees. This includes the hippocampus which merits its own detailed discussion later. Changes in serotonergic neuromodulation have been observed with maternally separated animals being found to have elevated dorsal raphe nuclei (DRN) basal firing rates in addition to weakened DRN sensitivity to noradrenaline (Gardner et al., 2009; Gartside et al., 2003). Changes in 5-HT_{1A} receptor expression have also been reported following ELS. However, a discrepancy exists whereby some studies report increased expression in the hippocampus (Diamantopoulou et al., 2018) while others report decreased hippocampal and PFC receptor levels (Harrison and Baune, 2014; Li et al., 2013; Ohta et al., 2014).

ELS has also been found to have powerful deleterious effects upon the dopaminergic system. One recent study reported that downregulation of dopamine receptor 3 (Drd3) in the lateral septum, a region involved in regulating behavioural responses to stress and processing emotional information, was sufficient to cause social dysfunctions in mice exposed to ELS (Shin et al., 2018). By restoring lateral septum Drd3 activity through either optogenetics or a selective agonist this was able to reverse social deficits in these mice. Another key finding in the dopaminergic system is the important of the Otx2 transcription factor within the VTA. By reducing Otx2 expression in neurones projecting from the VTA to lateral septum during adolescence but not adulthood this increases adulthood stress sensitivity in a similar manner to ELS while overexpression is able to ameliorate the effects of ELS (Peña et al., 2017).

Alterations of GABAergic signalling have also been suggested to be important in mediating the relationship between ELS and substance use disorders. Within human populations it has been found that specific haplotypes of GABRA2, the gene coding for the α2 subunit, are associated with decreased protein expression and an increased risk of developing substance use disorders following ELS (Enoch et al., 2010). This is similar to that seen in an animal model of ELS with LNBM mice found to have decreased GABA receptor α2 subunit expression in the nucleus accumbens compared to controls (Mitchell et al., 2018).

1.3.6.4 Altered connectivity

In addition to just focussing on a single brain region in isolation, effort has been undertaken to understand how ELS influences intra-region connectivity.

Increased structural connectivity between the amygdala and mPFC has been reported in rats bred using a LNBM model of ELS using diffusion tensor imaging, a technique allowing visualisation of white matter fibre tracts (Bolton et al., 2018). At a resting state this increased connectivity has also been reported functionally using fMRI (Johnson et al., 2018). However, another study found that that during stress (fear conditioning) there was decreased excitatory communication from the BLA to the mPFC in ELS animals (Ishikawa et al., 2015). Within humans it has been reported that emotional abuse was able to predict reduced resting state functional connectivity between the right amygdala and pregenual anterior cingulate cortex with this also predicting state anxiety after psychosocial stress (Fan et al., 2014). However, sex specific effects have been observed upon amygdala – ventromedial PFC (vmPFC) connectivity after ELS with it only being reported in females that greater ELS predicted amygdala-VmPFC functional connectivity and concurrent anxiety and depression symptoms (Burghy et al., 2012).

Altered hippocampal – BLA communication has also been measured through use of *in-vivo* electrophysiology. Rats that were maternally separated showed enhanced long term potentiation (LTP) and long term depression (LTD) in the hippocampal CA3 network following LTP/LTD induction in the BLA compared to control animals (Blaise et al., 2008). Increased connectivity between the amygdala and hippocampus has also been reported using resting-state functional magnetic resonance imaging (fMRI, Johnson et al., 2018) with this increased connectivity correlating with open-field behaviour in ELS animals.

Finally, there have been alterations in hippocampal – PFC communication reported in juvenile rats that have undergone maternal separation. Diminished cross-frequency coupling, a process whereby neural oscillation frequency bands interact to communicate information between brain regions, has been reported between the PFC and hippocampus (Canolty and Knight, 2010; Reincke and Hanganu-Opatz, 2017).

1.4 The Hippocampus

As previously mentioned the hippocampus is believed to be a key locus for mediating the link between early life stress and later psychiatric vulnerability through its high expression of glucocorticoid receptors, extensive postnatal development and key roles in both emotional processing and regulation of HPA activity (Fanselow and Dong, 2010; Tottenham, 2009; Tottenham and Sheridan, 2009).

The hippocampus is a neuronal network located within the medial temporal lobe (see Figure 1.6) First described in classical history as resembling "cornu ammonis" or the horn of a ram it was not until the 1950s where the function of the hippocampus started to be revealed. Famously the first glimpse of hippocampal function came from the case of patient HM who, following hippocampal resection to treat severe epileptic seizures, lost the ability to form episodic memories (Annese et al., 2014). Following the discovery of synaptic plasticity in the form of long term potentiation (LTP) within the hippocampus (Bliss and Collingridge, 1993), place cells which are able to selectively respond to specific environmental cues (Moser et al., 2015) and the importance of the hippocampus in emotional regulation (Fanselow and Dong, 2010) the region has become widely studied both for its important roles in health and disease and as a model brain circuit (Small et al., 2011).

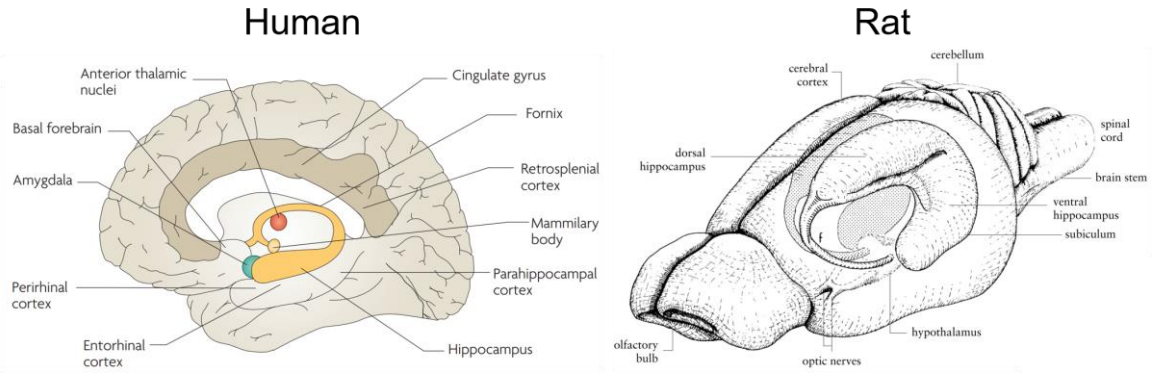


Figure 1.6. The hippocampus in man and rodents. Diagrams modified from Bird and Burgess, 2008 (human) and Amaral and Witter, 1995 (rat) showing the location of the hippocampus within the brain. Licensed from Springer Nature and Elsevier.

1.4.1 Hippocampal Function

As previously discussed, the longest standing association between the hippocampus and behavioural output has been its important role in memory, in particular episodic memory. Recent advances in technology have revealed that episodic memories are formed in the hippocampus through specific networks of cells which are connected by enhanced synaptic plasticity to form memory engrams (Tonegawa et al., 2015). Memory engrams are formed simultaneously in the hippocampus and neocortex but over time the neocortical engrams, with support from the hippocampus, mature while the hippocampal engram strength attenuates over time (Kitamura et al., 2017). Interestingly, through optogenetic manipulation of these hippocampal engram cells, it is possible to implant a false memory into mice whereby a fear memory was implanted to cause freezing in a previously neutral context (Ramirez et al., 2013).

Another characteristic hippocampal property is the ability of pyramidal neurones to form place fields. As an animal moves throughout an environment it has been found that specific cells fire in particular spatial locations with this being hypothesised to form an internal map of the external environment (Moser et al., 2015). Indeed, within London taxi drivers, spatial knowledge has been found to correlate with hippocampal volume (Maguire et al., 2006). This coding of space is also believed to combine with other information to form a compound memory of the place and information that occurred there (Moser et al., 2015). Additional cells that have spatial firing fields, known as grid cells, have been found in the entorhinal cortex (EC) alongside other cell types such as head direction cells and border cells that fire at the edges of the current environment (Moser et al., 2015).

In addition to memory, the hippocampus is strongly implicated in emotional processing and regulation of the HPA response to stressors. fMRI studies in humans have shown that the right hippocampus exhibits increased activity associated with the perception of emotion and the left hippocampus has a role in processing of fear perception (Lindquist et al., 2012). Other studies have reported hippocampal involvement in both fear conditioning and extinction (Shin and Liberzon, 2010). Conditioned emotional learning has also been found to have important roots in hippocampal information processing through collaboration with the amygdala (Labar and Cabeza, 2006). The hippocampus is also a key member of the default mode network, a highly co-ordinated network of brain

regions activated preferentially during rest which has important roles in memory, self-related emotion and decision making amongst a multitude of other responsibilities (Andrews-Hanna, 2012; Buckner et al., 2008). As previously discussed, the hippocampus has an inhibitory effect on HPA axis activity with direct electrical stimulation decreasing circulating corticosteroid concentrations and hippocampal resection having opposing effects (Jacobson and Sapolsky, 1991). The hippocampus is also able to control the ability of stressors to lead to a physiological response with hippocampal stimulation being sufficient to inhibit stress induced CORT release (Jacobson and Sapolsky, 1991). In addition to these important emotional constructs processed by the hippocampal formation, the region has an important function in supporting reward learning (Davidow et al., 2016; Le Merre et al., 2018).

1.4.2 The hippocampus and reward learning

By virtue of both its external connectivity with other regions involved with the processing of reward such as the amygdala and orbitofrontal cortex in addition to intrinsic hippocampal information processing the hippocampal network is an important locus for reward learning (Rolls and Xiang, 2005). In-vivo electrophysiological experiments in primates have found neurones that specifically respond to reward-location pairings with these neurones being able to remap following reward-location contingency reversal (Dupret et al., 2013; Hok et al., 2007; Rolls and Xiang, 2005). Dorsal hippocampus (DH) local field potential (LFP) activity in response to a whisker stimulus preceding reward has been found to generate increased activity over training with inactivation of dorsal hippocampus being able to impair task performance (Le Merre et al., 2018). Within a reward-induced conditioned place preference paradigm in mice, LeGates et al., 2018 found that LTP within hippocampus – nucleus accumbens synapses was integral for reward – location associations and that chronic stress, known to impair reward learning, attenuated synaptic strength. Interactions between the PFC and hippocampus have also been reported to be crucial in spatial reversal learning with unilateral but not ipsilateral inactivation of dorsal or ventral hippocampus and mPFC impairing both discrimination and reversal learning in rats performing a plus maze task (Avigan et al., 2020).

Within humans there is also evidence to the important role of the hippocampus in probabilistic reversal learning. Participants with severe hippocampal damage causing

amnesia were found to be able to learn the initial probabilistic discrimination but upon contingency outcome switching participants perseverated and were unable to switch behaviour to learn the new rule (Shohamy et al., 2009). It has been suggested that the role of the hippocampus during probabilistic reversal learning is to form an internal model of task structure and then use this structure to apply optimal strategies (Vilà-Balló et al., 2017). Patients with hippocampal sclerosis following anterior temporal lobe resection due to epilepsy were reported by Vilà-Balló et al., 2017 to be less able to anticipate the occurrence of reversals compared to controls leading to overall poorer task performance.

1.4.3 The circuit architecture of the Hippocampus

Hippocampal function is underlied by a complex array of brain circuitry consisting of both intra-hippocampal circuitry, allowing transformation of input signal into a computed output, in addition to rich connectivity with a range of brain regions where it acts in a synergistic fashion to co-ordinate complex behaviours.

1.4.3.1 The trisynaptic circuit

Along the dorso-ventral axis of the hippocampus a singular synaptic architecture is conserved, known as the trisynaptic circuit (see Figure 1.7, Andersen, 1975; Andersen et al., 1971. Neurones from within layer II/III of the entorhinal cortex (EC) project via the perforant pathway and synapse onto granule cells within the dentate gyrus (DG, Knierim, 2015; Senzai, 2019). This connection acts to convert a dense cortical signal from the EC into a sparser input for CA3 in a similar manner to how a high-pass filter functions (Cherubini and Miles, 2015). These granule cells give rise to mossy fibre projections which then project to CA3 and synapse onto pyramidal cells. The CA3 network is highly recurrent with 30-70% of pyramidal neurone projections synapsing onto another CA3 pyramidal neurone (Le Duigou et al., 2014). CA3 is believed to calculate associations between elements, therefore enabling representation of event sequences in episodic memory (Farovik et al., 2010). This is in addition to its role in pattern separation whereby overlapping inputs (such as similar contexts) are decoded into more dissimilar outputs (Rolls, 2013). CA3 pyramidal neurones, in addition to their recurrent projections, also project, via Schaffer collateral (SC) axons to the pyramidal neurones of CA1. CA1 also receives a direct input from the EC in the form of temporoammonic

(TA) pathway axons (Andersen et al., 2007). CA1 functions have been suggested to include context decoding in addition to the pattern completion of separate CA3 inputs (Allen et al., 2016; Rolls, 2013). CA1 pyramidal neurones then synapse onto pyramidal neurones in the subiculum, the main output of the hippocampus which in turn projects to a range of cortical and subcortical brain regions in addition to the EC (O'Mara, 2005).

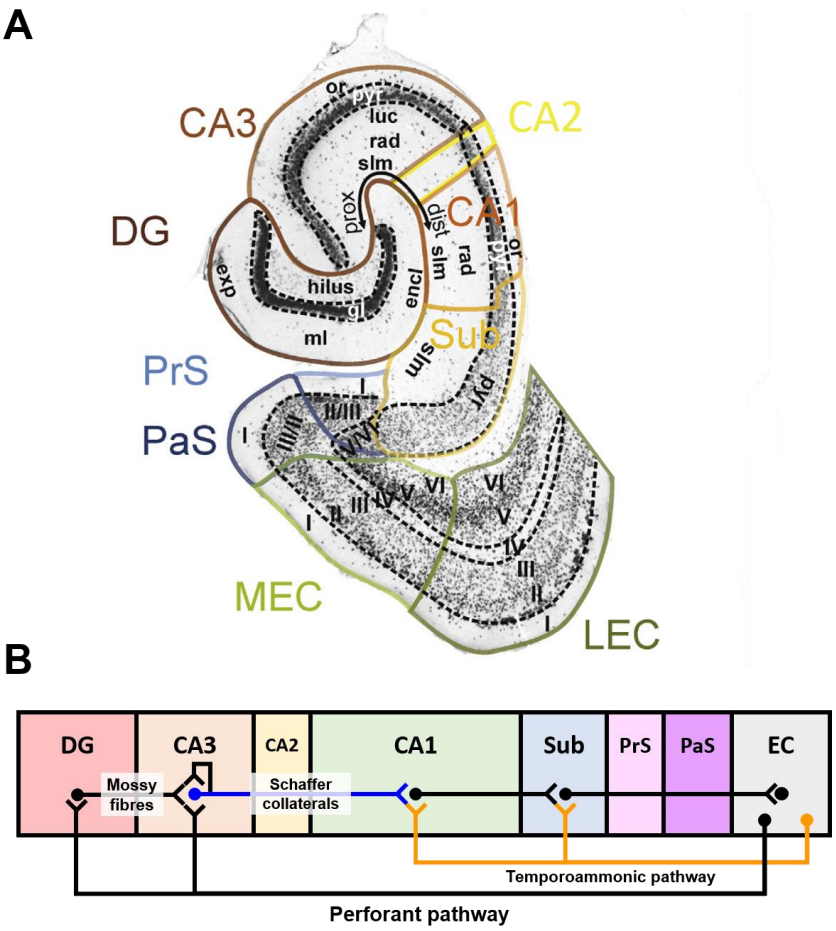


Figure 1.7 Circuit organisation of the hippocampus. (A) NeuN stained rodent hippocampal section showing key subfields and cellular layers. Diagram modified from Cappaert et al., 2015 and licensed from Elsevier. **(B)** Schematic of the trisynaptic circuit made from Anderson et al., 2007. Abbreviations: DG: dentate gyrus, CA3: cornu ammonis region 3, CA2: cornu ammonis region 2, CA1: cornu ammonis region 1, Sub: subiculum, PrS: Pre-subiculum, PaS: Parasubiculum, EC: entorhinal cortex, MEC: medial entorhinal cortex, LEC: lateral entorhinal cortex, py: stratum pyramidale, gl: granule cell layer, exp: exposed blade of the dentate gyrus, ml: molecular layer, or: stratum oriens, luc: stratum lucidem, rad: stratum radiatum, slm: stratum lacunosum moleculare, prox: proximal, dist: distal.

It should also be noted that in addition to the laminar organisation of the trisynaptic circuit along the longitudinal axis of the hippocampus there is also rich connectivity within the transverse axis between layers of the trisynaptic circuit (Knierim, 2015). The neurones of the different hippocampal subfields also exhibit strong coordinated activity patterns in the form of neural oscillations with the main frequencies produced by the hippocampus being theta and gamma waves alongside sharp wave ripples (Colgin, 2016).

1.4.3.2 Cellular organisation of the CA1 hippocampal subfield

As a key controller of hippocampal output, in addition to the amenability of CA3 - CA1 synaptic plasticity to be interrogated through patch-clamp electrophysiology, the CA1 subfield has been the most widely studied region of the hippocampus (Soltesz and Losonczy, 2018). Pyramidal neurones form the main computational unit of the CA1 subfield (see Figure 1.8) and have cell bodies located within a tight cell layer known as the stratum pyramidale (Cappaert et al., 2015). These neurones express axons which travel through the stratum oriens to reach their pyramidal cell targets in the subiculum. CA1 pyramidal neurones have both proximal and distal dendrites which lie in the stratum radiatum (sr) and stratum lacunosum moleculare (slm) respectively. CA1 is also richly innervated with GABAergic interneurons which target pyramidal neurones both proximally and distally. Proximal inhibition by parvalbumin (PV) containing interneurons can regulate CA1 spiking characteristics while distal inhibition by somatostatin (SST) containing GABAergic neurones is a powerful regulator of dendritic conductances and synaptic plasticity (Udakis et al., 2019).

1.4.3.3 Schaffer collateral and Temporoammonic pathways

As previously noted the Schaffer collateral (SC) pathway consists of synaptic projections from CA3 pyramidal cells to the sr of CA1 pyramidal neurones while the temporoammonic (TA) pathway follows a route from the EC to the CA1 slm (Speed and Dobrunz, 2009). SC synapses are rich in both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors and have been well characterised to have a powerful influence over cell output through long term potentiation or depression by a diverse range of mechanisms such as spike time dependent plasticity and dendritic plateau potentials (Collingridge et al., 1983; Palacios-Filardo and Mellor, 2019; Soltesz and Losonczy, 2018). These processes are also well known to be subject to

neuromodulation from a diverse range of mediators such as acetylcholine, noradrenaline, dopamine and 5-HT (see Palacios-Filardo and Mellor, 2019 for review).

The TA pathway, while also in its own right able to express LTP or LTD, albeit via differing mechanisms to the SC pathway (Bhouri et al., 2014), is able to modulate SC evoked spiking in addition to attenuating SC synaptic potentiation (Aksoy-Aksel and Manahan-Vaughan, 2013; Remondes and Schuman, 2002). Due to the larger distance of TA inputs from the soma compared to SC inputs and the attenuation of input signal by distance, dendritic spikes are believed to be the main form of communication between TA synapses and the cell body (Nicholson et al., 2006). These have the ability to be gated by SC synapses in the sr meaning a complex relationship exists between both SC and TA pathways alongside cellular output (Jarsky et al., 2005; Nicholson et al., 2006). TA synapses have been found to have different receptor expression compared to SC synapses with a higher ratio of NMDA to AMPA receptors (Otmakhova et al., 2002), increased expression of dopamine D_1/D_2 and noradrenaline α receptors (Otmakhova and Lisman, 2006) alongside lower pre-synaptic function of N-type Ca^{2+} channels compared to SC synapses (Ahmed and Siegelbaum, 2009). It should also be noted that TA axons also terminate onto inhibitory interneurons in CA1, meaning that it is not clear if the gross effect of TA input into the hippocampus is excitatory or inhibitory (Dvorak-Carbone and Schuman, 1999).

SC and TA pathways are believed to have different functions within the hippocampus with the SC pathway providing internal representations and the TA pathways being responsibly for inputting external representations (Aksoy-Aksel and Manahan-Vaughan, 2013; Eichenbaum and Lipton, 2008). Experimentally it has been found that SC input into CA1 is crucial for memory acquisition and the formation of new place fields while the TA pathways is more specific for memory consolidation and place cell maintenance (Bhouri et al., 2014).

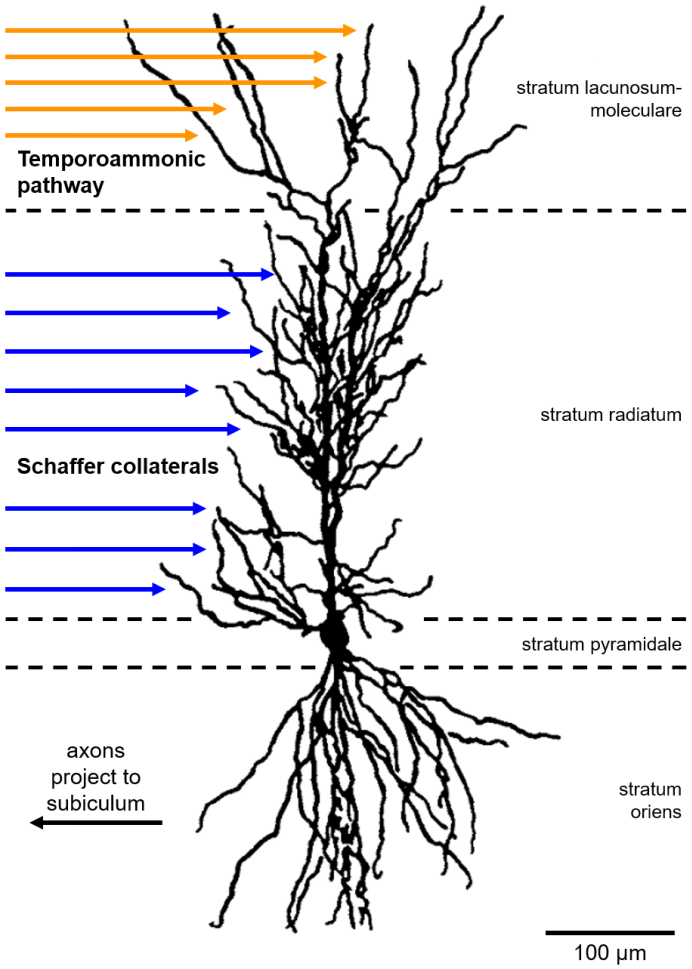


Figure 1.8. Morphology of a rat CA1 pyramidal cell. Adapted from Bannister and Larkman, 1995. Image licensed from Wiley. Arrows represent axon projections that synapse onto the pyramidal neurone. Inhibitory interneurons (not shown) also synapse both onto the cell body and the dendritic arbour (Udakis et al., 2019).

1.4.3.4 Spatial specialisation along the dorso-ventral axis of the hippocampus

While it was once believed that the hippocampus processed information using the tri-synaptic circuit in a unitary fashion along the dorso-ventral axis (rostro-caudal in humans) it is now well understood that there are both profound differences in functional connectivity and intrinsic circuit dynamics between the dorsal and ventral hippocampus (Fanselow and Dong, 2010; Grady, 2019). At a basic level the hippocampus is both responsible for cognition and memory with this primarily being reserved for the dorsal hippocampus (DH) alongside emotional processing and mediation of the stress response being the responsibility of the ventral hippocampus (VH, Fanselow and Dong, 2010). It should be noted however that it is not the case that each area is well defined, more that

there is a gradient of functional and structural change that occurs over the length of the hippocampus (see Figure 1.9).

This functional specification is partially driven by differential brain region connectivity along the dorso-ventral axis. The VH has a denser connectivity with the amygdala, hypothalamic nuclei and IL/PL cortices compared to the DH which has denser innervation with the anterior cingular and retrosplenial cortices (Grigoryan and Segal, 2016). Hippocampal outputs are also spatially segregated with subiculum axons terminating in a dorso-ventral axis in the EC (Moser and Moser, 1998). The VH is also subject to greater neuromodulation with richer cholinergic, dopaminergic, noradrenergic and serotonergic influence than the DH (Grigoryan and Segal, 2016).

In addition to functional connectivity there are also intrinsic differences in CA1 circuit dynamics between the different portions of the hippocampus. Differences in LTP have been found between SC inputs to CA1 pyramidal cells. However, changes have been seen in both directions with results appearing to be dependent upon multiple factors including LTP induction mechanism (Babiec et al., 2017; Kouvaros and Papatheodoropoulos, 2016; Malik and Johnston, 2017). There are also differences that have been reported in intrinsic excitability between the DH and VH with VH neurones being found to be more excitable, possessing higher input resistance and having a more depolarised resting membrane potential (Dougherty et al., 2012).

With regards to functional output, animals with DH but not VH lesions show impairments in spatial memory tasks such as the Morris water maze. Lesions in VH but not DH were found to increase open arm entry in the elevated plus maze (Fanselow and Dong, 2010). Ventral CA1 neurones have also been reported to store social memories with reactivation of a specific neuron being able to restore a previous social memory (Okuyama et al., 2016). It is also important to note that it has been suggested that there are important bilateral differences in hippocampal function whereby the left hippocampus stores spatial locations discretely and the right hippocampus stores space in a continuous manner (Jordan, 2020).

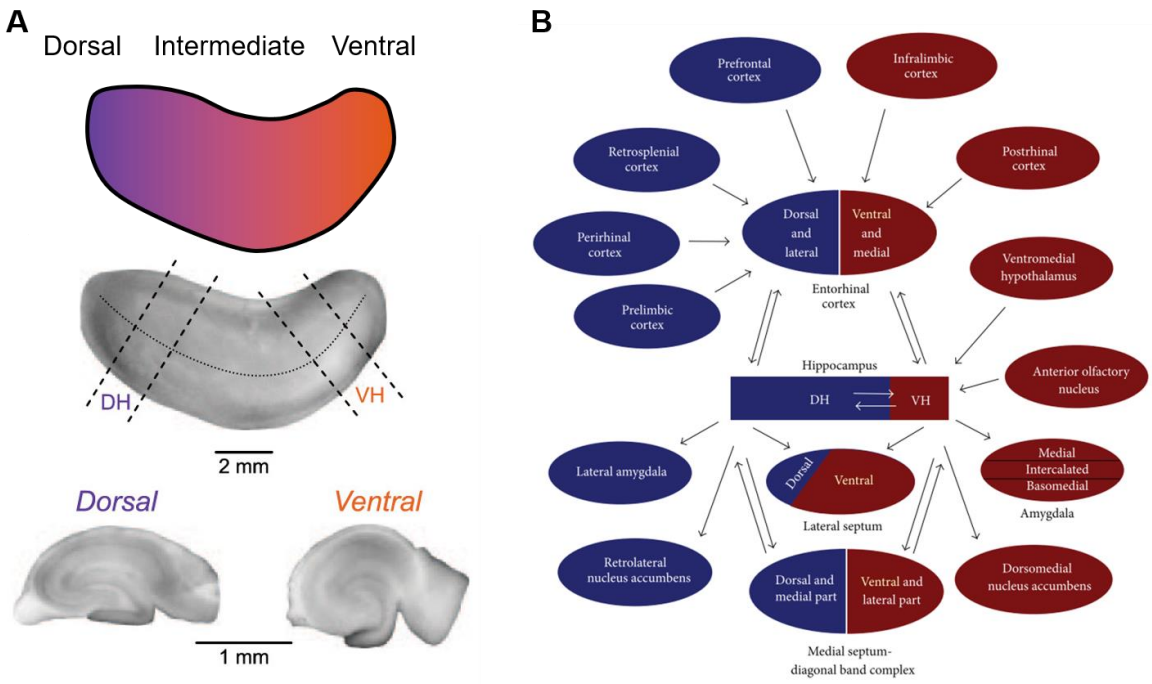


Figure 1.9. The dorso-ventral axis of the hippocampus. (A) Example photographs of rat hippocampal sections from the dorsal and ventral hippocampus alongside diagram showing gradient of dorsal-ventral changes across the brain region. Adapted from Tidball et al., 2017. **(B)** Schematic showing the differential connectivity between DH/VH and other brain regions. Reproduced from Grigoryan and Segal, 2016 under a creative commons license.

1.4.3.5 Hippocampal neurogenesis

The hippocampus, alongside the subventricular zone and olfactory bulb, is unique within the brain in possessing the ability to form new neurones in adulthood (Eriksson et al., 1998; Lledo and Valley, 2016). Progenitor cells within the subgranular zone (SGZ) of the DG divide and then their progeny migrate to the granule cell layer where they differentiate into mature neurones (Andersen et al., 2007). These adult born granule cells have been most heavily implicated as having an important role in pattern separation (Aimone et al., 2011). Evidence from animals performing hippocampal dependent tasks suggests that impairing neurogenesis has the greatest impact on performance when new or conflicting information is presented to animals (Toda et al., 2019). This conflicting input would be expected to require robust pattern separation to interpret. Other functions for neurogenesis have also been suggested including adult

born granule cells being able to encode environmental features from during their development, a period where they exhibit increased intrinsic excitability and plasticity (Aimone et al., 2011). Finally a role for neurogenesis in longer term memory has been suggested with mice exhibiting enhanced neurogenesis having memory deficits when required to remember a context over 6 weeks compared to controls with normal neurogenesis levels (Akers et al., 2014).

As previously discussed, neurogenesis has been implicated in the aetiology of MDD with mice manipulated to have inhibited adult neurogenesis showing a decreased immobility latency in the forced swim test and decreased sucrose preference (Brewer et al., 2011). Additionally, chronic conventional antidepressant administration has also been found to increase neurogenesis within the DG of adult rats (Malberg et al., 2000). Neurogenesis is also modulated in response to environmental input with rodents housed in enriched environments and provided voluntary exercise having being found to have increased neurogenesis and spatial learning ability (Kempermann et al., 1997; Toda et al., 2019).

1.4.4 Information processing alterations following ELS

As previously discussed the hippocampus has an extended developmental trajectory, not completing until adolescence, in addition to having high glucocorticoid receptor expression. This means that it is particularly susceptible to the effects of early life stress (Gómez and Edgin, 2016; Tottenham, 2009).

1.4.4.1 Morphological changes

One of the most striking effects of ELS upon the hippocampus that have been uncovered are changes in gross morphology. Reduced left hippocampal volume (Frodl et al., 2010; Vythilingam et al., 2002), CA3 and DG volume (Teicher et al., 2012), CA1 volume (Dahmen and Puetz, 2018) and reduced overall hippocampal volume (Pechtel and Pizzagalli, 2011; Rao et al., 2010) have all been reported in participants with a past history of childhood adversity. Within animal models similar findings have also been reported with a loss of left dorsal hippocampal volume found in rats bred using a LNBM model (Molet et al., 2016).

Underlying these changes in hippocampal volume are a wide range of microstructural changes resulting from stress exposure in early life. In addition to reduced neuronal counts and dendritic complexity in CA1 and CA3 (Cui et al., 2020; Molet et al., 2016), a reduction in overall synaptic density, increased dendritic turnover, reduced BDNF expression and reduced mossy fibre density has been reported in animal models of ELS (Bath et al., 2017; Huot et al., 2002; Maccari et al., 2014).

1.4.4.2 Basal neurotransmission

Effort has been undertaken to understand the effects of these morphological changes upon functional electrophysiological measures which underly information processing within the hippocampus. Intrinsic measures of excitable cell function such as AMPA / NMDA receptor ratio and spiking characteristics are important mediators of hippocampal function. A recent systematic review found no effects of ELS models upon basal neurotransmission in CA1 and the DG (Derks et al., 2017). However, such reviews are always limited by the necessity to combine multiple factors to allow comparison between studies. Individual studies have reported separate interesting findings which deserve review. A reduction in NMDAR mediated signalling relative to AMPAR function in CA1 has been reported following application of a LNBM model in mice (Pillai et al., 2018). Within the offspring of low licking-grooming rats there have also been region specific alterations in intrinsic excitability reported. Within the VH only low LG animals showed a more hyperpolarised resting membrane potential in addition to a more hyperpolarised action potential (ap) threshold and increased ap amplitude (Nguyen et al., 2015). These ventral neurones have also been found to be more excitable through analysis of excitatory postsynaptic potential to spike coupling (Nguyen et al., 2015). Alterations in GABAergic transmission within the hippocampus have also been observed both developmentally and in adulthood. Maternal separation appears to delay the GABA switch (Furukawa et al., 2017), the process by which GABAergic transmission changes from being excitatory to inhibitory in development, alongside also modulating expression of $GABA_A$ receptors on interneurons (Sterley et al., 2013; Yang et al., 2016).

ELS has also been found to affect hippocampal oscillations with postnatal stress being found to increase theta power in the ventral hippocampus during active behaviour (Murthy et al., 2019). This finding was coupled with reduced PV and SST cell densities in the ventral dentate gyrus with these neuronal types being crucial in regulating

hippocampal oscillations (Amilhon et al., 2015; Murthy et al., 2019; Stark et al., 2013). Increases in theta-gamma coupling were also observed, another process also reliant upon functional inhibitory transmission (Wulff et al., 2009). This increased theta power has also been observed during rapid eye movement sleep (Sampath et al., 2014). Many of these effects are also only apparent in adulthood suggesting complete neurodevelopment is required to unearth some of the impacts of ELS upon the hippocampus (Murthy and Gould, 2020).

1.4.4.3 Long term potentiation

Perhaps the most interesting line of investigation has been into the effects of ELS on both long-term potentiation (LTP) and long-term depression (LTD) due to these processes being believed to be crucial to the hippocampus's ability to store information (Collingridge and Bliss, 1987). The previously mentioned systematic review in respect of basal neurotransmission also assessed the effect of ELS models upon LTP in CA1 and the DG (Derks et al., 2017). This study concluded that ELS affected LTP in both CA1 and DG with the DG more affected in addition to observing differential effects of ELS model with LNBM and low-licking grooming animals showing impaired LTP while MS models had no effect. However, none of the included studies assessed the interaction between hippocampal aspect (DH vs VH) and due to the previously discussed crucial differences in LTP between DH and VH in order to understand the effect of ELS upon hippocampal LTP it is crucial that this is considered. Due to the relative recency of appreciation for this there are few studies that include this factor. Within the low LG model of ELS Nguyen et al., 2015 reported decreased LTP in the DH compared to controls in addition to enhanced LTP in the ventral hippocampus. This switch in plasticity from DH to VH has also been reported in a range of other stress models including pre-natal stress (Grigoryan and Segal, 2013), juvenile stress (Grigoryan et al., 2015; Maggio and Segal, 2011) and adulthood stress (Maggio and Segal, 2011). Changes in LTP appear to be stable and persist late into adulthood with one study finding impairments in SC synapse LTP in 70 week old rats bred in a MS model (Sousa et al., 2014). Post-mortem studies in an early parental deprivation model of ELS in marmosets have also observed decreased expression of genes associated with synaptic plasticity such as GAP-43 (Law et al., 2009). Another important protein involved in synaptic plasticity, GluN2B has also been found to decrease in expression following ELS in mice (Lesuis et al., 2019).

Interactions between LTP and modifying factors have also been investigated. Although Bagot et al., 2009 did not assess the role of hippocampal aspect, an interesting finding was that LTP in DG granule cells was lower in low LG offspring but that this was rapidly improved by CORT application or ß adrenoreceptor stimulation suggesting that ELS causes increased DG plasticity in response to stress. While not early life stress, interesting results were still observed in a model of juvenile stress where the action of isoproterenol (ß adrenoreceptor agonist) was enhanced in VH slices but impaired in DH slices compared to controls suggesting lasting changes in sensitivity to noradrenergic neuromodulation (Grigoryan et al., 2015). Increased cholinergic fibre density and acetylcholinesterase expression has also been observed in the CA1 of MD rats (Markovi et al., 2014) suggesting alterations in LTP regulation through cholinergic neuromodulation (Palacios-Filardo and Mellor, 2019).

These data suggest that stress in early life is able to reduce LTP in the DH while concurrently leading to enhancement in the VH when using a high frequency stimulation induction protocol. However, due to the marked impact of induction protocol upon LTP outcome (Babiec et al., 2017; Buchanan and Mellor, 2007; Malik and Johnston, 2017) it is not possible to state with any confidence what the effects of ELS are when a more physiologically relevant LTP induction protocol is applied.

1.4.4.4 Neurogenesis

Decreases in neurogenesis within the dentate gyrus have also been extensively observed in animal models of ELS (Korosi et al., 2012; Naninck et al., 2015; Oomen et al., 2010; Stuart et al., 2019). Interestingly transient increases in neurogenesis have been reported in both MS and MD models at weaning; however these changes rapidly reverse (Korosi et al., 2012). The effects of ELS upon neurogenesis appear to be both region specific and stage specific to neurogenesis. Maternal separation has been found to reduce cell proliferation but not survival and differentiation while maternal deprivation has been reported to decrease proliferation across the whole hippocampus while decreases in survival and differentiation were selective to the ventral hippocampus (Korosi et al., 2012).

1.4.4.5 Functional activity

Hippocampal dependent tasks in animal models allow the assessment of the ultimate output of the hippocampal formation: behaviour. LNBM animals have been found to have impairments in hippocampal processing tasks such as location memory (Short et al., 2019), novel object recognition (Rice et al., 2008) and Y-maze tasks (Hoeijmakers et al., 2015; Wang et al., 2011). Interestingly, utilising the Morris water maze, a popular hippocampal dependent task there is a lack of consistent results with multiple studies reporting impairments and a similar number the contrary (Kosten et al., 2012). Interestingly this suggests that the effect of ELS might be specific to different hippocampal functions as opposed to a general impairment.

Within humans, fMRI has been able to provide additional insights into hippocampal function following ELS. Children previously exposed to violence were reported to have reduced hippocampal activation when asked to remember contexts paired with angry faces (Lambert et al., 2017). Changes in hippocampal activity compared to controls have also been reported during emotional processing, responding to socio-affective stimuli and memory (Kraaijenvanger et al., 2020). For example, maltreated children have been found to exhibit reduced hippocampal activation when asked to recall positive autobiographical memories (McCrory et al., 2017). With regards to memory, children exposed to violence have been found to show associative memory impairments that correlate with reduced hippocampal recruitment (Lambert et al., 2019).

At present there have not been any studies conducted in man investigating the functional activity of the hippocampus during reward learning within participants with a history of early life stress.

1.5 Summary

Major depressive disorder is a debilitating disease both for society and the individual with a complex aetiology formed of multiple interweaving causative factors. These factors include genetics, neurotrophic changes and inflammation; however, it appears that stress in early life has the highest importance. Early life stress re-programs the developing brain with the hippocampus being a key region of interest due to its high susceptibility to glucocorticoids and having an important role in reward processing. Multiple alterations in hippocampal processing are known to be a result of stress in early life with these being hypothesised to impact reward learning ability. Reward learning impairments, as a result of hippocampal dysfunction in addition to prefrontal and basal ganglia alterations, are hypothesised to directly lead to the development of depression in populations exposed to early life stress but who are otherwise healthy. By further investigating the links between hippocampal function, reward learning and stress in early life this will provide valuable insights into the mechanisms of depression aetiology that may lead to either future prophylactic or treatment strategies.

1.6 Thesis Aims

- Mice provide an ideal model species for neuropsychological research due to their low cost and ease of genetic modification. However little research has been conducted into the effects of ELS on reward learning within this species and the disease models are poorly validated. In Chapter 2 it was aimed to assess the phenotype of mice bred in a limited nesting and bedding material paradigm in addition to assessing neurogenesis as a biomarker of ELS. Reward learning was also assessed, utilising the 2vs1 affective bias task as a well validated task measuring reward-induced learning biases.

- Translational behavioural tasks allow the comparison of behavioural responses between man and model species such as rodents. The probabilistic reversal learning task (PRLT) is a translational reward learning task that has been used both in humans and to a limited degree in rodents. In Chapter 3 it was aimed to pharmacologically evaluate the PRLT with a range of antidepressant, dopamine modulating and pro-depressant compounds in rats in order to further understand the neurobiology underlying this behavioural task in both humans and animals.

- Early life stress has been found to lead to wide ranging alterations in hippocampal information processing which may underly ELS mediated reward learning impairments. However previous studies have never investigated this phenomenon in detail with a lack of appreciation of crucial factors such as hippocampal aspect, sex and input pathway. In Chapter 4 it was aimed to assess both basal neurotransmission and synaptic plasticity in ELS rats bred in a maternal separation paradigm. To assess successful induction of an ELS phenotype a brief behavioural and biochemical characterisation of the model was also undertaken.

- Reward learning impairments have been seen in participants with a history of early life stress, however this has never been assessed using the probabilistic reversal learning task. In Chapter 5 it was aimed to assess the reward learning ability and feedback sensitivity of human participants with a history of ELS in the PRLT compared to controls with no ELS in addition to the response bias probabilistic reward task as a comparator.

Chapter 2

Behavioural and physiological characterisation of the limited nesting and bedding material model of early life stress in mice

2.1 Introduction

Animal models of early life stress are useful to understand the mechanisms underlying the predisposition to mental health disorders following ELS. Within rodents, the maternal separation and maternal deprivation models have been the most widely applied, however most research has been carried out in rats. Mice provide several advantages over rats for neuropsychological research including their ease of genetic modification, availability of transgenic lines and lower cost (George et al., 2010). Nevertheless, mice present a challenge for use in ELS research as previous studies have suggested that maternal separation does not produce consistent behavioural changes congruent with those seen in rats (Millstein and Holmes, 2007). Notably this includes a lack of increased anxiety like behaviour measured by the elevated plus maze, open field test and light-dark box in addition to no difference in immobility latency in the forced swim test. A key driver of this lack of efficacy appears to be that maternal separation in mice leads to increased maternal care upon cessation of separation (Millstein and Holmes, 2007) which appears able to counteract the negative effects of the separation.

In order to overcome these limitations, Rice et al., 2008 developed the limited nesting and bedding material (LNBM) model as a naturalistic model of scarcity induced ELS. Within the model, animals are exposed to sparse environmental resources through reduced nesting material and use of a metal grid cage floor from postnatal day (PND) 2 to PND9. This leads to fragmented maternal care with dam sorties from the nest correlating with pup basal plasma CORT concentrations (Rice et al., 2008). However, while the phenotype resulting from maternal separation in rats is well characterised, including increased anxiety, decreased granule cell proliferation in the dentate gyrus and HPA axis hyperactivity (Bonapersona et al., 2019; Francis et al., 2002; Mirescu et al., 2004; Stuart et al., 2019), there is much less consensus in the mouse LNBM literature, hampered by a lack of studies. Previous studies have reported inconsistent effects upon anxiety, neurogenesis and HPA axis activity (Arp et al., 2016; Kanatsou et al., 2017; Kohl et al., 2015; McIlwrick et al., 2016; Naninck et al., 2015; Rice et al., 2008; van der Kooij et al., 2015; Wang et al., 2012; Youssef et al., 2019). There is also a lack of studies investigating the effects of the LNBM model upon reward learning or affective processing, an interesting omission considering the crucial role of ELS in predisposing for affective disorders. However, it should be noted that subsequent to the current study, Goodwill et al., 2018, reported that limited nesting and bedding material during

development leads to reversal learning deficits that are female specific. LNBM females required more trials to meet the criterion for changing task phase and subsequently made more errors following this rule change.

As discussed in detail (see chapter 1) impaired reward learning is hypothesised to be an intermediate phenotype in ELS animals. The affective bias task (ABT) is a task normally used to compare the relative value of the same reward under different affective manipulations but has been readily modified to assess reward induced positive biases by requiring animals to learn two different reward magnitude-substrate combinations (2vs1 reward-learning assay (RLA)). Animals are then provided with both substrates equally rewarded and show a bias towards the previously more highly rewarded substrate (Stuart et al., 2013). Rats bred under a maternal separation paradigm have been found to lack this ability to form a reward induced positive bias (Stuart et al., 2019; Stuart and Robinson, 2016). These findings are similar to those observed in animals chronically treated with either the immune modulator interferon-alpha or pro-depressant treatment with retinoic acid (Stuart et al., 2017). This task has also been previously adapted for use in mice (data unpublished) and presents an opportunity for assessment of reward learning deficits in LNBM mice.

In this chapter a cohort of mice were bred to undergo the LNBM of ELS. In order to validate that LNBM mice show a similar phenotype to previous rat models of ELS, animals completed a battery of behavioural tests followed by biochemical analysis similar to experiments previously carried out in rats (Stuart et al., 2019). To assess reward learning ability, LNBM mice were also trained and tested in the RLA. By validating the LNBM mouse model as aetiologically relevant to the core hypothesis then this would enable its use in future electrophysiological and behavioural experiments where it would be possible to dissect in detail the links between early life stress, reward learning and hippocampal electrophysiological dysfunctions.

2.2 Chapter Aims

- Generate a cohort of animals exposed to the limited nesting and bedding material model of early life stress for validation experiments

- Biochemically and behaviourally assess the arising phenotype and compare findings with other models of ELS

- Assess if LNBM mice have a reward learning deficits as previously observed in maternally separated rats.

This work was completed between 2016 and 2017.

2.3 Methods

2.3.1 Animals

A total of 12 C57BL/6J mice (Charles River, UK) aged between PD56 and PD62 and their 47 offspring were used. Animals were housed in a temperature-controlled room with a 12:12h lighting cycle (lights off at 19:00) in addition to ad-libitum access to food (Purina, UK) and water. After weaning ELS and control animals were housed in groups of 2 to 3 by sex and by litter under reverse lighting (lights on at 20:00) with all behavioural experiments being carried out during the animal's active phase (08:00 – 20:00). Animals were not provided with enrichment except for a red Perspex house due to evidence that environmental enrichment can reverse the phenotype of ELS (Francis et al., 2002). For RLA experiments animals were mildly food restricted (3g per mouse daily) to not less than 90% of their free-feeding weight matched to a normal growth curve. All experiments were undertaken in accordance with local institutional guidelines and the UK Animals (Scientific procedures) Act of 1986.

2.3.2 Early Life Stress Procedure

Mice were bred in-house using the limited nesting and bedding material (LNBM) model of ELS as previously described by Rice et al., 2008. 4 male and 8 female animals were housed as trios (1 male and 2 females per cage) and left undisturbed for 14 days. Once animals were visibly pregnant females were individually housed. Dams were observed every 12 hours for the birth of pups with PND0 being termed the day of birth. On the morning of PND2 all litters were culled to no more than 6 pups before randomisation to either control or experimental cages (see Figure 2.1A for overview of LNBM protocol).

Control cages contained standard amounts of sawdust bedding (≈650ml) in addition to a 5x5cm square of nestlet bedding material (see Figure 2.1B). LNBM cages contained an aluminium mesh platform elevated 2.5cm above the cage floor covering the entire floor area of the cage. Below this platform was a small amount of sawdust bedding (≈60ml) and cages were also provided with 2/3 by area of the nestlet bedding material compared to control cages. Animals were left completely undisturbed from PND2 to PND9 and on the morning of PND9 all animals were transferred to fresh control cages before being weaned at PND21 and housed by sex and by litter.

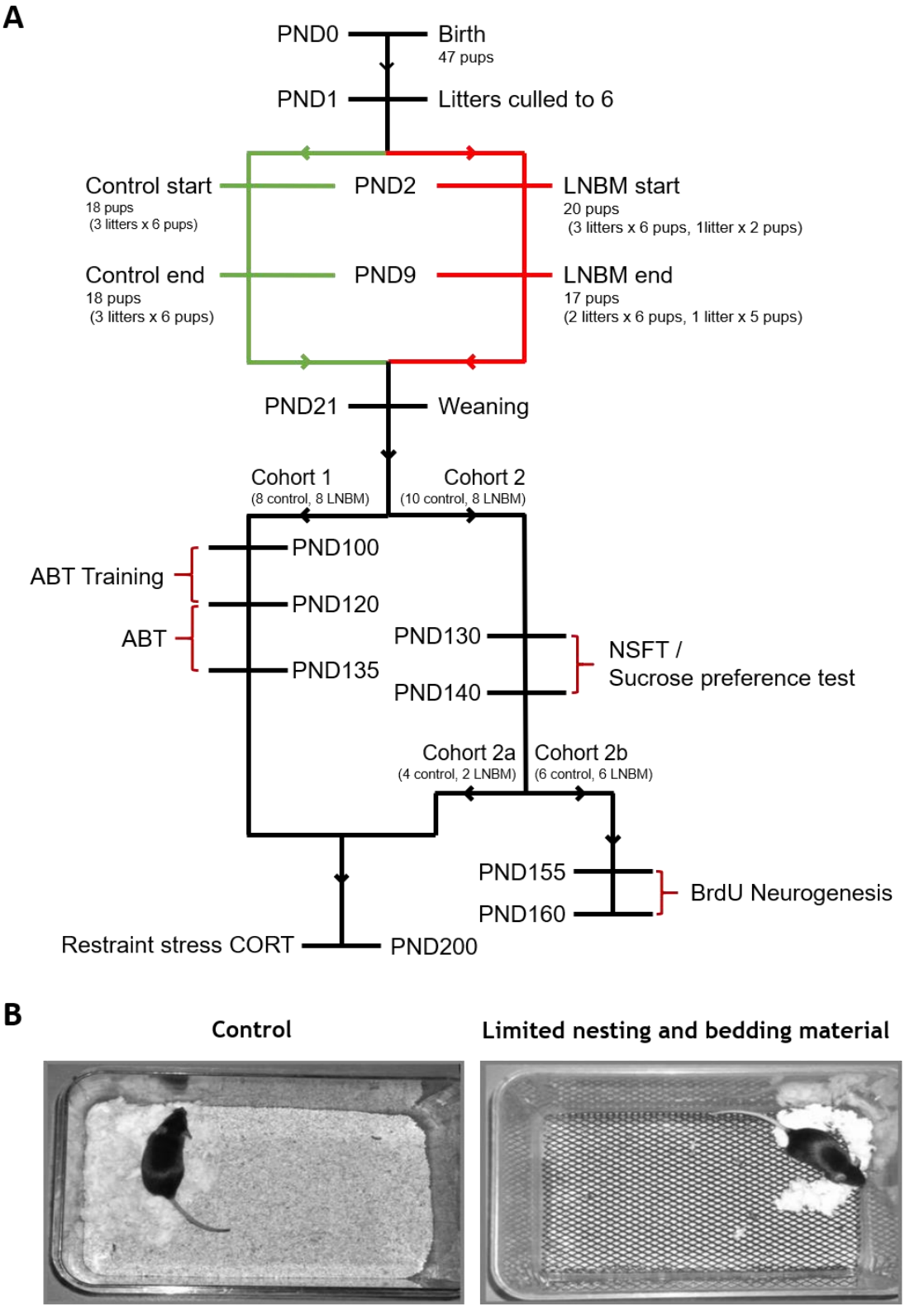


Figure 2.1 Overview of the LNBM model and study design. (A) Overview of the experimental protocol for animals in this study showing the approximate ages of animals when they completed each experiment. **(B)** Example photographs of a control (left) and a limited nesting and bedding material (right) cage reproduced from Rice et al., 2008. Licensed from Oxford University press.

Three mice from the ELA group were found dead between PND2 and PND9. From PND30 all animals were weighed weekly and at PND100 animals were split into two experimental cohorts (see Figure 2.1A). Cohort 1 was trained in the RLA while cohort 2 completed the sucrose preference test (SPT) and novelty supressed feeding test (NSFT). A subset of cohort 2 animals were killed following BrdU injections to measure neurogenesis and the remaining cohort 2 animals combined with cohort 1 to be used in terminal restraint stress corticosterone (CORT) measurements. From PND100 onwards the experimenter was blind to the condition of the animals for all experiments and cohort allocation was random.

2.3.3 Behavioural Testing of LNBM Animals

2.3.3.1 Sucrose Preference

Animals were provided with two bottles (Anacare, UK) per cage of 1% sucrose in water for two days before then being returned to standard water bottles for a single day. Animals were then water restricted for 4 hours and singly housed for an hour before testing. Mice were then provided with one bottle of 1% sucrose in water and one bottle standard drinking water for an hour with bottle positions being swapped after 30 minutes. Sucrose preference was defined as:

$$\text{Sucrose preference } (\%) = \frac{\text{Sucrose consumed}}{\text{Sucrose consumed+water consumed}} \times 100 \qquad \text{Eq 2.1}$$

2.3.3.2 Novelty Supressed Feeding Test

Animals were tested in a novel 40cm^2 Perspex arena containing a clear glass bowl filled with rodent chow (Purina, UK) with a tray liner covered floor. Animals were food deprived for 24 hours prior to testing. Individual animals were placed in the corner of the arena and the latency to approach the bowl and the latency to eat from the bowl were recorded. Animals were removed from the arena either 30 seconds after eating or after a cut off time of 10 minutes had elapsed. Between animals the tray-liner was changed, and the bowl cleaned and refilled.

2.3.3.3 Reward learning assay

Cohort 1 animals were trained in the RLA following the training protocol previously described by Stuart et al., 2013. Animals were trained in a modified arena (40cm2) containing a dividing wall placed between two bowls. Following habituation to the arena, mice first had to learn to dig in sawdust contained in two bowls to receive 20 mg reward pellets (Test Diet, Sandown Scientific, UK). Animals then proceeded to discrimination training as the next phase of the protocol. In this stage animals were presented with two substrates and had to learn to dig in only one of these to receive reward. If animals dug in the rewarded substrate 6 times consecutively (up to one omission was allowed as part of this criteria) then this concluded their session otherwise animals completed 20 trials. On the first session of discrimination training animals were presented with paper bedding containing a reward pellet and wool which served as the blank substrate. On session 2 animals had to learn a new substrate reward pairing (moss: 1 pellet, beech leaves: blank). Due to the poor session 2 performance of animals they were permitted to explore both bowls for the first 6 trials on session 3 to improve performance. Animals that met criterion on session 3 (10/16 animals) were moved to a new substrate-reward pairing on session 4 (coconut fibre: 1 pellet, beech leaves: blank) and then on session 5 all animals were switched pairings again so that animals that previously had a coconut fibre – 1 pellet pairing moved to moss – 1 pellet and vice versa for those animals remaining on the old moss – 1 pellet pairing. For sessions 4 and 5 animals were only permitted to explore both bowls for the first trial. Animals then proceeded to the main RLA protocol (see Figure 2.2).

On day 1 of the RLA animals were placed into the arena where one bowl contained a specific digging substrate (substrate A) containing 2 pellets and the other contained a different substrate containing only a crushed sugar pellet (blank substrate) to prevent scent-based discrimination. An individual trial is defined as the mouse being placed into the arena and making a choice of which substrate to dig in. Once a decision has been made the other substrate is taken away and the mouse given time to find the pellet (or explore the blank) before the mouse is then removed from the arena. Animals were allowed up to 20 trials to meet the criterion of 6 consecutive choices for the substrate paired with reward and any trial where mice took over 15s to make a choice was classed as an omission. On day 2 this is repeated with the same blank substrate but using substrate B containing only one pellet instead of substrate A. On days 1 and 2 animals could explore both substrates for the first trial only. These two pairing days were repeated to give pairing days 3 and 4 (see Figure 2.2). On day 5 mice were presented with both substrate A and substrate B for 30 trials with animals given a free choice

between substrates and the latency to dig recorded. Again, the unchosen substrate was taken away. During the preference test each trial was randomly reinforced with one pellet (1 in 3 reward probability per substrate) so that the objective reward value of each substrate during the preference test was identical. Following the preference test an additional two pairing sessions and a preference test was conducted to alleviate the possibility that animals had not had enough pairing sessions to learn the reward induced cognitive bias. All variables were fully counterbalanced between animals and sessions.

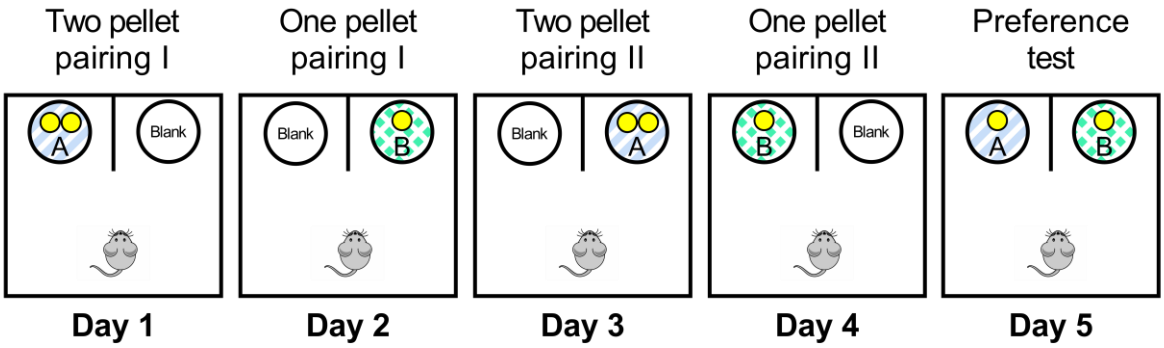


Figure 2.2 Overview of the reward learning assay. Animals learn on days 1 and 3 to associate substrate A with high reward and on days 2 and 4 to associate substrate B with low reward. On day 5 animals are preference tested and should show a bias to the more highly rewarded substrate. All bowl positions, pairing order and substrate-reward pairings are completely counterbalanced between animals.

2.3.4 Biochemical Analysis of LNBM animals

2.3.4.1 Neurogenesis

A subset of animals from cohort 2 (6 control, 6 ELS) were intraperitoneal (i.p.) injected 4 times with 50mg/kg 5-bromo-2'-deoxyuridine (BrdU) in 0.9% saline every 2 hours before then being trans-cardiac perfused 24 hours after the final injection with first phosphate buffered saline (PBS) then 4% paraformaldehyde (PFA) in PBS. Brains were removed, post fixed in 4% PFA for 24 hours and then sunk in 30% sucrose prior to sectioning. Brains were sliced into coronal sections using a freezing microtome (Bregma = -1.00 to –4.00 mm) before slices were stored in PBS containing 0.9% sodium azide prior to immunohistochemistry.

Slices were first washed in PBS containing 0.2% tween20 (PBS-T) before being incubated in 2M HCl at 37°C for 30m. Sections were then incubated in 0.1M sodium tetraborate (2

x 5m) before being again washed in PBS-T (3 x 5m) and then incubated in 0.05M glycine for 30m. After another PBS-T wash (3 x 5m) sections were blocked in immunobuffer (3% goat serum in PBS-T) for 90 minutes before then being incubated overnight in primary antibody (rat anti-BrdU @ 1:1000 dilution in immunobuffer, ab6326 (abcam, UK)). After the overnight incubation slices were again washed in PBS-T (3 x 5m) before then being incubated for 2 hours in secondary antibody (goat anti-rat alexa fluor 488 @ 1:1000 in immunobuffer, ab150157 (abcam, UK)). Finally, slices were washed (3 x 5m) in PBS-T again, incubated in 166ng/ml 4′,6-diamidino-2-phenylindole (DAPI) in PBS for 30m and washed again in PBS (3 x 5m) before being mounted and imaged. $BrdU^+$ cells were counted manually in both hippocampi from each section and additionally the dorso-ventral location of each slices was determined by comparing slice morphology individually to a brain atlas (Franklin and Paxinos, 2007).

2.3.4.2 Restraint Stress Corticosterone

Animals were either assigned to receive 30m restraint stress or no stress in a between subject study design. Animals assigned to the stress condition (6 control, 5 ELA) were placed into a modified 50ml falcon tube containing breathing holes in a clean standard cage for 30 minutes and then were removed and immediately stunned and exsanguinated with blood collected into tubes containing 0.5M EDTA. Animals under control conditions (6 control, 5 ELA) were immediately removed from their home cage and killed in the previously described way. Blood samples were stored on ice before being centrifuged at 6000rpm for 15 minutes to collect plasma which was then immediately snap frozen in dry ice. The Thymus gland and both adrenal glands were dissected from mice and then weighed. Plasma Corticosterone was measured using a radio-immuno binding assay as has been previously described (George et al., 2017).

2.3.5 Statistical Analysis

Sample size was estimated from a previous study in rats doing a similar experimental design (Stuart et al., 2019). Statistical analysis was carried out using in GraphPad Prism 8 (GraphPad, US) and IBM SPSS Statistics 24 (IBM, US). All graphs were constructed using GraphPad Prism 8 and data is presented as mean ± standard error. Outlier detection was conducted blind to condition using Grubbs' test. The experimental unit was always taken as an individual mouse.

Unless otherwise stated, where graphs are presented as four groups (control male, control female, LNBM male, LNBM female) data were first assessed for normality utilising the Shapiro-Wilk and Kolmogorov-Smirnov tests before being analysed as two-way ANOVAs with the factors sex and condition. Post-hoc analysis was conducted using Sidak's correction. Where data were not normally distributed efforts were first made to transform data to normality and where this was not possible data were analysed utilising Mann-Whitney tests.

Bodyweight data were fit using a Gompertz growth curve via non-linear least squares fitting then the extra-sum of squares test was used to assess if data were better fit by a single joint fit or as separate fits for each condition. For sucrose preference test and RLA outputs, data were compared to a hypothetical mean by use of one-sample t-tests. RLA pairing data were analysed as a three-way repeated measures ANOVA with factors condition, sex and session (repeated). Before any repeated measures analysis all data were first assessed for violations of Sphericity with Mauchly's test. Where repeated measures data were not normally distributed a Friedman test was used instead. Win-stay and lose-shift behaviours were calculated (see chapter 3.3.5 for more detail) as the probability that if an animal received reward it returned to the same substrate in the RLA for the next trial and conversely if it received no reward that it chose the opposite substrate for the next trial respectively. Correlations between RLA preference tests and between hippocampal bregma level and $BrdU^+$ cells were carried out using linear least squares regression. Finally, 3-way ANOVAs were used to analyse overall $BrdU^+$ cell populations (factors: sex, condition and region (within subject)) and CORT response to restraint stress (factors: sex, condition, stress). All data is shown as mean ± SEM. * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

2.4 Results

2.4.1 Body mass through development

The bodyweight of mice was monitored weekly from approximately PND30 to PND120. Both male (Figure 2.3A) and female (Figure 2.3B) animals bred under the LNBM protocol had significantly different growth curves compared to their control counterparts (Extra sum of squares test; Males: $F_{3,158} = 14.21$, $p < 0.0001$; Females: $F_{3,152} = 54.16$, $p < 0.0001$). When animal weights were compared at PND100, an approximate timepoint where mice are widely considered adults (Jackson et al., 2017), LNBM animals again had a reduced body weight (2-way ANOVA; main effect of condition: $F_{1,28} = 11.06$, $p = 0.002$; main effect of sex: $F_{1,28} = 137.5$, $p < 0.0001$).

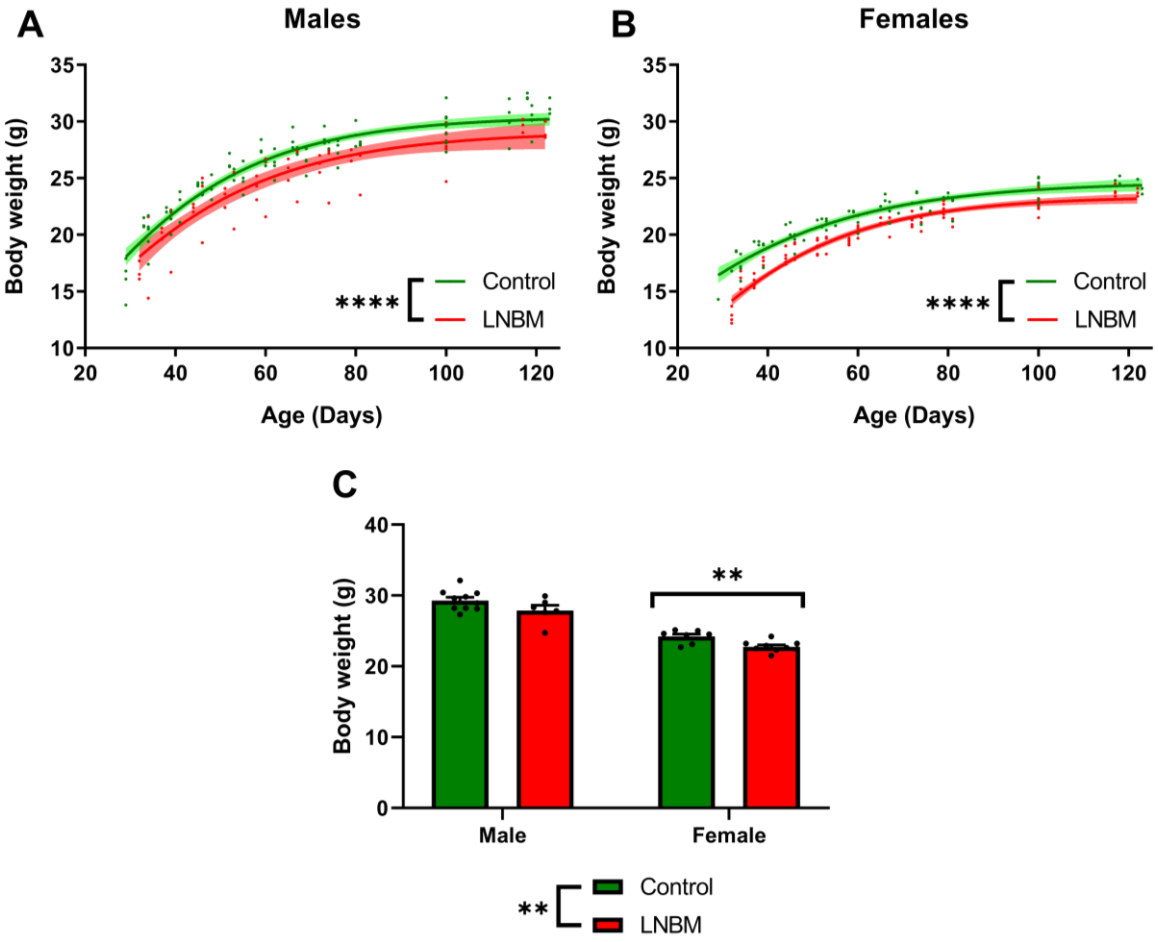


Figure 2.3 Body weight across development in LNBM and control mice. Body mass between approximately PND30 and PND120 in male **(A)** and female **(B)** mice. Data were fit with a Gompertz growth curve. The shaded area indicates 95% confidence interval of the line of best fit. **(C)** Body mass at PND100. N = 17 control (10 male, 7 female) and 15 LNBM (6 male, 9 female).

2.4.2 Novelty supressed feeding test

Animals completed the novelty supressed feeding test, a common test of anxiety requiring animals to overcome neophobia to receive food. There was a trend for LNBM animals to require more time to approach the bowl (Figure 2.4A, 2-way ANOVA, $F_{1,13}$ = 3.88, p = 0.071) while there was no difference between groups in the latency to feed (Figure 2.4B).

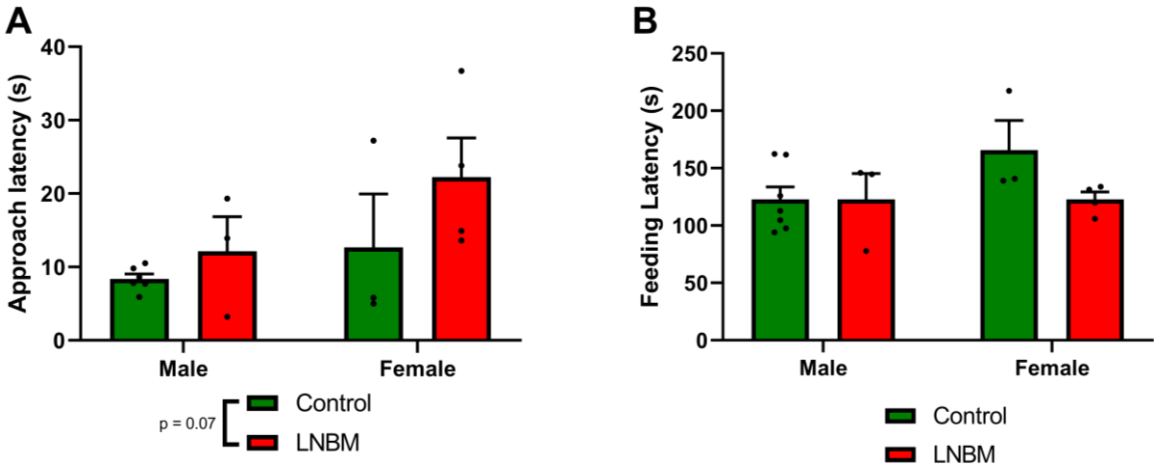


Figure 2.4 Novelty supressed feeding test in LNBM and control mice. (A) Latency to approach the bowl. **(B)** Latency to start consumption of food within the bowl. N = 9 control (6 male, 3 female) and 7 LNBM (3 male, 4 female).

2.4.3 Sucrose Preference Test

Animals completed the sucrose preference test as a measure of hedonic processing. There was no difference between control and LNBM groups in preference for sucrose (Figure 2.5A). When each group's sucrose preference was compared against 50% preference, LNBM animals had a significant preference towards consuming sucrose (one-sample t-test, t_1=46.7, p = 0.014) while control animals had a trend towards having a sucrose preference (one-sample t-test, t_1 = 8.58, p = 0.074). There was no difference in total consumption of fluid (Figure 2.5B) between LNBM and control groups over the course of the 60 minute preference test.

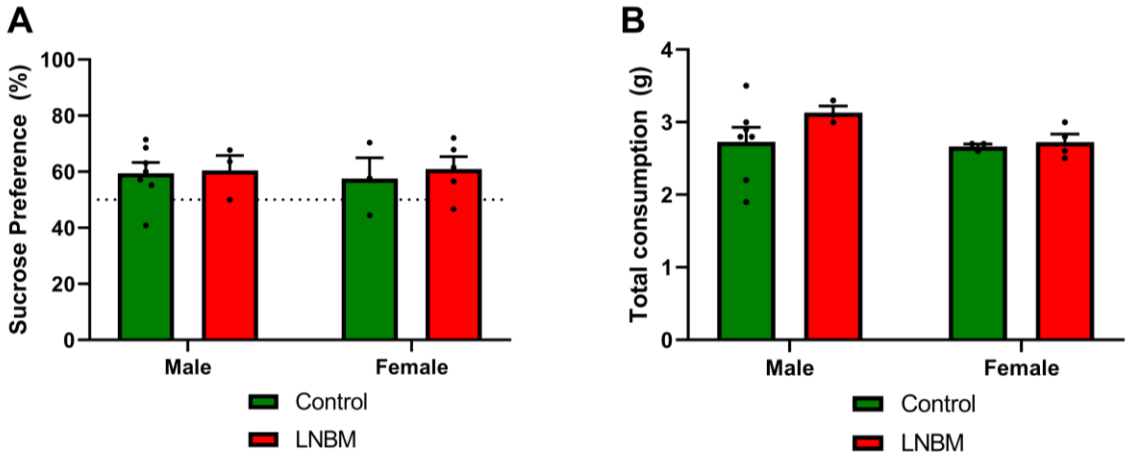


Figure 2.5 Sucrose preference test in LNBM and control animals. (A) Sucrose preference after 1hr of consumption by animals. The dotted line indicates 50% preference where animals prefer neither sucrose nor water. **(B)** Total consumption of fluid by animals over the preference test. N = 10 control (7 male, 3 female) and 8 LNBM (3 male, 5 female).

2.4.4 Reward learning assay

As part of training for the RLA animals completed a week of discrimination training. On the first session of discrimination no animals reached the criteria (Figure 2.6A) of 6 consecutive correct choices (allowing a single omission).

Additionally, animals were equally likely to choose the rewarded substrate as the blank substrate (Figure 2.6B, one-sample t-test against 50%; control: $t_1 = 0.22$, $p = 0.86$; LNBM: $t_1 = 0.23$, $p = 0.086$). Average latencies were well below the cut-off of 15s (Figure 2.6C) and there was no difference between groups in any of these measures for session 1. By session 5 all but one animal met the criteria (Figure 2.6D) with animals performing significantly better than chance (Figure 2.6E, one-sample t-test against 50%; control: $t_1 = 86$, $p = 0.007$; LNBM: $t_1 = 14.99$, $p = 0.04$). There was again no difference between groups for latencies (Figure 2.6F) however females from the LNBM cohort appeared to perform the task poorer than their control counterparts with them requiring a higher number of trials to reach criterion (Mann-Whitney, $U = 2.5$, $p = 0.032$) and having lower task accuracy (Mann-Whitney, $U = 0$, $p = 0.008$).

Animals then progressed to the main RLA protocol where they completed pairings between substrates and either 2 pellet or 1 pellet rewards depending on the day. As measured by the trials required to meet criterion animals successfully learnt the required pairings (Figure 2.7A) all animals combined: Friedman test, $x^2 = 24.4$, $p = 0.0002$). Animals' accuracy also increased over the pairing sessions (Figure 2.7B, 3-way ANOVA, main effect of session, $F_{3.4,41.3} = 4.34$, $p = 0.0072$). Additionally, animals became quicker to dig in a substrate over the period (Figure 2.7C, 3-way ANOVA, main effect of session, $F_{5,60} = 2.8$, $p = 0.024$).

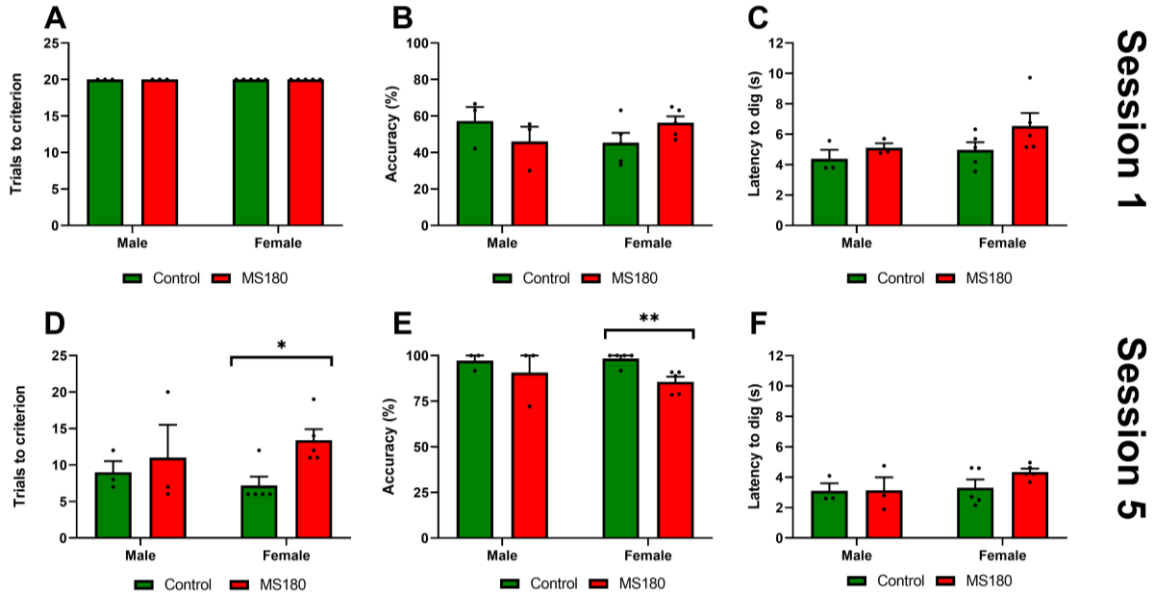


Figure 2.6 Discrimination training in the RLA. (A-C) Session 1. **(A)** Trials to criterion of 6 consecutive correct responses (allowing a single omission). **(B)** Accuracy. **(C)** Latency to dig. **(D-F)** Session 5. **(D)** Trials to criterion. **(E)** Accuracy. **(F)** Latency to dig. N = 8 control (3 male, 5 female) and 8 LNBM (3 male, 5 female).

No effect of the LNBM model was observed in the pairing phase of the RLA protocol. Animals completed a preference test after pairing session 4. Overall, animals did not show a bias towards the 2-pellet paired substrate (Figure 2.8A) and there was no effect of condition in the overall ANOVA analysis. However, in exploratory analysis male LNBM animals showed a significantly reduced 2vs1 bias compared to control males (t-test, $t_4 = 3.32$, $p = 0.029$). Animals also did not show a bias towards any specific substrate (Figure 2.8B) or spatial location (Figure 2.8C). There was also no difference between groups in

latency to dig (Figure 2.8D). Positive and negative feedback sensitivity (PFS and NFS) were also analysed as measures of animals' response to probabilistic feedback within a session. PFS (Figure 2.8E, win stay probability) and NFS (Figure 2.8F, lose shift probability) were no different between groups.

Due to evidence that further pairing sessions can strengthen the 2vs1 bias (Stuart et al., 2015) another preference test was conducted following two more pairing sessions. Again, animals did not show a preference overall for the 2-pellet paired substrate (Figure 2.9A) with no group difference also. Animals again did not have any substrate or spatial biases (Figure 2.9B and 2.9C) nor were there differences between groups in latency to dig (Figure 2.9D) or NFS (Figure 2.9F). When win-stay behaviour was analysed an interaction between condition and sex emerged (Figure 2.9E, 2-way ANOVA, $F_{1,12} = 6.6$, $p = 0.025$) which appeared to be specific to the male cohort (Sidak's multiple comparison, $t_{12} = 2.74$, $p = 0.036$).

In order to assess if individual animals' 2vs1 bias was due to a genuine bias or chance 2vs1 bias was compared between the two preference tests There was a correlation in 2vs1 bias (Figure 2.10A, linear regression, $F_{1,14} = 5.9$, $p = 0.03$, $R^2 = 0.3$) and substrate bias (Figure 2.10B, linear regression, $F_{1,14} = 10.3$, $p = 0.006$, $R^2 = 0.42$) between the two test sessions, however side bias (Figure 2.10C) did not correlate. The gradient of the best-fit line for 2vs1 bias and substrate bias was 0.65 and 0.74 respectively whereby both biases were of weaker strength in the second preference test compared to the first.

2.4.5 BrdU Neurogenesis

Brain sections from animals subject to repeat BrdU injections were used to measure $BrdU^+$ neurones in the subgranular zone (SGZ) of the dentate gyrus (see Figure 2.11A and B for example photomicrographs). There was no difference between groups when the total number of $BrdU^+$ cells per animal was analysed (Figure 2.11E) although there was a main effect of region with ventral sections having a greater number of $BrdU^+$ cells (3-way ANOVA, $F_{1,8} = 7.52$, $p = 0.025$).

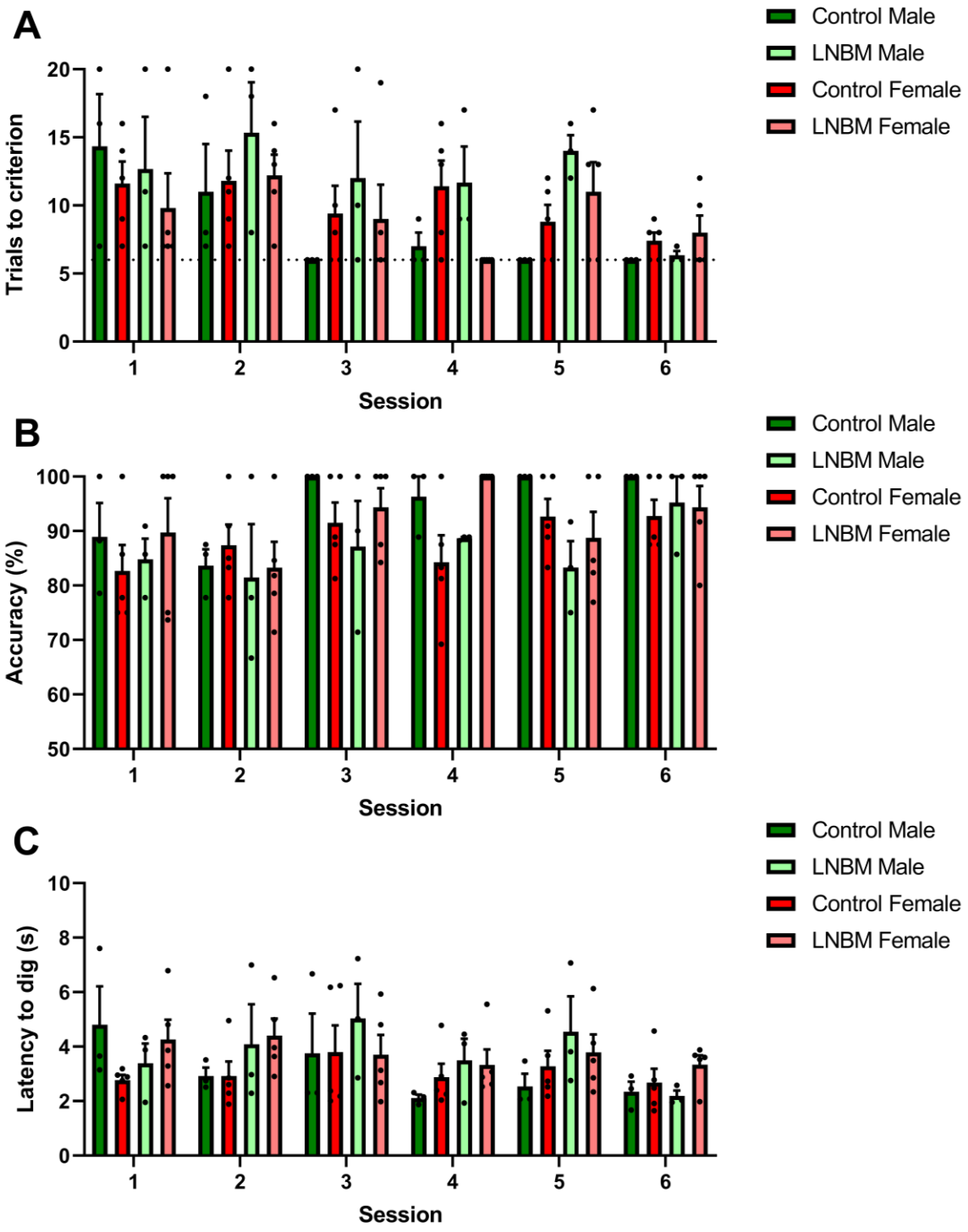


Figure 2.7 Reward substrate pairing in the RLA. (A) Trials to criterion, **(B)** task accuracy and **(C)** latency to dig across all 6 sessions of reward-substrate pairing. Between sessions 4 and 5 a preference test was conducted and the dotted line on (A) shows the criterion of 6 correct consecutive trials allowing a single omission. N = 8 control (3 male, 5 female) and 8 LNBM (3 male, 5 female).

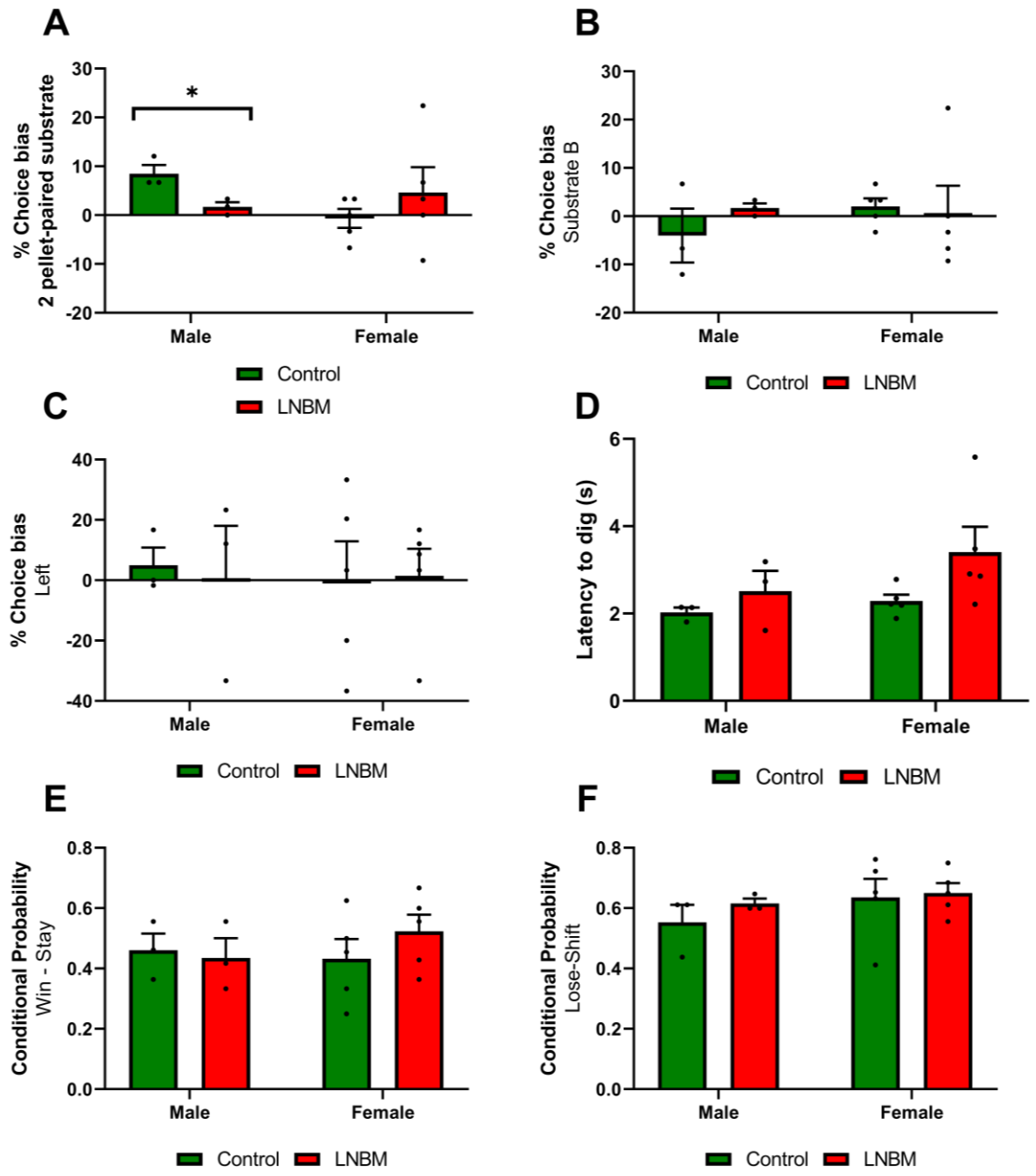


Figure 2.8 Preference test 1 in control and LNBM mice. **(A)** Choice bias toward 2 pellet paired substrate (2vs1 bias). **(B)** Choice bias towards substrate B. **(C)** Choice bias towards left direction. **(D)** Latency to dig. **(E)** Conditional probability: Win-stay. **(F)** Conditional probability: Lose-shift. N = 8 control (3 male, 5 female) and 8 LNBM (3 male, 5 female).

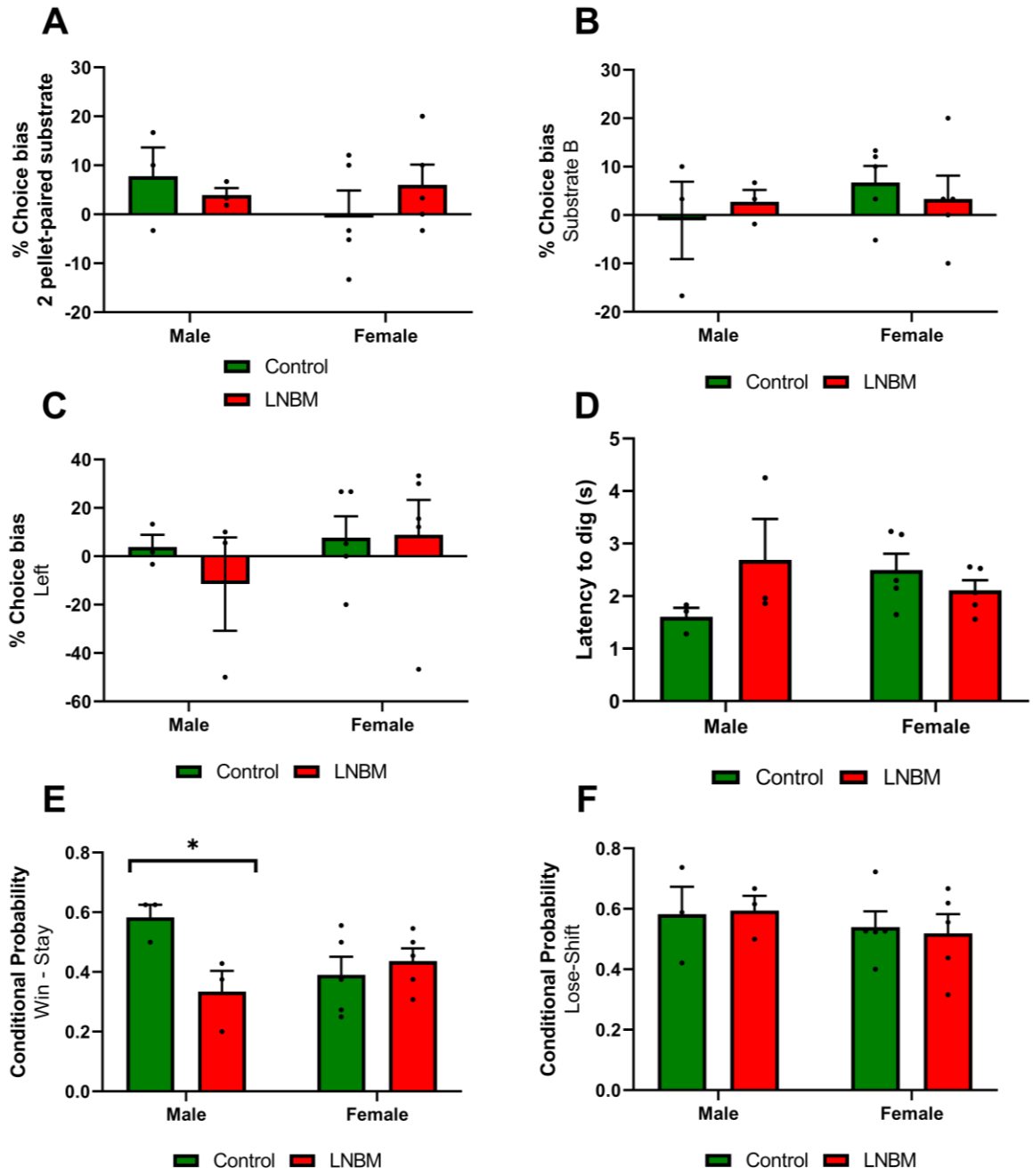


Figure 2.9 Preference test 2 in control and LNBM mice. **(A)** Choice bias toward 2 pellet paired substrate (2vs1 bias). **(B)** Choice bias towards substrate B. **(C)** Choice bias towards left direction. **(D)** Latency to dig. **(E)** Conditional probability: Win-stay. **(F)** Conditional probability: Lose-shift. N = 8 control (3 male, 5 female) and 8 LNBM (3 male, 5 female).

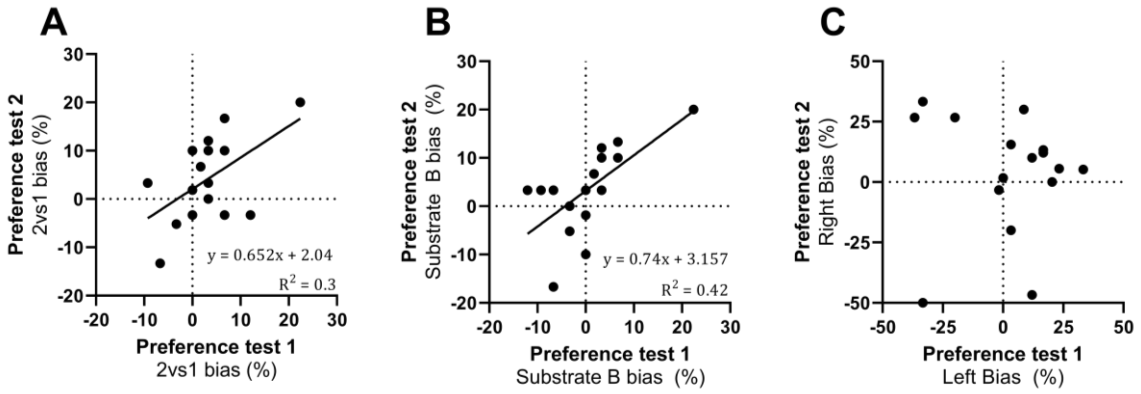


Figure 2.10 Correlation between preference tests in the RLA. (A) Correlation in 2 pellet paired substrate bias between preference test 1 and 2. **(B)** Correlation between substrate bias between the two test sessions. **(C)** Correlation between both sessions regarding spatial location bias. For each graph the R^2 value and equation shown are the result of the least squares linear regression fit. N = 18.

The number of $BrdU^+$ cells was correlated across the hippocampus in order to observe if there was a difference between control and LNBM animals in the relationship between hippocampal region and $BrdU^+$ cell count in a more granular way than splitting the hippocampus into two regions. For male animals there was no difference between groups and a weak correlation between bregma and $BrdU^+$ cell count in both groups (control: $F_{1,29} = 5.54$, $p = 0.026$, $R^2 = 0.16$; LNBM: $F_{1,29} = 8.2$, $p = 0.008$, $R^2 = 0.22$). In the cohort of female animals there was no correlation in the control group ($R^2 = 0.002$) contrasting to the LNBM group showed a weak correlation between bregma and $BrdU^+$ cell count (linear regression, $F_{1,33} = 7.8$, $p = 0.009$, $R^2 = 0.19$). When the lines of best fit for control and LNBM females were compared there was a trend towards a common line of best fit not being adequate (extra sum of squares test, $F_{2,69} = 2.76$, $p = 0.071$).

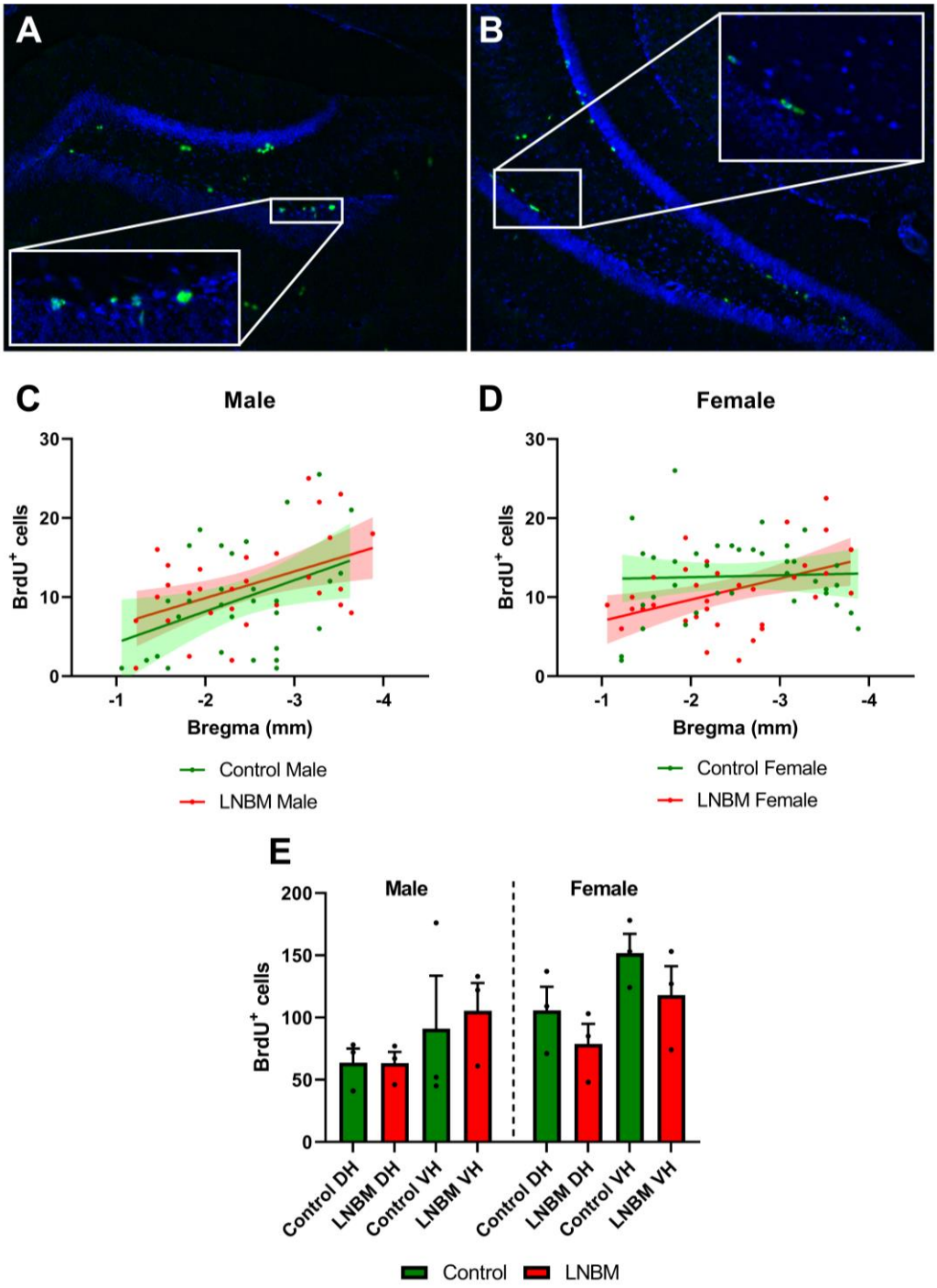


Figure 2.11 BrdU Immunohistochemistry in control and LNBM animals. Example photomicrographs of dorsal **(A)** and ventral **(B)** hippocampal sections. DAPI is shown in blue and BrdU is shown in green. Correlations between hippocampal region, as shown by bregma, in **(C)** male and **(D)** female animals. Each datapoint indicates a single brain section. Lines show linear least squares best fits and the shaded areas indicate 95% confidence intervals for these fits. **(E)** Total $BrdU^+$ cells counted per animal. DH: dorsal hippocampus, VH: ventral hippocampus. N = 6 control (3 male, 3 female) and 6 LNBM (3 male, 3 female).

2.4.6 HPA Axis

Restraint stress elicited a robust increase in plasma corticosterone in both control and LNBM animals (Figure 2.12A, 3-way ANOVA, main effect of stress: $F_{1,13} = 62.7$, $p<0.0001$) although there was no difference between groups. There was also no difference between groups when adrenal and thymus glands were dissected from animals and weighed (Figures 2.12B and 2.12C respectively) but main effects of sex were observed for both measures (two-way ANOVA, adrenal: $F_{1,16} = 5.54$, $p = 0.03$; thymus: $F_{1,16} = 4.81$, $p = 0.043$) with females having heavier thymus and adrenal glands when normalised for bodyweight.

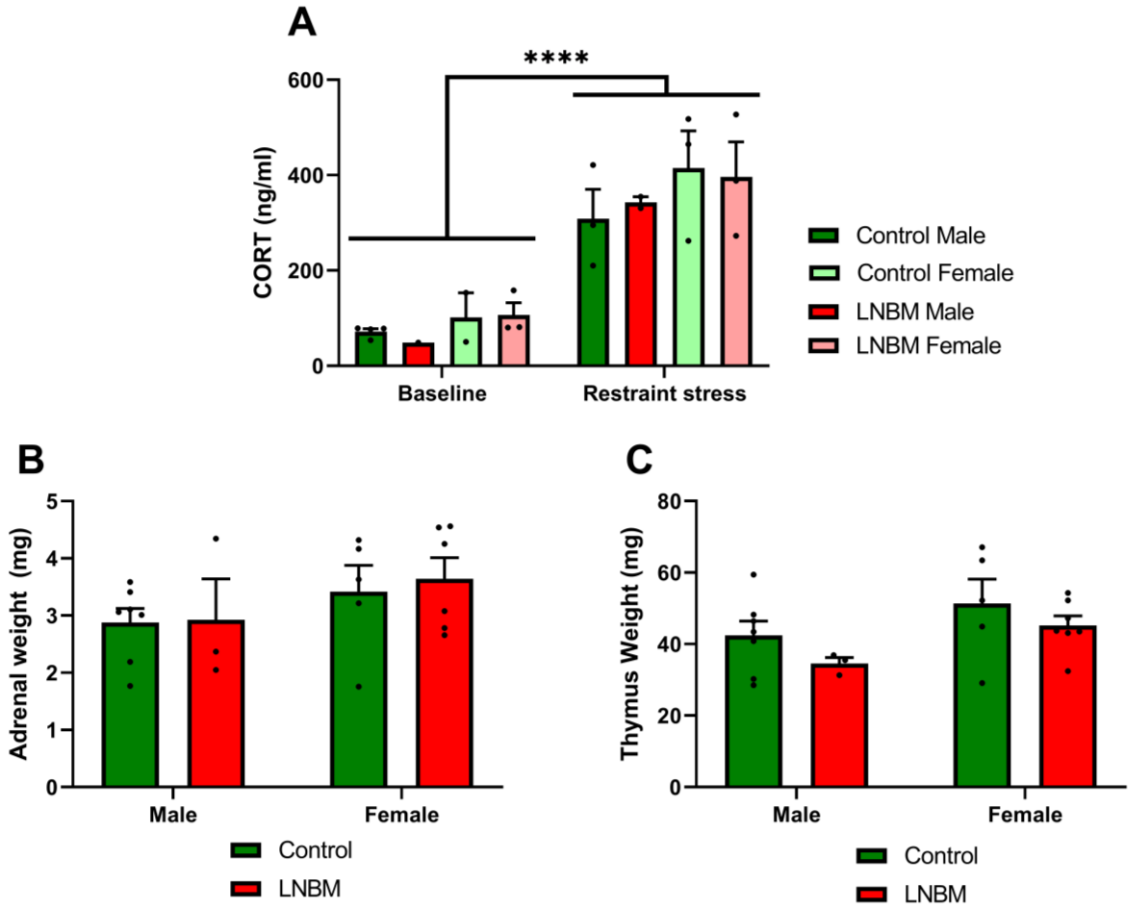


Figure 2.12. HPA activation in control and LNBM animals. (A) Plasma corticosterone measurements from animals either killed directly from the home cage or subject to 30m restraint stress. **(B)** Adrenal and **(C)** thymus gland weights dissected from animals. For RS CORT: Baseline: N = 6 control (4 male, 2 female) and 4 LNBM (1 male, 3 female), RS: N = 6 control (3 male, 3 female) and 5 LNBM (2 male, 3 female). For adrenal and thymus weighs N = 12 control (7 male, 5 female) and 9 LNBM (3 male, 6 female).

2.5 Discussion

2.5.1 Bodyweight

As has been previously reported (Kohl et al., 2015; Naninck et al., 2015; Rice et al., 2008; Walker et al., 2017; Wang et al., 2012) animals bred in the present study under the limited nesting and bedding material paradigm had a lower bodyweight throughout development, although there is disagreement between studies as to whether this is maintained to adulthood (Rice et al., 2008). This compares to other ELS models such as maternal separation (Stuart et al., 2019) and maternal deprivation (Derks et al., 2016) where no changes in bodyweight are seen postweaning within rats. Interestingly it has been reported that within female rats, maternal separation actually leads to increased obesity when fed a high fat diet post weaning (Murphy et al., 2017) which ties into the ability of ELS to increase the risk of developing metabolic disorders (Joung et al., 2014). Due to bodyweight being correlated to quantity of maternal care (Guerra and Nunes, 2001) these data suggest that the LNBM model in mice is potentially a more severe model of ELS than other commonly used models.

2.5.2 Anxiety behaviours

Although the effects on bodyweight of the LNBM model in mice in this study were greater than observed in other models there was no visible effect on anxiety-related behaviour in the novelty supressed feeding test, one of the best phenotypic observations in rats following application of an ELS model (Bonapersona et al., 2019). A similar lack of effects of the LNBM model upon anxiety behaviours has also been observed utilising the open field test (Kanatsou et al., 2017; Kohl et al., 2015; Rice et al., 2008; van der Kooij et al., 2015; Wang et al., 2012) and elevated plus maze (Kohl et al., 2015; van der Kooij et al., 2015; Wang et al., 2012). Maternal separation in mice has also been found to not affect anxiety behaviours in either of these assays (Wang et al., 2020). Animals' latency to feed in the present study was also similar to previously published data (Mineur et al., 2006), suggesting no overall baseline anxiety increase across both control and ELS cohorts. Interestingly increased anxiety has been observed in the light dark box where animals showed reduced time in the lit compartment (Wang et al., 2012). Changing the time period where the mice pups experienced deprivation also appears to be important for the development of anxiety behaviours with mice deprived between PND 10 and

PND17 showing reduced centre time in the open field test compared to those deprived in the standard PND2 to PND9 time period (van der Kooij et al., 2015).

2.5.3 Sucrose preference test

The sucrose preference test has been extensively used as a test for anhedonia in rodents (Liu et al., 2018). Similar to the NSFT, no difference in sucrose preference was observed between control and LNBM animals in this study. This is a similar finding to that reported by Hsiao et al., 2016 who also found no difference between control and LNBM groups. However, there appear to be species differences with reduced sucrose preference being observed within rats raised using the LNBM model (Bolton et al., 2018). A similar lack of sucrose preference has also been observed following maternal separation in rats (Bai et al., 2012; Frank et al., 2019). However other studies utilising similar protocols have not observed any changes in sucrose preference (Stuart et al., 2019; Wei et al., 2018). Interestingly it has previously been reported that C57BL/6J mice, the same strain as used in the present study, have a mean sucrose preference of 92.5% when presented with a choice of a 1% sucrose solution or water (Pothion et al., 2004). Mice in the present study had a much lower preference than this, both groups were equally around 60%. Indeed this is a similar level of preference seen to animals who have experienced 28 days of chronic mild stress exposure (Liu et al., 2018). Due to the fact that animals drank comparable amounts of fluid compared to other studies (Alves-dos-Santos et al., 2020), implying overcoming of neophobia and engagement with the bottles, this potentially suggests that both groups of mice showed anhedonic like behaviours.

2.5.4 Reward learning assay

Neither controls nor LNBM mice in the present study were able to form a reward induced memory bias in either the initial preference test or after further pairing sessions and another preference test. While animals biasing toward one of the digging substrates could explain the lack of a 2vs1 bias, animals in the present study did not exhibit any substrate bias. However, animals did show large spatial biases with 6 animals in the first preference test showing a side preference greater than 20%. A potential explanation for this is that animals were not using their prior memories to guide decision making within the preference test but were instead trying to use an alternative strategy devised during the session. This could be supported by insufficient quality of learning within the pairing

sessions which did not persist between sessions. This suggestion is supported by evidence from when performance was correlated between the two preference test sessions. There was only a weak correlation in 2vs1 bias between the two sessions (R^2 = 0.3) with the line of best fit showing a gradient of below one suggesting that animals bias was weaker upon retesting compared to when initially tested. This also implies that animals' choices were modulated by a large amount of noise when performing the preference test and that 2vs1 bias was only driving a part of their behaviour. How mice responding to positive and negative feedback was also inconsistent with LNBM males showing reduced positive feedback sensitivity (PFS) compared to controls in the second preference test only.

With these data suggesting that mice from both groups failed to exhibit biased learning as a function of reward it was interesting to observe that female LNBM mice showed reduced learning during discrimination training compared to controls with this effect not persisting into pairing training. Learning and memory has been consistently shown to be impaired in the mouse LNBM model with mice performing poorer compared to their control peers in the Morris water maze and novel object recognition tasks (Rice et al., 2008). This appears to be more specific to spatial learning and memory with multiple studies reporting impairments (Bath et al., 2017; Kanatsou et al., 2017; Naninck et al., 2015; Rice et al., 2008; van der Kooij et al., 2015; Wang et al., 2011) while there are much more mixed results for fear conditioning with two studies reporting no difference between groups (Heun-Johnson and Levitt, 2016; Kanatsou et al., 2017) while one study a piece reported either impaired (Arp et al., 2016) or improved performance (Bath et al., 2017). However, it should be noted that within MS180 rats being trained in the RLA there was no difference in performance compared to controls (Stuart et al., 2019) which matches well with meta-analytic evidence that MS180 animals do not show deficits in non-stressful learning (Bonapersona et al., 2019).

2.5.5 Biochemical measures

Within the biochemical measures collected there was no difference between either dentate gyrus neurogenesis or HPA axis reactivity between control and LNBM animals. Within the LNBM model there is considerable heterogeneity between reported results concerning differences in the HPA axis. Studies have reported either decreased (Arp et al., 2016; Youssef et al., 2019) or increased (Arp et al., 2016; Hsiao et al., 2016) basal CORT concentrations while many have found no difference between ELS and control

groups (McIlwrick et al., 2016; Naninck et al., 2015; Wang et al., 2012). When HPA axis reactivity has been assessed in response to restraint stress the most consistent finding has been no change in reactivity (McIlwrick et al., 2016; Wang et al., 2012; Youssef et al., 2019) while one study reported attenuated CORT release in response to stress (Hsiao et al., 2016). These findings broadly are consistent with data from humans where no difference in basal CORT or reactivity has been observed (Bunea et al., 2017; Fogelman and Canli, 2018). However this differs from other rat models of ELS such as maternal separation (Stuart et al., 2019), maternal deprivation (Penke et al., 2001) and the offspring of low licking grooming rats (Liu and Meaney, 1997; Sánchez et al., 2001) where exaggerated CORT responses to stress are seen following restraint stress. However it is important to note that other studies have reported basal CORT concentrations to be around 20ng/ml in males and 40ng/ml in females while in the present study control males had a basal CORT of 71.8 ng/ml and females 101.8 ng/ml on average (Hsiao et al., 2016; Naninck et al., 2015; Rice et al., 2008). Following stress control males within this study exhibited CORT values of on average 308.8 ng/ml with females being higher at 414.9 ng/ml. This is markedly higher than previously reported values after 30m restraint stress in mice which range between 60 and 200 ng/ml (Hsiao et al., 2016; Wang et al., 2012). While mice in the present study all appear to have a hyperactive HPA axis it is interesting that when the weights of adrenal and thymus glands, measures of long-term HPA activity, are compared to previous data there is little difference in weights with the exception of control female adrenal gland weights. As a percentage of bodyweight thymus glands were around 10% smaller in control male and female mice compared to those from Naninck et al., 2015 while when adrenal glands were compared there was an effect of sex with male adrenal glands being around 25% smaller in the present study and female glands being around 50% smaller. However, there is wide variation between radio immune assays in addition to there being variation in how dissections are carried out to measure thymus and adrenal gland weights between studies. This means that comparison between experiments is troublesome at best and it is therefore difficult to interpret the effects of the LNBM model upon the HPA axis.

There are multiple methodologies employed to investigate hippocampal neurogenesis and these include the endogenous markers Ki67 which stains proliferating cells and doublecortin (DCX) which is used to mark young differentiating neurones (Pan et al., 2013). Intercalation of the exogenous thymidine analogue BrdU into replicating DNA is widely used to study neurogenesis with the time period between injection and collection

of tissue dictating whether cell labelling better reflects proliferation or cell survival (Wojtowicz and Nohjin, 2006). Within the present study a 24-hour time period between final injection and tissue collection means that cell labelling better reflects proliferation as opposed to other phases of neurogenesis. Within the mouse LNBM model it has previously been reported that ELS mice show no difference in cell proliferation or differentiation as measured by Ki67 and DCX respectively (Naninck et al., 2015). However, decreases in cell survival were observed in males only when BrdU was injected 30 days prior to tissue collection. It should be noted that one additional study carried out these same experiments and found no differences in any measure (Kanatsou et al., 2017). While the results from the present study are consistent with other reports upon the LNBM model in mice it has been observed that the same BrdU injection protocol reveals robust decreases in proliferation within the maternal separation model of ELS (Mirescu et al., 2004; Stuart et al., 2019). This finding has also been observed using Ki67 to label proliferating cells, although interestingly effects have been observed to be localised to the ventral hippocampus only (Hulshof et al., 2011). It should be noted that if BrdU administration is used to observe cell survival in MS180 rats then no difference between ELS and control groups has been observed in concordance with DCX results (Greisen et al., 2005; Hulshof et al., 2011).

2.5.6 Limitations

There are several limitations of this study which should be noted. Firstly the study is relatively underpowered which means that interpretation of results needs viewing through this prism (Button et al., 2013). Although previous studies have used similar sample sizes they did not attempt to include both sexes in analysis (Abbink et al., 2017; Chocyk et al., 2013; Stuart et al., 2015). Secondly control animals throughout the study did not perform in a manner consistent with that previously observed suggesting that it is challenging to describe them as true control animals. This means that any comparisons between controls in this study and the LNBM animals are potentially flawed. One of the best ways of assessing the successful application of the LNBM model is through measuring the number of sorties the dam makes from the nest with dam sorties correlating with plasma CORT concentrations in pups at PND9 (Gunn et al., 2013; Rice et al., 2008). This did not occur in the present study due to concerns over experimenter induced stress in the dams however it would have added valuable evidence as to whether the model application had successfully developed the correct phenotype in LNBM but not control

pups. Finally, the failure of animals to exhibit a 2vs1 bias in the RLA suggests that they did not successfully learn the task and the results of further exploratory analysis therefore needs to be taken with caution.

2.5.7 Conclusions

Overall, there are two broad strands of conclusion that can be drawn from this study's data. Firstly, control mice showed deficits in reward processing as evidenced by sucrose preference and RLA data alongside also having a hyperactive HPA axis as shown by increased basal CORT. This could be due to methodological reasons meaning that control animals were exposed to high enough levels of stress during the critical neurodevelopmental period that this was able to overcome the stress hyporesponsive period to cause these impairments. However, it is equally probable that these observations were due to methodological issues with assessing these behaviours such as animals not being successfully trained in the RLA and differences in CORT radio-immunoassay sensitivity.

A second tentative conclusion, subject to the previously discussed limitations, is that the limited nesting and bedding material model of ELS within mice does not re-capitulate the affective processing alterations and reward learning deficits seen in the rat maternal separation model of ELS. Evidence from the present study combined with that of previous authors suggests that the mouse LNBM model is extremely successful at causing spatial learning deficits but has lower consistency in other behavioural endpoints. In order for valid conclusions to be made for future work it is important to have an animal model which is both well validated and displays these key reward learning deficits which are hypothesised to be the intermediate phenotype between ELS and MDD development.

Chapter 3

Pharmacological characterisation of the rodent probabilistic reversal learning task

3.1 Introduction

As previously discussed, (see sections 1.3.4 and 1.2.3) reward learning impairments are present both in patients with depression and people with a history of early life stress. These impairments are hypothesised to be a key link between ELS and the aetiology of MDD (Admon and Pizzagalli, 2015; Hanson et al., 2017; Pechtel and Pizzagalli, 2013; Vrieze et al., 2013). Probabilistic reversal learning tasks (PRLTs) and probabilistic reward tasks (PLTs) have been widely used to study reward learning and feedback sensitivity in both humans and animals (see section 1.2.2, Bari et al., 2010; Murphy et al., 2003; Slaney et al., 2018).

Through translation of human probabilistic learning paradigms into rodent tasks this allows the mechanistic links between reward learning, depressive behaviour and early life stress to be investigated in a system that allows much greater manipulability and controllability than in man. Within animal studies, the PRLT offers multiple benefits compared to other PLTs in that it is substantially easier to train animals in (Bari et al., 2010; Der-Avakian et al., 2013), does not require lengthy re-baselining between manipulations and the task also incorporates a degree of cognitive flexibility which is believed to depend upon prefrontal cortex neuronal populations (Bartolo and Averbeck, 2020; Verharen et al., 2020). The task has also been successfully translated into mice (Ineichen et al., 2012) although investigations have been much more limited in this species.

Efforts, albeit limited, have been undertaken to understand the neurotransmitter systems and brain circuits that underly performance in the PRLT through pharmacological manipulation of animals performing the task. Bari et al., 2010 observed that acute administration of the selective serotonin reuptake inhibitor (SSRI) citalopram bidirectionally modulated reversal performance and negative feedback sensitivity (NFS) depending on dose while also increasing positive feedback sensitivity (PFS) when given at 5mg/kg chronically. Drozd et al., 2018 and Rychlik et al., 2017 explored the assay using a range of antidepressant compounds and found that ketamine decreased sensitivity to misleading negative feedback while mirtazapine, an α2 adrenoreceptor and 5-HT2/3 receptor antagonist, decreased overall reward learning. However, Drozd et al., 2018 did not find any effect of the SSRI escitalopram upon any output measure. There is also evidence for a dopaminergic influence upon task performance with nucleus accumbens shell inactivation found to reduce reversal performance and PFS (Dalton et al., 2014). Genetic knockdown of the dopamine transporter

DAT has also been found to increase reversal performance in the mouse PRLT alongside also improving motivation (Milienne-Petiot et al., 2017).

Within tasks investigating cognitive affective biases acute manipulations of affective state have been well established to influence aspects of reward learning (Lewis et al., 2019; Robinson and Roiser, 2016; Slaney et al., 2018). In the affective bias test (ABT), an assay of reward induced memory biases during learning, acute conventional antidepressant treatment is able to positively bias the recall of a reward-paired memory while manipulations known to induce a negative affective state such as LPS, corticosterone or IFN-α cause the formation of a negative bias (Hinchcliffe et al., 2017; Stuart et al., 2013, 2015, 2017). However, in the judgement bias test (JBT), an ambiguous cue interpretation task, animals only bias towards the more highly rewarded cue following chronic but not acute conventional antidepressant administration (Hales et al., 2017). However acute rapid-acting antidepressants (RAAD) are able to lead to a judgement bias in addition to blocking the recall of a previously created negative bias in the ABT (Hales et al., 2017; Stuart et al., 2015). Taken together these results suggest these tasks are able to dissociate between both affective state manipulations and different classes of antidepressant treatment. However, little is known about whether these manipulations have dissociable effects in the PRLT. By understanding both the effects of affective state upon behaviour in the PRLT and if the task dissociates between conventional and rapid-acting antidepressants this will enable greater insights into the processes by which affective state, reward learning and feedback sensitivity interact to lead to depressive behaviour.

In this chapter it was aimed to characterise the probabilistic reversal learning task more fully in response to acute pharmacological manipulation. Both conventional and rapid-acting antidepressants were tested following acute administration in normal animals. Animals were also probed with the broad-spectrum dopamine receptor antagonist flupentixol in addition to the dopamine, noradrenaline and 5-HT releasing agent amphetamine due to the well-known importance of dopamine in reward learning (Schultz et al., 1997). The effect of negative state induction was also investigated through administration of the monoamine depleting agent tetrabenazine (TBZ) and glucocorticoid receptor agonist corticosterone (CORT). Data were also analysed utilising a computational reinforcement learning model. This additionally allows the estimation of parameter changes caused by pharmacological treatment which map onto computational differences in the brain. This approach has

previously been applied to both human probabilistic reward learning tasks (Grogan et al., 2017) and the PRLT in rodents (Alsiö et al., 2019; Noworyta-Sokolowska et al., 2019).

3.2 Chapter Aims

- Assess the effects of bidirectional dopaminergic manipulation upon reward learning and feedback sensitivity

- Assess how acute changes in affective state through pharmacological manipulation affect the way animals learn from reward and respond to positive and negative feedback in the PRLT.

- Compare the effects of conventional and rapid-acting antidepressants upon task performance in the PRLT

- Examine pro-depressant manipulations in the PRLT to understand if acute modulation of reward learning within the PRLT forms a part of their mechanism.

- Analyse data with a Q-learning reinforcement learning model to provide further insights into the effects of pharmacological treatment upon underlying neural substrates of reward learning.

Part of this chapter formed the basis of the publication:

> Wilkinson MP, Grogan JP, Mellor JR and Robinson ESJ. 2020. Comparison of conventional and rapid-acting antidepressants in a rodent probabilistic reversal learning task. Brain and Neuroscience Advances; 4:1-11

The reinforcement learning model used was written by John Grogan (Grogan et al., 2017) who provided assistance in applying it to PRLT data alongside Claire Hales.

This work was completed between 2017 and 2018

3.3 Methods

3.3.1 Animals

Male lister-hooded rats (n=12, Harlan, UK) were pair housed in enriched laboratory cages (55 x 35 x 21 cm) containing paper bedding, sawdust, cotton rope, carboard tubes and red Perspex houses (30 x 17 x 10 cm). Animals were accommodated in temperature (21 ± 1 °C) and humidity (45-65%) controlled conditions with a reverse light-dark cycle (12:12h, lights off at 08:00) and had access to *ad-libitum* water. Experiments were carried out in the animals' active phase between 09:00 and 18:00. Animals weighed approximately 270g and 420g at the start of training and the start of drug study experiments respectively. Rats were mildly food restricted to no less than 90% of their free-feeding weight matched to a normal growth curve (≈18g of food per rat/day laboratory chow (LabDiet, PMI Nutrition International)) and sample size was estimated from previous studies using similar tasks and manipulations (Bari et al., 2010). All studies were carried out following local institutional guidelines (University of Bristol Animal Welfare and Ethical Review Board), the UK Animals (Scientific procedures) Act of 1986 and the European Parliament and Council Directive of 22 September 2010 (2010/63/EU).

3.3.2 Apparatus

All behavioural testing was carried out in operant boxes equipped with a three-panel touchscreen (see Figure 3.1 for diagram, Med Associates, USA)). Boxes contained a tone generator, house light, magazine (45mg reward pellets (Test Diet, Sandown Scientific, UK))) and infrared touchscreen panel. Operant boxes were controlled by KLimbic software (Conclusive Solutions Ltd, UK) which created output files that were subsequently decoded into behavioural output measures by a bespoke MATLAB program (MathWorks Inc version R2017a, USA).

3.3.3 Probabilistic Reversal Learning Task

The probabilistic reversal learning task was adapted for a touchscreen operant system (original task design: Bari et al., 2010) with spatial nose poke locations being remapped into touchscreen locations and the addition of an initiation stage to start each trial.

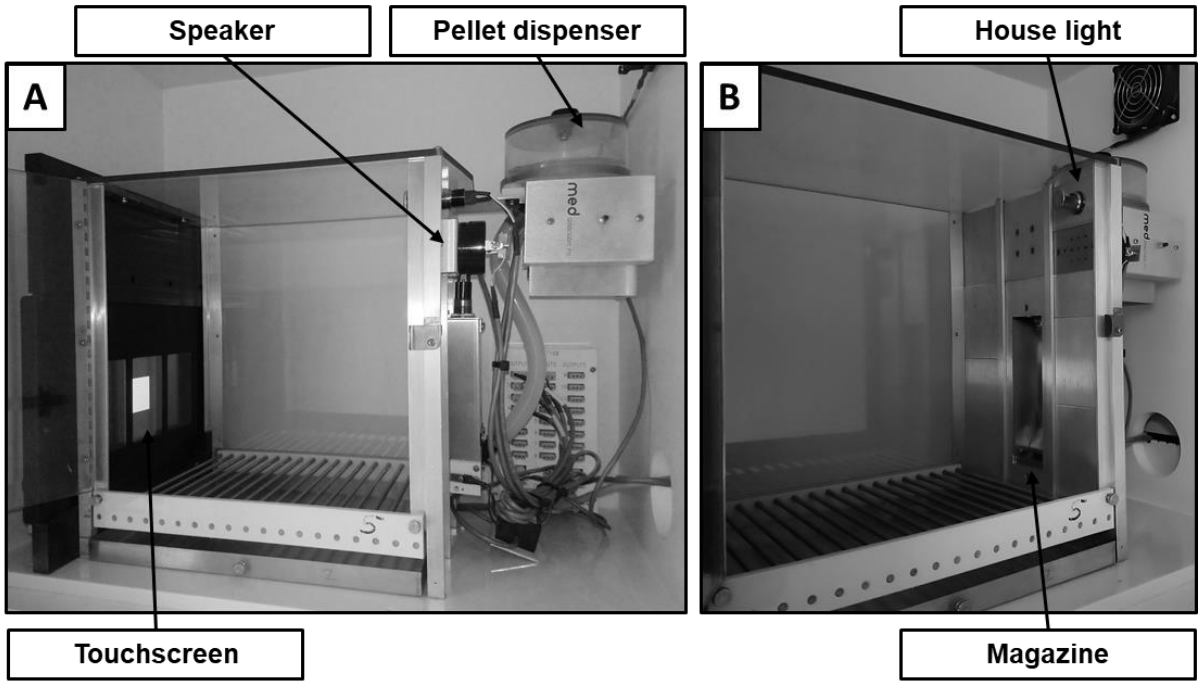


Figure 3.1. Operant system configuration for the PRLT. (**A**) and (**B**) show the same box from either a touchscreen facing or magazine facing perspective respectively.

Before the commencement of drug study experiments animals were trained in the task through a three-phase process. Phase 1 required animals to learn to touch an initiation square presented in the centre touchscreen window to receive a single reward pellet. The session ended when animals either first reached completion of 120 trials or 30m had elapsed. The criterion for passing phase 1 of training was successful completion of all 120 trials within a session for 2 consecutive sessions (mean time to train: 6.7 ± 0.51 sessions). Within stage 2, animals first had to again press the initiation square but then subsequently had to press either of the left or right touchscreen windows to receive reward (max 200 trials or 40 minutes). Animals progressed to phase 3 of training once they completed 80% of presented trials within a session for 2 consecutive sessions (mean time to train: 2.25 ± 0.13 sessions).

Phase 3 of training consisted of animals being moved to performing the main task until they had reached a suitable level of performance for drug studies to commence, deemed as when performance was stable in rule changes, win-stay probability, lose-shift probability and initiation reaction time over 5 consecutive sessions. In the main probabilistic reversal learning task (see Figure 3.2 for overview) animals were first, as in stage 1 and 2 of training, required to initiate their own trial by pressing the initiation stimulus presented in the centre

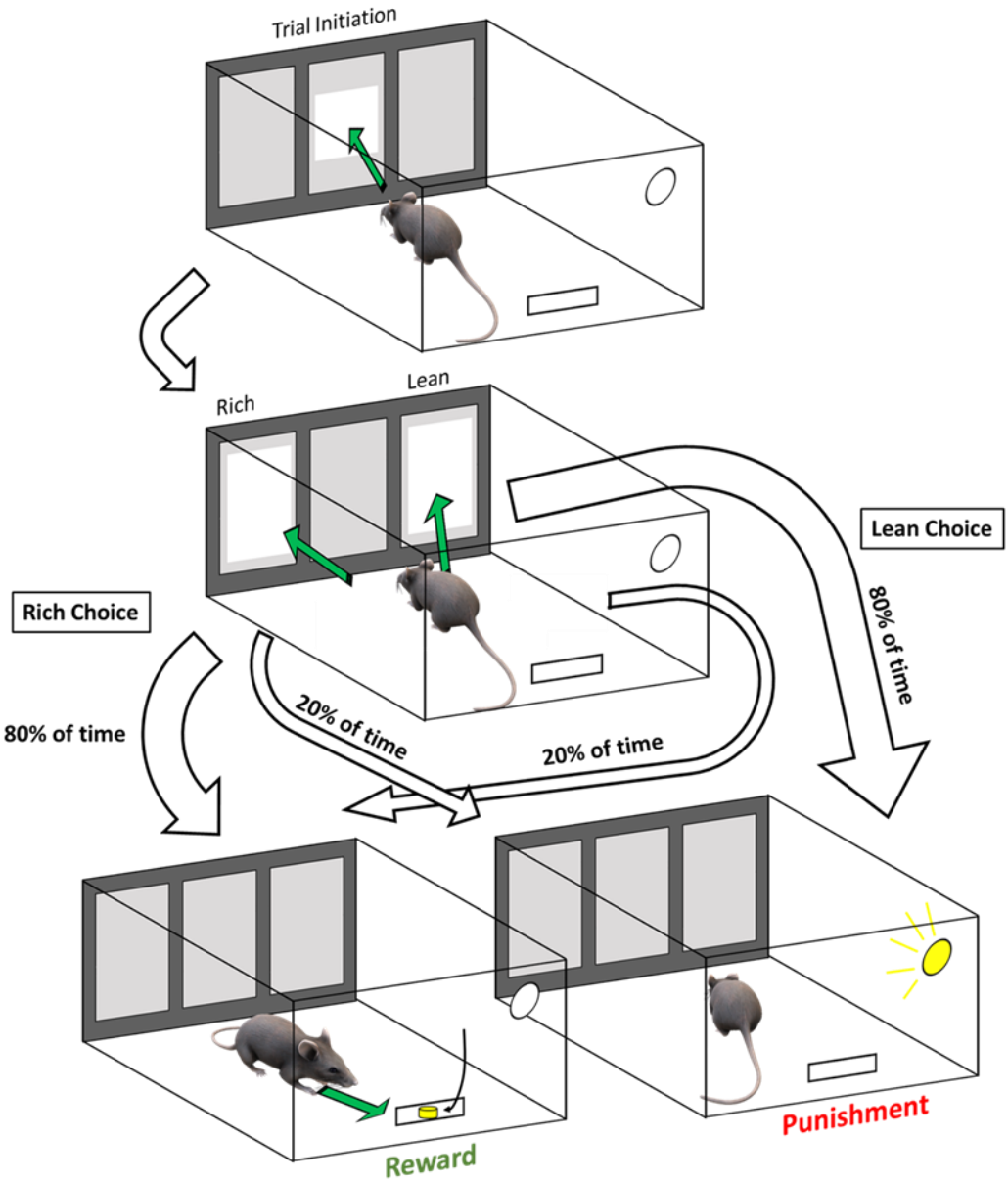


Figure 3.2. Overview of trial routes in the probabilistic reversal learning task. Flowchart of all trial routes within the PRLT task for a fully completed trial. The probabilities of each outcome are depicted by the width of each arrow. White arrows show transfer from one stage of a trial to the next while green arrows depict an animal making action. If no response was detected within 10s of an animal pressing the initiation square then this was classed as an omission and animals received a 5s timeout.

window of the touchscreen before then responding to either a left or right spatial stimulus. There was no time cut-off for animals to start a trial, allowing animals to self-pace within a session. Upon selection of a stimulus by an animal they were rewarded depending upon the reward contingency of the selected option. One spatial stimulus was classed as the "rich" stimulus and was rewarded 80% of the time while the other stimulus had a reward probability of 20% and was called the "lean" stimulus. Once a stimulus choice was made animals either had to retrieve a reward pellet from the magazine and could then immediately start the next trial or if they were not rewarded were punished with a timeout of 5s and bright illumination from the house light. Omissions were classed as if animals did not make a stimulus choice within 10s and led to the same "punishment" as a lack of reward. Following 8 consecutive "rich" stimulus choices the contingencies switched so that the spatial location previously associated with the "rich" stimulus was now associated with the "lean" stimulus and vice-versa. Animals were allowed to reverse as many times as able within a session which lasted for a maximum of 200 trials or 40 minutes (which ever was reached first). The spatial location of the "rich" stimulus at the start of a session was consistent across sessions and counterbalanced across animals.

3.3.4 Experimental design

The effects of acute pharmacological treatment on animals' performance in the PRLT was studied by way of a blinded, within-subject, fully counterbalanced design with all animals receiving every dose of every drug. Treatment groups were allocated through use of a fully randomised design containing 4 treatment groups (except for the scopolamine study where 3 groups were used) with each group having the treatments in a different order. Pharmacological treatments (see table 3.2 for details) were administered by a low stress dosing technique (Stuart and Robinson, 2015) prior to testing. Drug doses and pre-treatment times were chosen as to be clinically relevant and were based upon previous behavioural studies (see table 3.2). All studies were carried out to a common design (see Figure 3.3 for overview) whereby animals completed a baseline day before a test day where treatment was administered with a complete week of baseline sessions between different pharmacological treatments. Animals were food restricted for all behavioural testing. In order to assess the effect of this on task performance animals completed a session where they were provided with their standard food ration 1hr before testing (this was counterbalanced over two sessions). Animals also had the first rule they had to learn reversed in order to assess the important of this to task performance. While this design has

considerable benefits with respect to implementation of the 3Rs (Russel and Burch, 1959) and time efficiency it does open the possibility of drugs such as ketamine having long-term carry over effects (Duman, 2018) that influence the result of following experiments.

Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Baseline	Test	Off	Baseline	Test	Off	Off
Baseline	Test	Off	Baseline	Test	Off	Off
Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline

Figure 3.3. Example schedule for a study of a single pharmacological compound. Animals received pharmacological treatment on test days, completed the task with no treatment on baseline days then did not complete the task on "off" days.

3.3.5 Data Analysis

Parameters to be analysed were based on previous studies employing the PRLT (see table 3.1 for a summary, Bari et al., 2010; Noworyta-Sokolowska et al., 2019; Rychlik et al., 2017). Completed rule changes were analysed as the main behavioural output measure of reward learning within the task and were defined as the number of times an animal was able to successfully switch reward contingencies in a session. The trials to first rule change parameter was described as the number of trials each animal took to reach the criterion for a rule change within a session. Positive feedback sensitivity, how likely animals were to change their behaviour as a function of positive feedback, was assessed through animals' win-stay probability which is described as the likelihood for animals to stay at a stimulus following reward (Eq 3.1).

$$p(winstay) = \frac{win{-}stay\ trials}{total\ rewarded\ trials} \qquad \text{Eq 3.1}$$

Lose-shift behaviour (Eq 3.2), the probability of animals shifting responding following punishment at a stimulus, was analysed as a proxy of negative feedback sensitivity (NFS). NFS describes how sensitive animals are to change behaviour as a function of punishment.

$$p(loseshift) = \frac{lose{-}shift\ trials}{total\ punished\ trials} \qquad \text{Eq 3.2}$$

Measure	Description	Measures
Rule changes	How many times an animal was able to meet the rule change criteria within a session	RL
Trials to first rule change	How many trials an animal required to meet criteria for a rule change for the first time in a session	RL
Trials completed	How many trials (correct and incorrect) an animal was able to complete within a session	Motivation
Win-stay probability	The average probability within a session that if an animal were rewarded it would return to the same stimulus for the next trial	Feedback sensitivity
Lose-shift probability	The average probability within a session that if an animal were punished (not rewarded) it would shift to the opposite stimulus for the next trial	Feedback sensitivity
Initiation reaction time	The average latency from when the initiation square appeared to an animal pressing it to initial a trial	Motivation
True vs misleading win-stay	Win-stay probability subdivided by whether feedback matched (true) or clashed (misleading) with the underlying rich/lean contingencies at the time	Feedback sensitivity
True vs misleading lose-shift	Lose-shift probability subdivided by whether feedback matched (true) or clashed (misleading) with the underlying rich/lean contingencies at the time	Feedback sensitivity
Better fitting Qlearn model	Whether the data from each animal better fit (using BIC comparison) to a single learning rate (one learning rate for all feedback) or dual learning rate Qlearn model (separate learning rates for positive and negative feedback)	Reward learning strategy
Learning rate	Model free learning rate from the Qlearn1 model	RL
Theoretical accuracy	The accuracy of an animal compared to a perfect strategy predicted by the Qlearn1 model	RL
β	The degree to which the Qlearn1 model suggested an animal's strategy was deterministic or random.	RL

Table 3.1 Overview of parameters analysed in the PRLT.

Drug	Class	Doses (mg/kg)	Vehicle	t-	Route	Order	Supplier	Ref
Citalopram	SSRI	1, 3, 10	0.9% saline	30	i.p.	1	Hellobio, UK	[1]
Venlafaxine	SNRI	1, 3, 10	0.9% saline	30	i.p.	2	Hellobio, UK	[1,2]
Flupentixol	$D_1/D_2/D_3$ antagonist	0.03, 0.1, 0.3	0.9% saline	60	i.p.	3	Merck, UK	[1]
Reboxetine	NRI	0.1, 0.3, 1	0.9% saline	30	i.p.	4	Merck, UK	[1]
Ketamine	NMDAR antagonist	1, 3, 10	0.9% saline	60	i.p.	5	Merck, UK	[3]
Scopolamine	Muscarinic antagonist	0.03, 0.1	0.9% saline	60	i.p.	6	Tocris, UK	[2]
Amphetamine	DA/NA releasing agent	0.1, 0.3, 1	0.9% saline	15	i.p.	7	Merck, UK	[4]
Corticosterone	GR/MR agonist	1, 3, 10	5% DMSO, 95% sesame oil	30	s.c.	8	Merck, UK	[5]
Sertraline	SSRI	1, 3, 10	5% DMSO, 10% CrEL, 85% saline	30	i.p.	9	Tocris, UK	[6]
Tetrabenazine	MA depleting agent	0.1, 0.3, 1	10% DMSO, 20% CrEL, 70% saline	90	i.p.	10	Merck, UK	[7]
Citalopram	SSRI	3	0.9% saline	30	i.p.	11	Hellobio, UK	[1]

Table 3.2 Details of acute pharmacological treatment in the PRLT. Summary of all treatments animals received in the PRLT, order refers to the order in which studies occurred in and t- is the pre-treatment time in minutes. Ref refers to the source of doses and pre-treatment times. [1]: Stuart et al., 2013, [2]: Hinchcliffe et al., 2017, [3]: Jones and Higgins, 1995, [4]: Stuart et al., 2017, [5]: Refsgaard et al., 2016, [6]: Hales et al., 2017, [7]: Phelps et al., 2015. Abbreviations: i.p. intraperitoneal, s.c. subcutaneous, SSRI selective serotonergic reuptake inhibitor, SNRI serotonergic noradrenergic reuptake inhibitor, NRI noradrenergic reuptake inhibitor, NMDAR N-methyl-D-aspartate receptor, GR glucocorticoid receptor, MR mineralocorticoid receptor, MA monoamine, CrEL Cremophor EL.

Feedback was also classified into whether it matched or clashed with the underlying rule of the task. This is known as true and misleading feedback. If a rat was rewarded for choosing the "rich" stimulus then this feedback matches with the underlying task rule and is true whereas if it was rewarded at the "lean" stimulus this would be misleading. Motivation to complete the task was assessed using the time it took for rats to initiate their own trial, the time taken between presentation of the initiation square and it being pressed.

3.3.6 Qlearn reinforcement learning model

Behavioural outputs measured directly from animal performance in the PRLT are the result of underlying reward learning computational processes within the brain. By fitting drug study data with a reinforcement learning model this enables estimation of parameter changes that underly modifications of reward learning seen in the task. One of the most common reinforcement learning models is the Q-learning model which uses the information presented to an animal in each trial and iterates trial by trial making decisions to maximise total reward. A pre-existing model used in Grogan et al., 2017 was adapted for use with the PRLT based upon the Q-learning model (Sutton and Barto, 1998). As described in Grogan et al., 2017 the model assumes that animals estimate an expected reward associated with choosing a stimulus (Q(i)) for each stimulus (i) which is updated every trial (t) after animals receive feedback from their decision. The updated value is modulated by learning rate (α) and the reward prediction error (δ).

$$Q_{t+1}(i) = Q_t(i) + \alpha\delta \qquad \text{Eq 3.3}$$

Reward prediction error is described as the difference between an animal's expected (Q(i)) and received reward (r = 1 or r = 0)

$$\delta = r - Q_t(i) \qquad \text{Eq 3.4}$$

Animals are assumed to choose stimuli with the highest estimated reward value such that the probability of choosing the stimulus (i) on a trial (t) is dependent upon the animal's estimation of the stimuli value. The decision of which stimulus to choose is also dependent upon the parameter ß (the inverse temperature of the softmax equation) which describes

how deterministic stimulus selection is. High ß values mean that choices are made towards stimuli with higher estimated values while low ß values essentially mean that choices are random.

$$P(i) = \frac{e^{\beta Q_t(i)}}{\sum_{i=1}^{n} e^{\beta Q_t(i)}} \qquad \text{Eq 3.5}$$

Due to evidence that both animals and humans tend to learn from positive and negative prediction errors at different rates (Alsiö et al., 2019; Grogan et al., 2017; Noworyta-Sokolowska et al., 2019) data were also fit to a model containing separate learning rates for positive and negative prediction errors.

$$\begin{cases} Q_{t+1}(i) = Q_t(i) + \alpha_+ \delta & \delta > 0 \\ Q_{t+1}(i) = Q_t(i) + a_- \delta & \delta < 0 \end{cases} \qquad \text{Eq 3.6}$$

By iterating through each trial as described using the same input information as that animals received it is possible to create a computer generated "optimal strategy" which can be compared with behavioural outputs to create an estimation of absolute reward learning compared to a perfect strategy. This has been termed theoretical accuracy. Data from both training and acute drug studies were fitted by the model to generate outputs. For acute drug studies data for each individual rat were fit to both single learning rate (Qlearn1) and dual learning rate (Qlearn2) models in order to generate the learning rate and ß parameters. The best fitting model was assessed from vehicle data using Bayesian Information Criterion (Schwarz, 1978, BIC) with the model with the lowest BIC always chosen. Once the model had been fit, starting parameters were used to individually fit each dose and animal separately to create the theoretical accuracy output parameter.

3.3.7 Statistical analysis

A custom MATLAB program (MathWorks Inc version R2017a, USA) was used to decode KLimbic output files into outcome measures. This program was also used to run the Q-learning model. Following decrypting of data, statistical analysis was conducted in SPSS (IBM version 26, USA) and graphics were constructed using GraphPad Prism 8 (GraphPad Software, USA). Previous studies in the PRLT were used to estimate the sample size required for the present study (Bari et al., 2010). All analysis and outlier exclusion was carried out blind to treatment

with Grubbs' test being used to identify outliers. All drug studies were analysed as independent entities. Animals were also excluded from analysis for a session if they did not complete over 50 trials, however the rule changes and trials to first rule change were kept in the analysis as this was less influenced by low trial completion.

All parameters from principal drug study experiments were analysed using a repeated measures one-way ANOVA (factor: treatment(repeated)) with the exception of true and misleading feedback which was analysed as a repeated measures two-way ANOVA (factors: treatment (repeated) and feedback type (repeated)). Post-hoc comparisons were made utilising Sidak's correction. All data were assessed for normality using Shapiro Wilk and Kolmogorov-Smirnov tests with Friedman's test coupled to Bonferroni corrected Wilcoxon Signed Ranks tests being carried out where this assumption was not met. Data that passed the assumptions of normality was also assessed for violations of Sphericity using Mauchly's test and where this assumption was violated then the Huynh-Feldt correction was used to adjust the degrees of freedom.

Image reversal data were analysed as a two factor repeated measures ANOVA with the factors session (repeated) and reversal. Pre-feeding and single citalopram dose experiments alongside comparisons in output measures before and after extensive behavioural testing were analysed using paired t-tests where data were normally distributed and with Wilcoxon signed rank tests where this was not the case. For comparison of model fit the difference between Qlearn1 and Qlearn2 BIC output values was compared to 0 using one-sample Wilcoxon tests. Model data throughout training was fitted by either least squares linear regression or by least-squares fitting of a Gompertz growth equation. Comparisons between theoretical and task accuracy were made utilising the extra sum of squares test.

Dotted lines indicate separate drug studies. A bracket and star/s over multiple bars indicates a main effect of treatment while star/s over a single bar indicate a post-hoc significant difference compared to vehicle treatment for that drug study. All data is shown as mean ± SEM. * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

3.4 Results

3.4.1 Effects of pre-feeding and starting image upon task performance

In order to understand the effects of food restriction upon performance animals were pre-fed before undergoing the task. Pre-feeding had no effect on the main outcome measure of rule changes but did decrease motivation as measured by initiation reaction time (Figure 3.4C, paired t-test, t_{11} = 4.6, p = 0.0008). Pre-feeding also had no effect upon feedback sensitivity but decreased the ability of animals to follow an optimal reward learning strategy as measured by theoretical accuracy (Figure 3.4F, paired t-test, t_{11} = 2.97, p = 0.013).

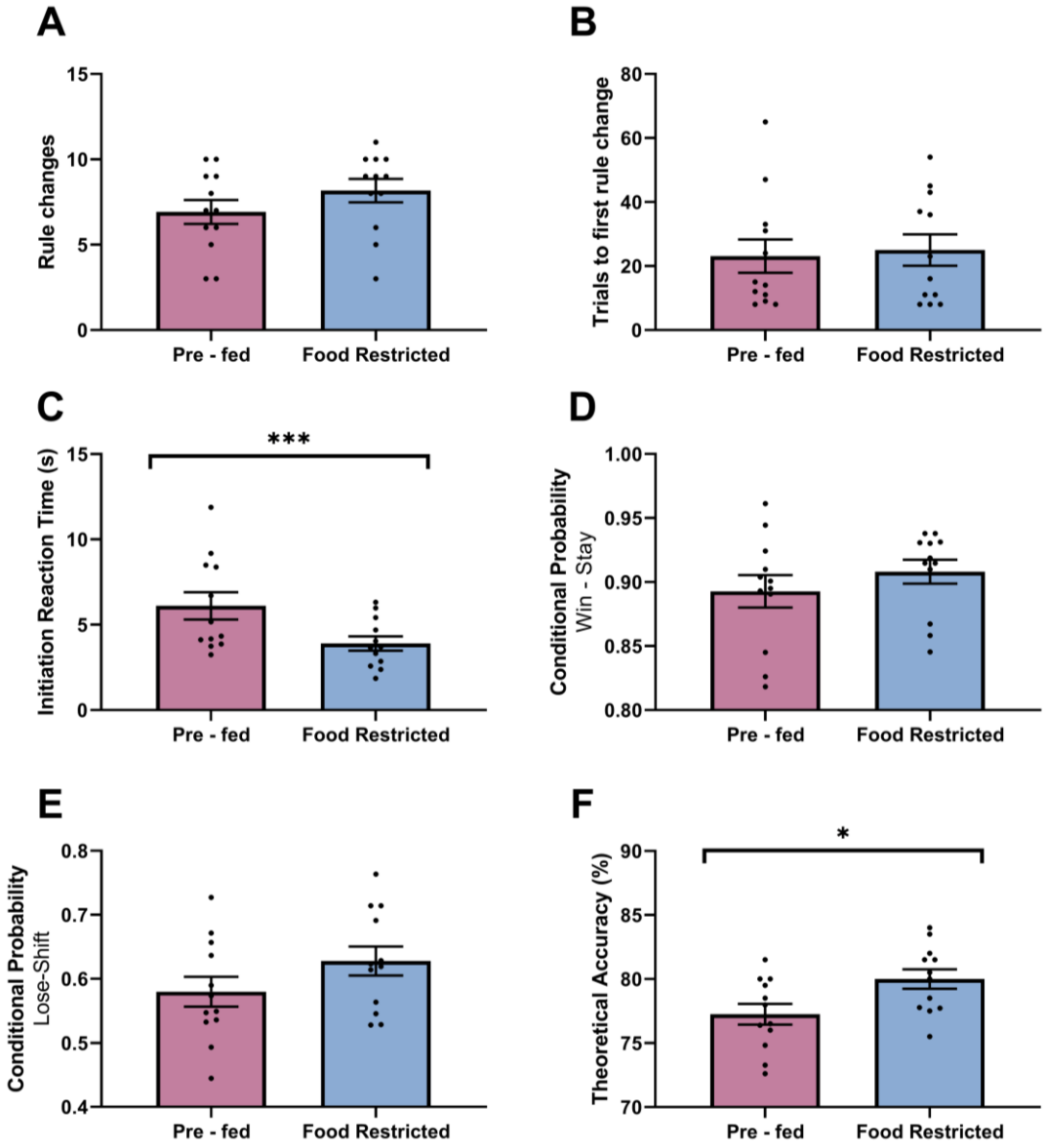


Figure 3.4 The effect of pre-feeding animals upon performance in the PRLT. (A) Rule changes, **(B)** trials to first rule change, **(C)** Initiation reaction time, **(D)** win-stay probability, **(E)** lose-shift probability and **(F)** theoretical accuracy. N = 12 rats

Animals always started each session having the same spatial directions for the "rich" and "lean" stimuli between sessions. In order to ascertain the importance of this for task performance the starting contingencies for each animal were reversed ("image reversal"). Image reversal had no effect upon the main output measure of rule changes but did increase the number of trials required by animals to learn the first rule (Figure 3.5B, two-way RM-ANOVA, main effect of reversal, $F_{1,10} = 6.21$, $p = 0.032$) and decreased theoretical accuracy (Figure 3.5F, two-way RM-ANOVA, main effect of reversal, $F_{1,11} = 10.03$, $p = 0.009$).

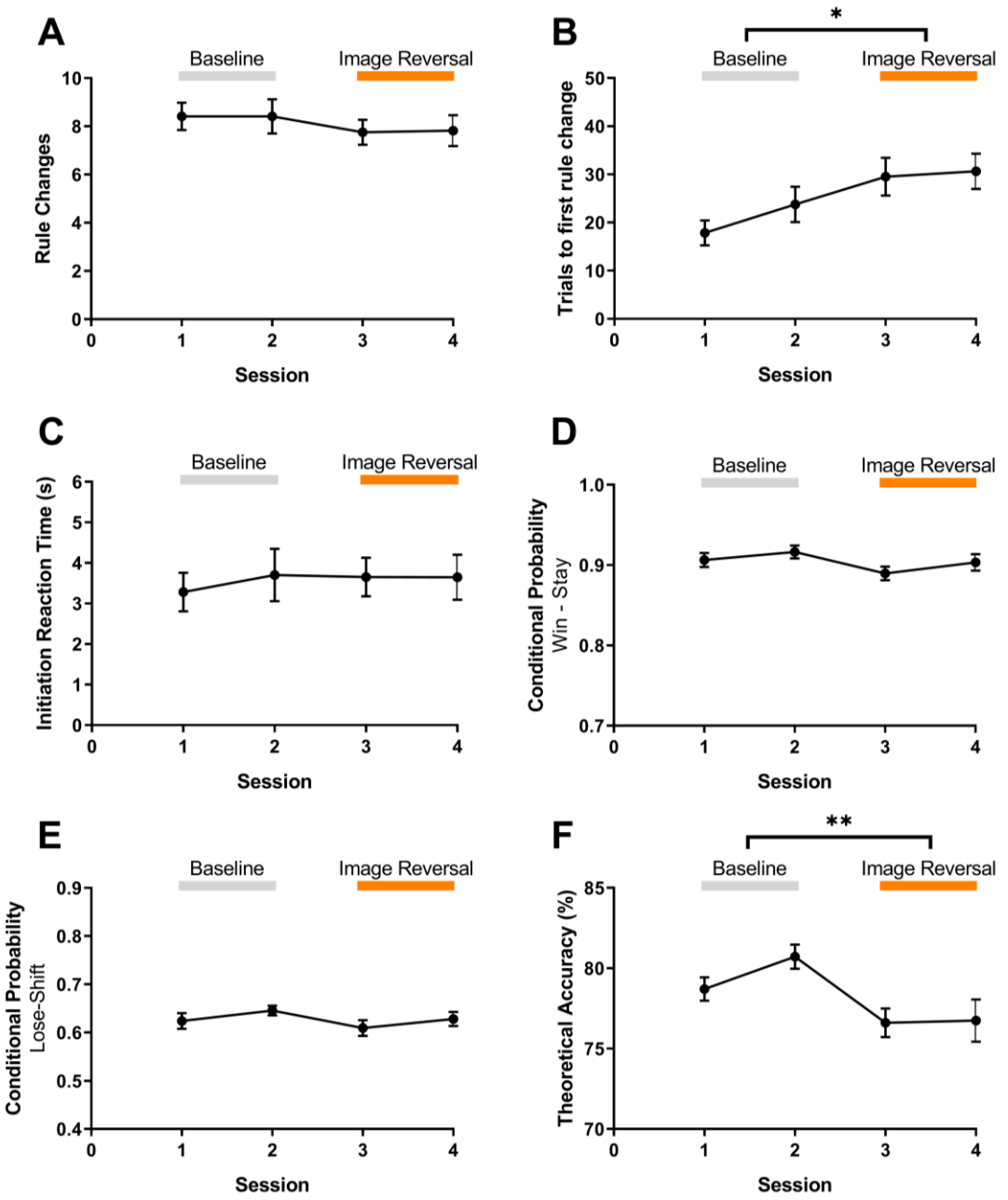


Figure 3.5. Effects of reversing starting image contingencies upon animal performance. **(A)** rule changes, **(B)** trials to first rule change, **(C)** initiation reaction time, **(D)** win-stay probability, **(E)** lose-shift probability and **(F)** theoretical accuracy. N = 12 rats

3.4.2 Evaluation of Qlearn reinforcement learning model fit

Data were fit with both a single (Qlearn1) and dual (Qlearn2) learning rate Qlearning model to understand the effects of pharmacological treatment upon underlying reward learning computational parameters. Animal data from training was fit into the model and compared to behavioural outcome measures in order to reassure that the model has captured reward related processing within the task. Throughout training, the learning rate derived from the Qlearn1 model followed a close relationship with the behavioural parameter rule changes (Figure 3.6A) with there being a correlation across all sessions between learning rate and rule changes (Figure 3.6B, linear regression, $F_{1,207} = 180.9$, $p < 0.0001$, $R^2 = 0.47$). Throughout training, both experimentally derived accuracy and theoretical accuracy followed a similar relationship (Figure 3.6C), however experimental accuracy always remained below theoretical accuracy (extra sum of squares test, $F_{3,424} = 15.9$, $p < 0.0001$). Across all training sessions there was a strong correlation between the two accuracies (Figure 3.6D, linear regression, $F_{1,212} = 453.3$, $p < 0.0001$, $R^2 = 0.68$). The gradient of this best fit line ($m = 0.79$) suggests that there is approximately a consistent 20% difference between theoretical and experimental accuracy. For every drug study Qlearn1 and Qlearn2 model fit was compared for vehicle data with the Qlearn1 model always the better fitting model (Figure 3.6E, statistics in figure legend).

3.4.3 The PRLT is sensitive to modulation of dopamine neurotransmission

Due to the longstanding understanding that intact dopamine neurotransmission is integral for reward learning (Schultz, 2016), animals were treated with the broad spectrum dopamine receptor antagonist flupentixol and the serotonin, noradrenaline and dopamine releasing agent amphetamine before testing in the PRLT.

Flupentixol treatment reduced the number of rule changes animals were able to make within a session (Figure 3.7A, Friedman test, $x^2(3) = 17.0$, $p = 0.0007$) while concurrently reducing the number of trials animals completed (Figure 3.7C, Friedman test, $x^2(3) = 25.98$, $p<0.0001$). Flupentixol treatment also impacted positive feedback sensitivity with a decrease in win-stay probability (Figure 3.7D, RM-ANOVA, $F_{1.422,14.22} = 24.5$, $p = 0.0001$) seen.

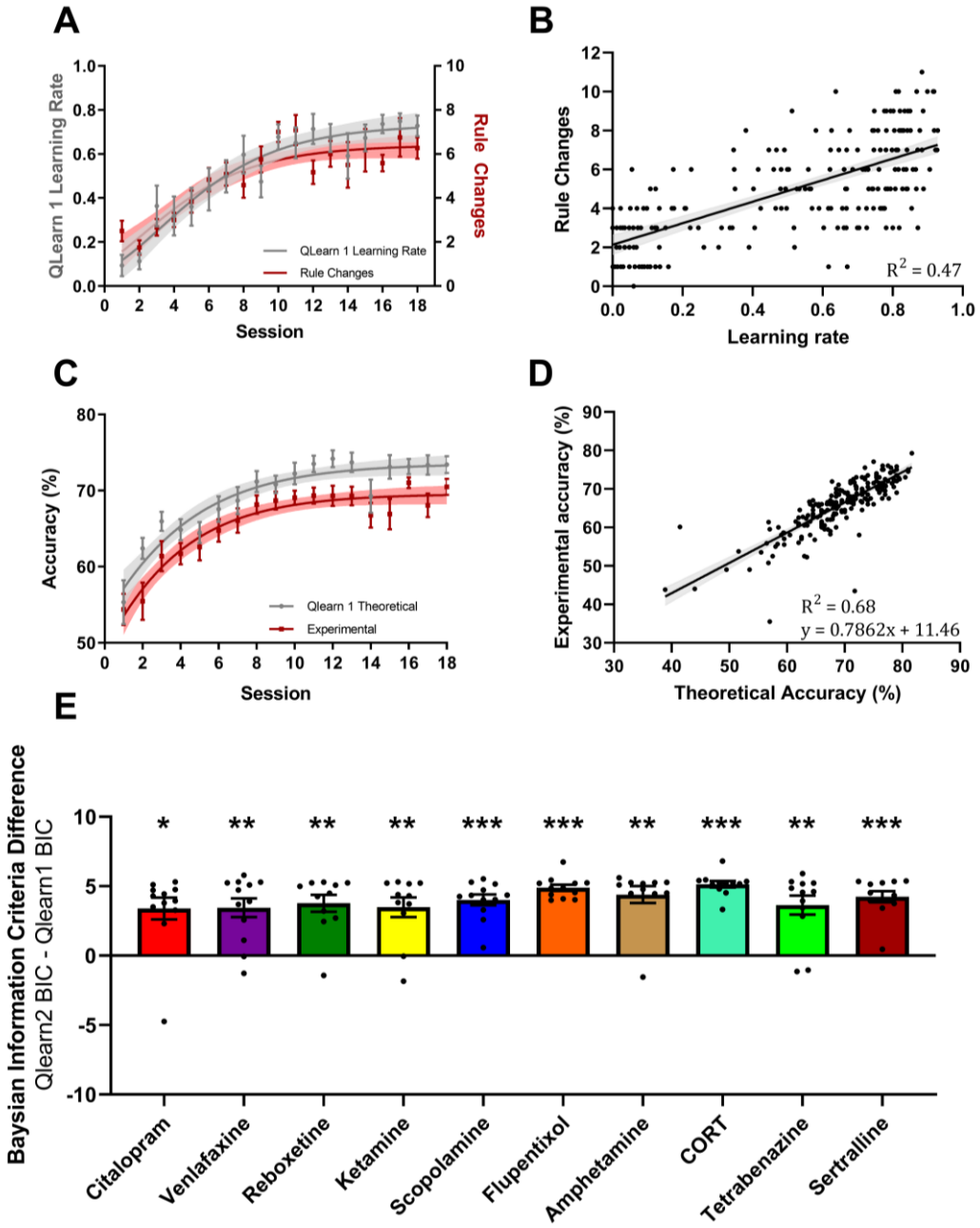


Figure 3.6 Evaluation of Qlearn model fit using PRLT data. **(A)** Correlation between rule changes and learning rate over the first 18 sessions of training in the PRLT. **(B)** Correlation between rule changes and learning rate. **(C)** Correlation between absolute accuracy and accuracy compared to a model predicted perfect strategy during training. **(D)** Correlation between experimental accuracy and theoretical accuracy from training data. **(E)** Comparison between Qlearn1 and Qlearn2 using BIC, positive values indicate Qlearn1 fitting better while negative values indicate Qlearn2 fitting better. Model fit comparisons were made using one-sample Wilcoxon tests; citalopram: p = 0.016, venlafaxine: p = 0.003, reboxetine: p = 0.002, ketamine: p = 0.005 scopolamine: p = 0.0005, flupentixol: p = 0.0005, amphetamine: p = 0.002, corticosterone: p = 0.0005, tetrabenazine: p = 0.002, sertraline: p = 0.0005. N = 11-12 rats depending on study. N = 12 rats

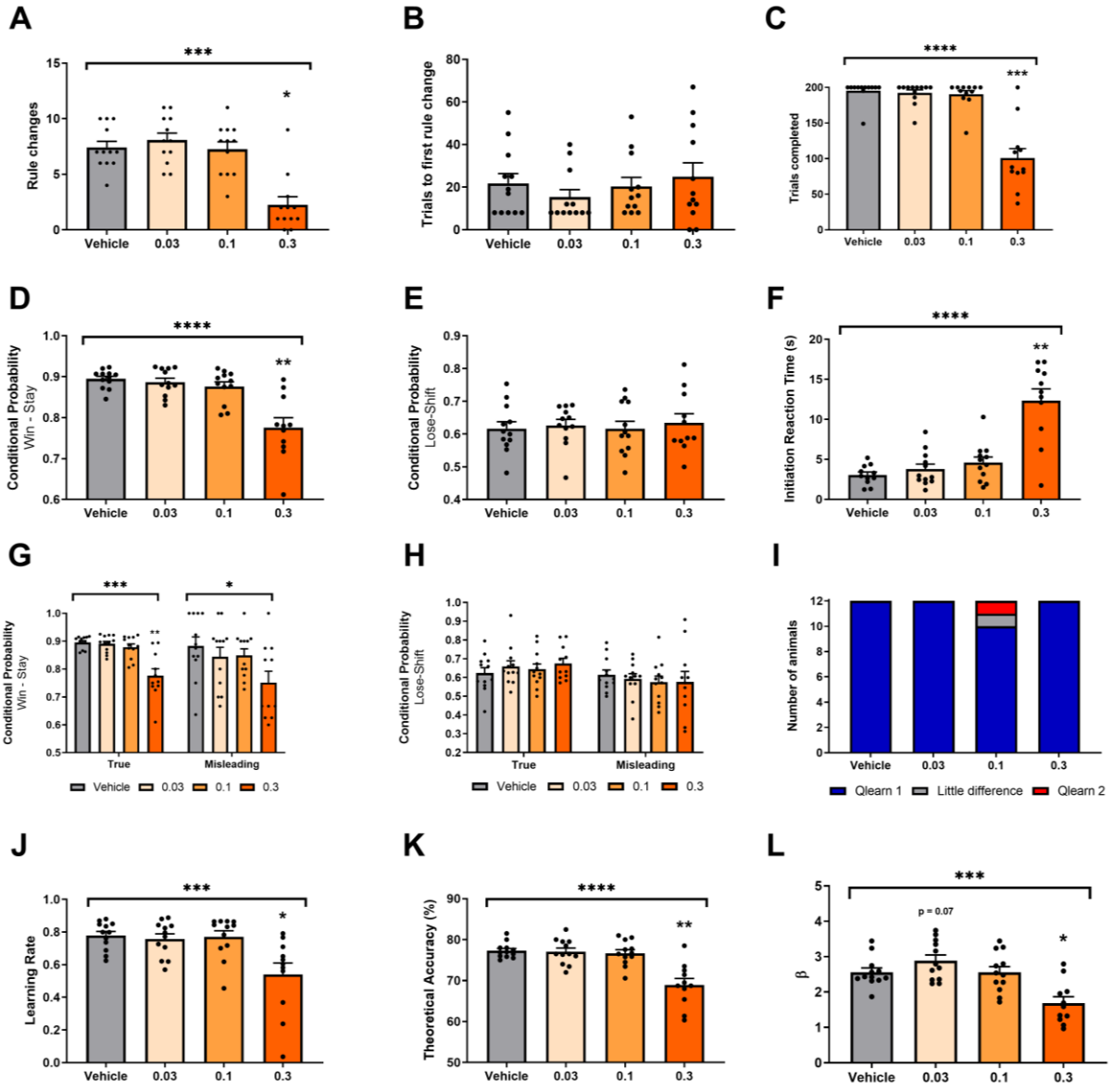


Figure 3.7 Effects of flupentixol upon reward learning and feedback sensitivity in the PRLT. (A) rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** ß, the inverse SoftMax temperature. N = 12 rats. All doses are shown in mg/kg i.p.

A trend towards an interaction between feedback type (true vs misleading) and treatment was also seen (Figure 3.7G, 2-way ANOVA, $F_{3,30} = 2.80$, $p = 0.057$). Upon further investigation both true and misleading win-stay behaviour appeared to be attenuated by flupentixol treatment (true: RM-ANOVA, $F_{1.47,14.73} = 21.6$, $p = 0.0001$; misleading: RM-ANOVA, $F_{3,30} = 4.02$, $p = 0.016$). Decreased motivation was also seen following treatment (Figure 3.7F, RM-ANOVA, $F_{1.64,14.8} = 28.9$, $p < 0.0001$). When data were analysed in the Qlearn1 model it revealed that dopamine antagonism led to a strong attenuation of reward learning with decreases in learning rate (Figure 3.7J, Friedman test, $x^2(3) = 17.1$, $p = 0.0006$) and theoretical accuracy (Figure 3.7K, RM-ANOVA, $F_{3,30} = 16.55$, $p < 0.0001$) observed which were coupled with a decrease in deterministic decision making (Figure 3.7L, RM-ANOVA, $F_{2.01,20.08} = 15.47$, $p < 0.0001$). Interestingly there was a trend towards treatment increasing ß at the 0.03 mg/kg dose (Sidak corrected post-hoc, $p = 0.07$), however this effect was reversed at the high 0.3mg/kg (Sidak corrected post-hoc, $p = 0.041$).

Amphetamine treatment interestingly decreased both the number of rule changes animals performed in a session (Figure 3.8A, RM-ANOVA, $F_{3,33} = 7.01$, $p = 0.0009$) and the number of trials it took for animals to learn the first probabilistic rule (Figure 3.8B, Friedman test, $x^2(3) = 14.62$, $p = 0.002$). Amphetamine also had similar effects on both positive and negative feedback sensitivity causing a decrease in both (Figure 3.8D and 3.8E respectively, win-stay: RM-ANOVA, $F_{3,30} = 39.9$, $p < 0.0001$; lose-shift: RM-ANOVA, $F_{3,30} = 4.1$, $p = 0.015$). Motivation was also modulated by treatment (Figure 3.8F, RM-ANOVA, $F_{3,27} = 3.08$, $p = 0.044$) with this appearing to be driven by a trend towards increased motivation at the 0.1 mg/kg dose (Sidak corrected post-hoc, $p = 0.056$) which then returned to baseline at the 1mg/kg dose. Surprisingly when amphetamine data were fit into the Qlearn1 and Qlearn2 models then the model fits compared it appeared that treatment made it more likely animals' data would fit better into the Qlearn2 model (Figure 3.8I, Friedman test, $x^2(3) = 13.1$, $p = 0.005$). This appeared entirely driven by the 1 mg/kg dose (Dunn's multiple comparison test, $Z = 3.3$, $p = 0.003$) suggesting that animals learnt at different rates from positive and negative stimuli under the 1mg/kg dose of amphetamine. When the parameters resulting from these model fits were analysed it was found that amphetamine treatment impaired both learning rate (Figure 3.8J, RM-ANOVA, $F_{1.43, 14.26} = 42.5$, $p < 0.0001$) and theoretical accuracy (Figure 3.8K, RM-ANOVA, $F_{3,30} = 36.1$, $p < 0.0001$).

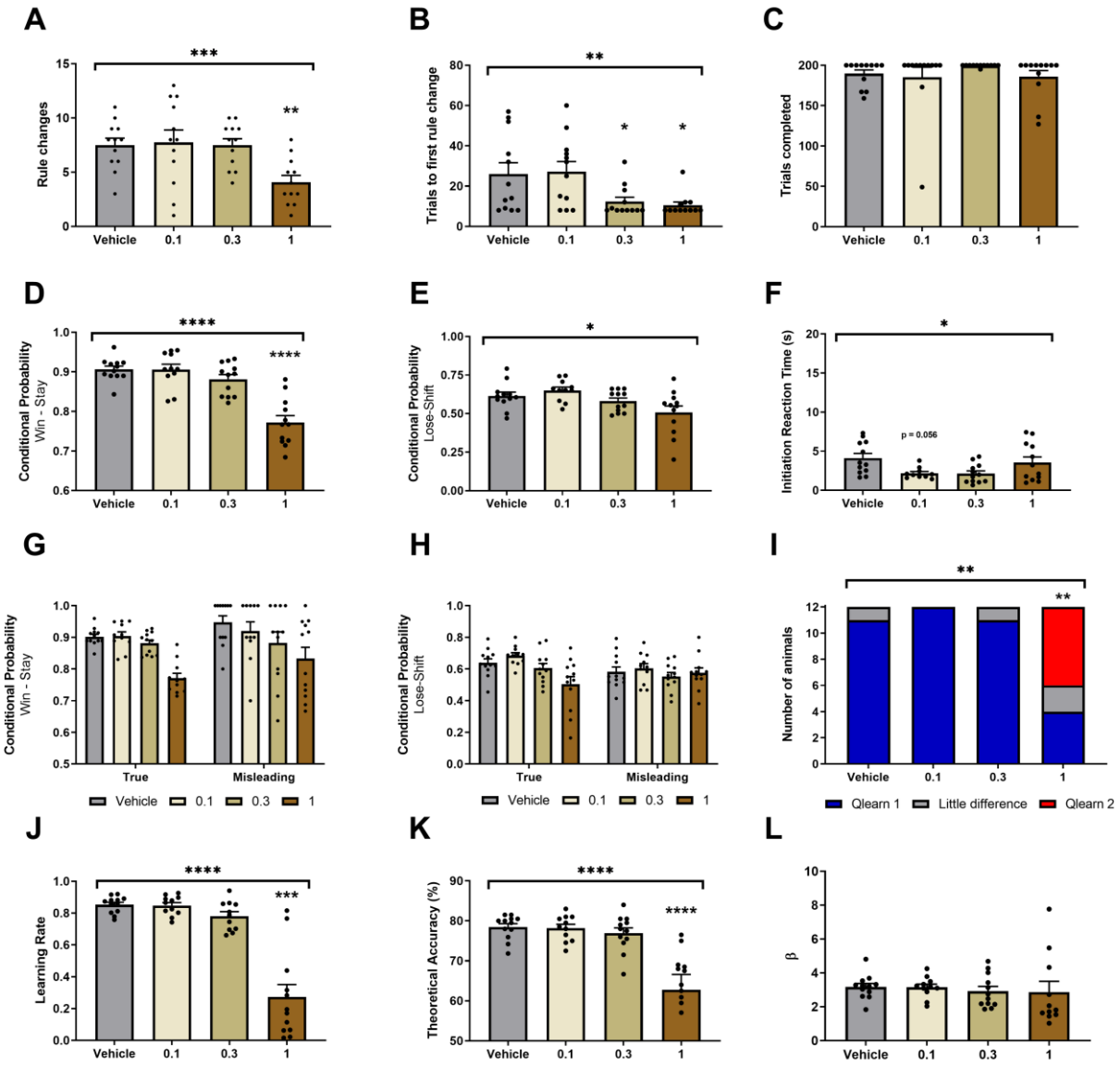


Figure 3.8 Effects of amphetamine upon reward learning and feedback sensitivity in the PRLT. (A) rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** ß, the inverse SoftMax temperature. N = 12 rats, one animal excluded from 0.1mg/kg dose for completing < 50 trials. All doses are shown in mg/kg i.p.

3.4.4 Modulation of reward learning and feedback sensitivity by conventional antidepressant treatment

Animals were acutely administered the conventional antidepressants citalopram, venlafaxine, reboxetine and sertraline before testing in the PRLT. Citalopram trended toward increasing rule changes over the first three doses (Figure 3.9A, RM-ANOVA, $F_{2,22}$ = 2.91, p = 0.076) while also decreasing the number of trials animals required to learn the first probabilistic rule (Figure 3.9B, Friedman test, $x^2(3)$ = 8.50, p = 0.037). Citalopram treatment also modulated positive feedback sensitivity with an increase in win-stay behaviour seen after administration (Figure 3.9D, RM-ANOVA, $F_{3,33}$ = 3.81, p = 0.019). Treatment also led to animals taking longer to initiate a trial, a proxy of motivation (Figure 3.9F, $F_{3,33}$ = 8.02, p = 0.0004), but caused animals to make more deterministic stimulus choices as measured by the ß parameter from the Qlearn1 model (Figure 3.7L, RM-ANOVA, $F_{3,33}$ = 7.24, p = 0.0007).

Reboxetine decreased the number of rule changes animals were able to make in a session (Figure 3.10A, RM-ANOVA, $F_{3,30}$ = 3.31, p = 0.033) while also trending towards increasing the number of trials required to complete a first rule change (Figure 3.10B, Friedman test, $x^2(3)$ = 7.022, p = 0.071). Reboxetine treatment also decreased the number of trials animals completed within a session (Figure 3.10C, Friedman test, $x^2(3)$ = 18.0, p = 0.0004). Other effects of reboxetine treatment included, surprisingly for an antidepressant, decreased positive feedback sensitivity (Figure 3.10D, RM-ANOVA, $F_{3,24}$ = 6.16, p = 0.003), and decreased motivation (Figure 3.10F, RM-ANOVA, $F_{1.25,\ 12.48}$ = 11.17, p = 0.004). When reboxetine derived data were analysed using the Qlearn1 model it was revealed that treatment decreased accuracy compared to a model derived optimal strategy (Figure 3.10K, RM-ANOVA, $F_{3,30}$ = 3.14, p = 0.04) and that animals made less deterministic stimulus choices following treatment (Figure 3.10L, RM-ANOVA, $F_{3,30}$ = 4.13, p = 0.014).

Venlafaxine and Sertraline (Figure 3.11 and Figure 3.12 respectively) had no effect upon any behavioural or reinforcement learning model parameter analysed apart from Sertraline increasing the Qlearn1 ß parameter (Figure 3.12L, mixed-effects model, $F_{3,31}$ = 3.47, p = 0.028), meaning stimulus choices were less random.

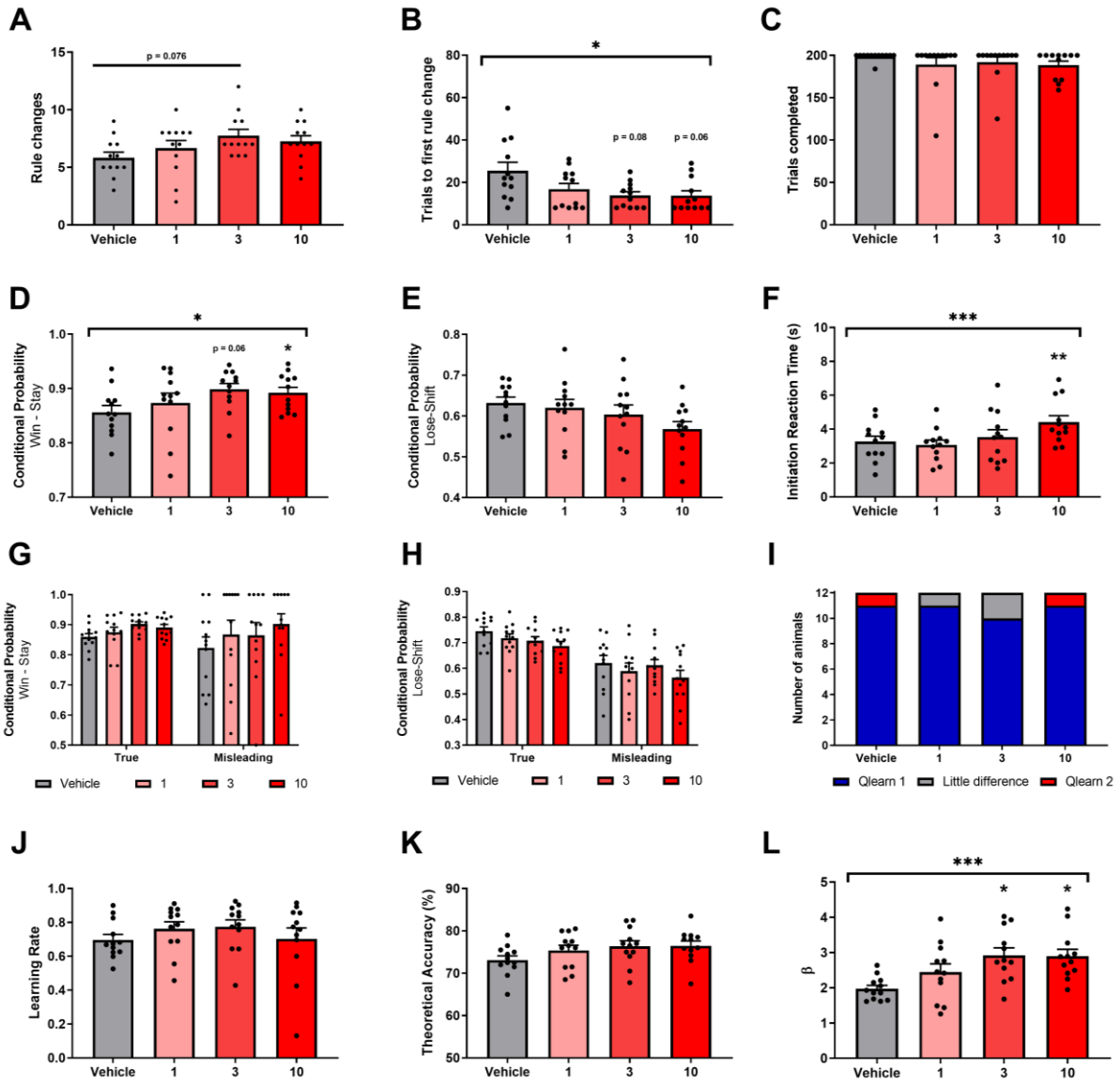


Figure 3.9 Effects of citalopram upon reward learning and feedback sensitivity in the PRLT. **(A)** rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** ß, the inverse SoftMax temperature. N = 12 rats. All doses are shown in mg/kg i.p.

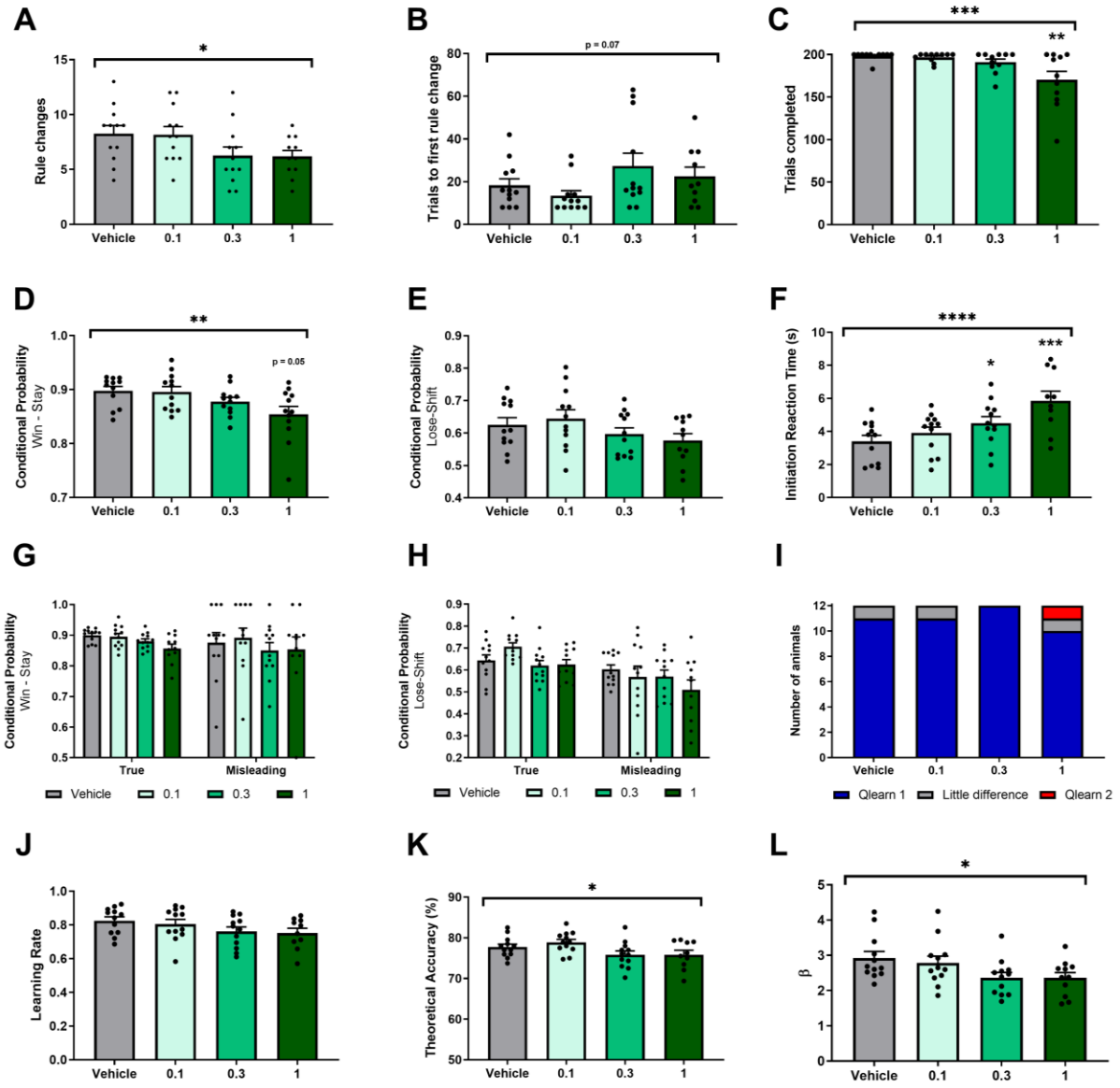


Figure 3.10 Effects of reboxetine upon reward learning and feedback sensitivity in the PRLT. **(A)** rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** β, the inverse SoftMax temperature. N = 12 rats. All doses are shown in mg/kg i.p.

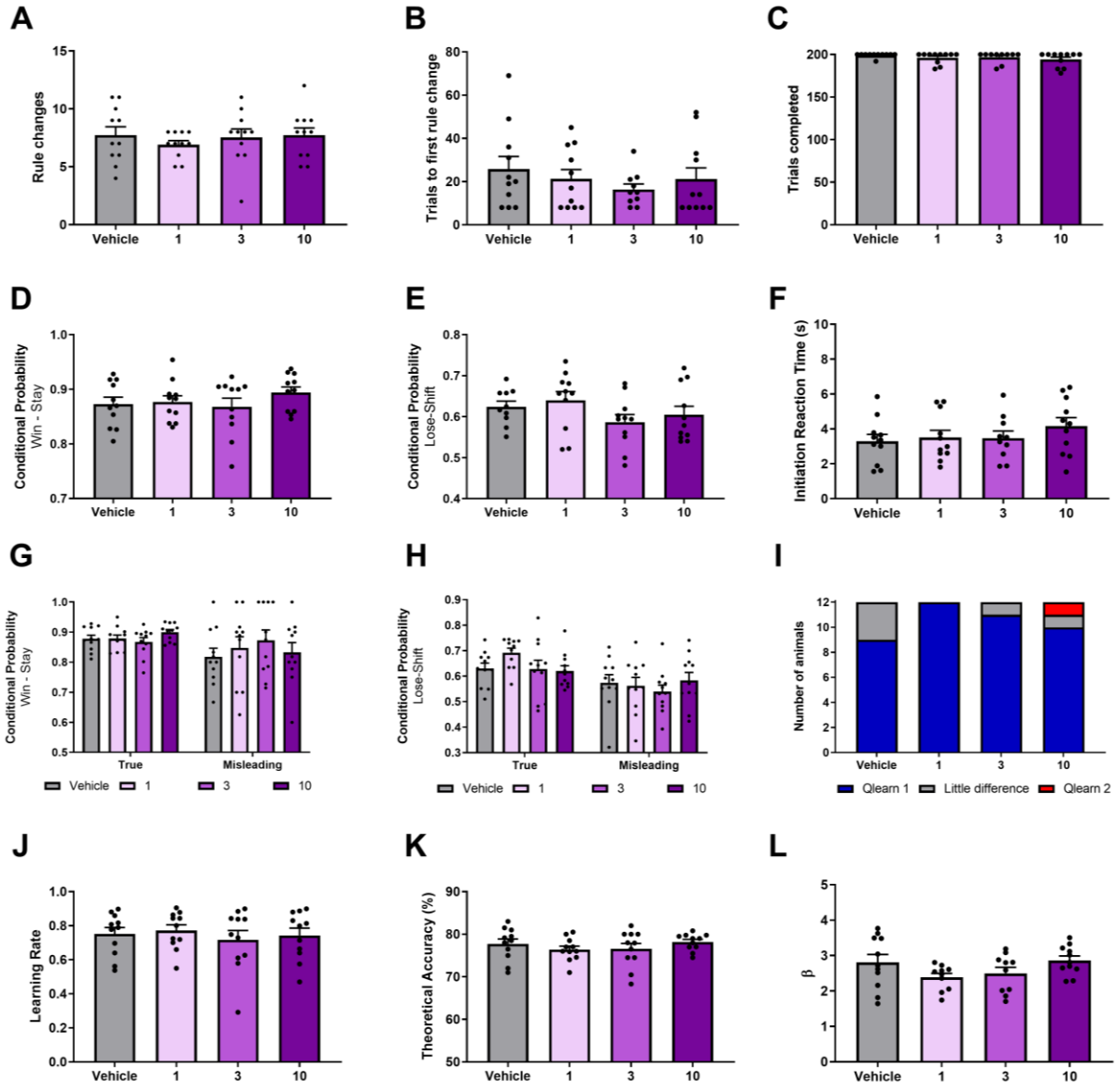


Figure 3.11 Effects of venlafaxine upon reward learning and feedback sensitivity in the PRLT. (A) rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** ß, the inverse SoftMax temperature. N = 11 rats. All doses are shown in mg/kg i.p.

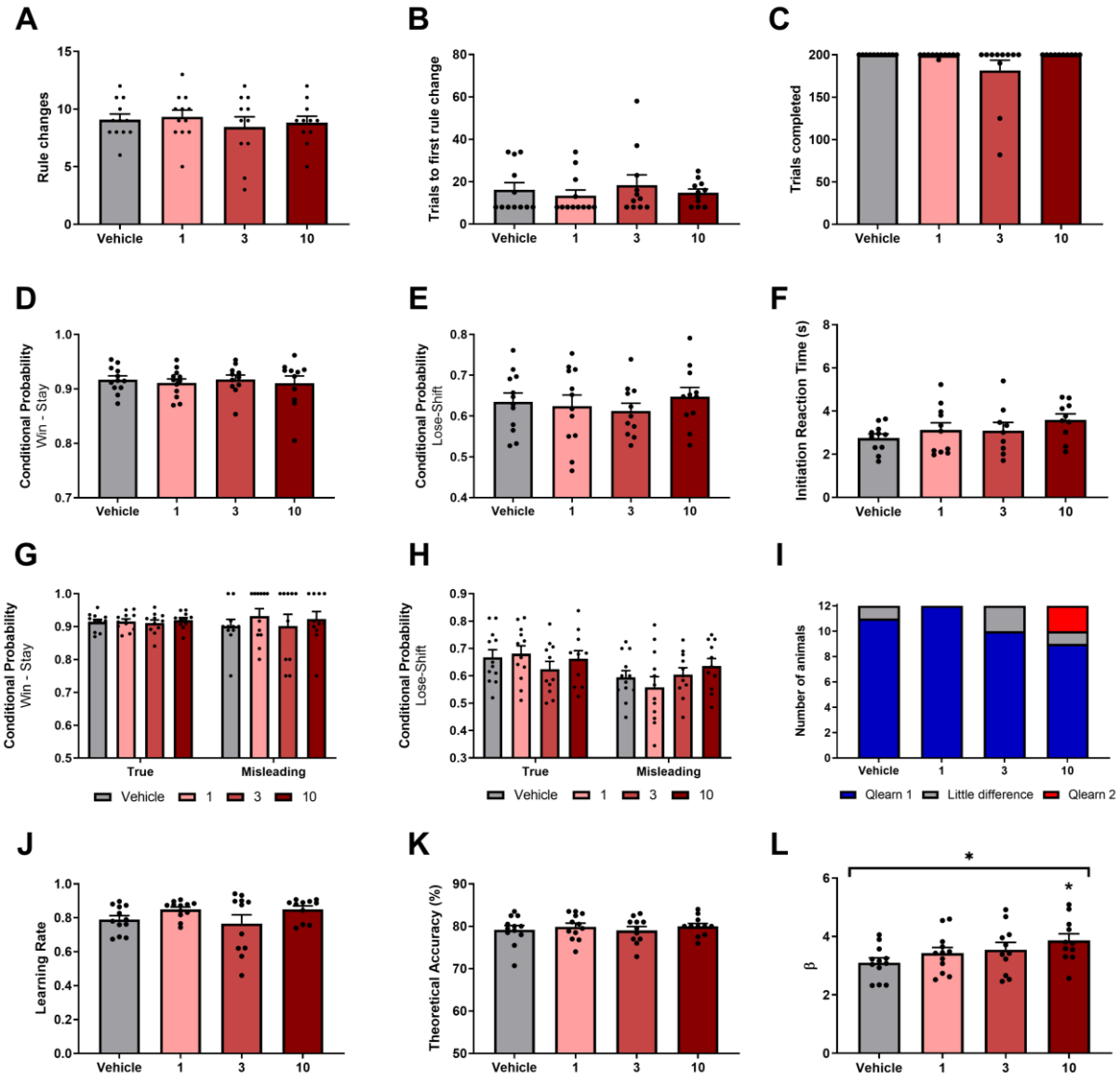


Figure 3.12 Effects of sertraline upon reward learning and feedback sensitivity in the PRLT. **(A)** rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** β, the inverse SoftMax temperature. N = 12 rats, one rat excluded from 3mg/kg and another from 10mg/kg for partial doses. All doses are shown in mg/kg i.p.

3.4.5 Impairment of reward learning by rapid acting antidepressant treatment in the PRLT

Prior to testing animals were administered with the rapid-onset antidepressant compounds ketamine and scopolamine. Ketamine reduced both the number of rule changes (Figure 3.13A, RM-ANOVA, $F_{3,33} = 5.697$, $p = 0.003$) and trials completed within a session (Figure 3.13C, Friedman test, $x^2(3) = 19.08$, $p = 0.0003$). Ketamine treatment also decreased positive feedback sensitivity as measured by win-stay probability (Figure 3.13D, RM-ANOVA, $F_{1.97, 19.66} = 3.928$, $p = 0.037$) alongside also decreasing motivation (Figure 3.13F, RM-ANOVA, $F_{1.08, 9.68} = 7.36$, $p = 0.021$) as measured by time taken to initiate a trial. An interaction between ketamine treatment and feedback type was observed for animal's lose-shift response to true and misleading feedback (Figure 3.13H, 2-way ANOVA, $F_{3,27} = 3.565$, $p = 0.027$). Further analysis revealed no effect of ketamine treatment on true lose-shift behaviour but a trend toward decreased sensitivity to NFS following misleading feedback emerged (RM-ANOVA, $F_{3,27} = 2.69$, $p = 0.066$). Ketamine also had the effect of reducing learning rate derived from the Qlearn1 model (Figure 3.13J, RM-ANOVA, $F_{1.31, 13.10} = 7.41$, $p = 0.013$).

Similarly to ketamine, scopolamine decreased the number of rule changes and trials completed within a session (Figure 3.14A and 3.14C respectively, rule changes: RM-ANOVA, $F_{2,22} = 16.23$, $p < 0.0001$; trials completed: Friedman test, $x^2(2) = 14.6$, $p = 0.0007$). Scopolamine also decreased positive feedback sensitivity (Figure 3.14D, RM-ANOVA, $F_{2,14} = 8.36$, $p = 0.004$) but increased initiation reaction time (Figure 3.14F, Friedman test, $x^2(2) = 7.75$, $p = 0.021$). When analysed with the reinforcement learning model it was apparent that scopolamine decreased both learning rate (Figure 3.14J, RM-ANOVA, $F_{2,14} = 12.36$, $p = 0.0008$) and theoretical accuracy (Figure 3.14K, RM-ANOVA, $F_{1.247,8.728} = 8.77$, $p = 0.013$) but had no effect on β (Figure 3.14L).

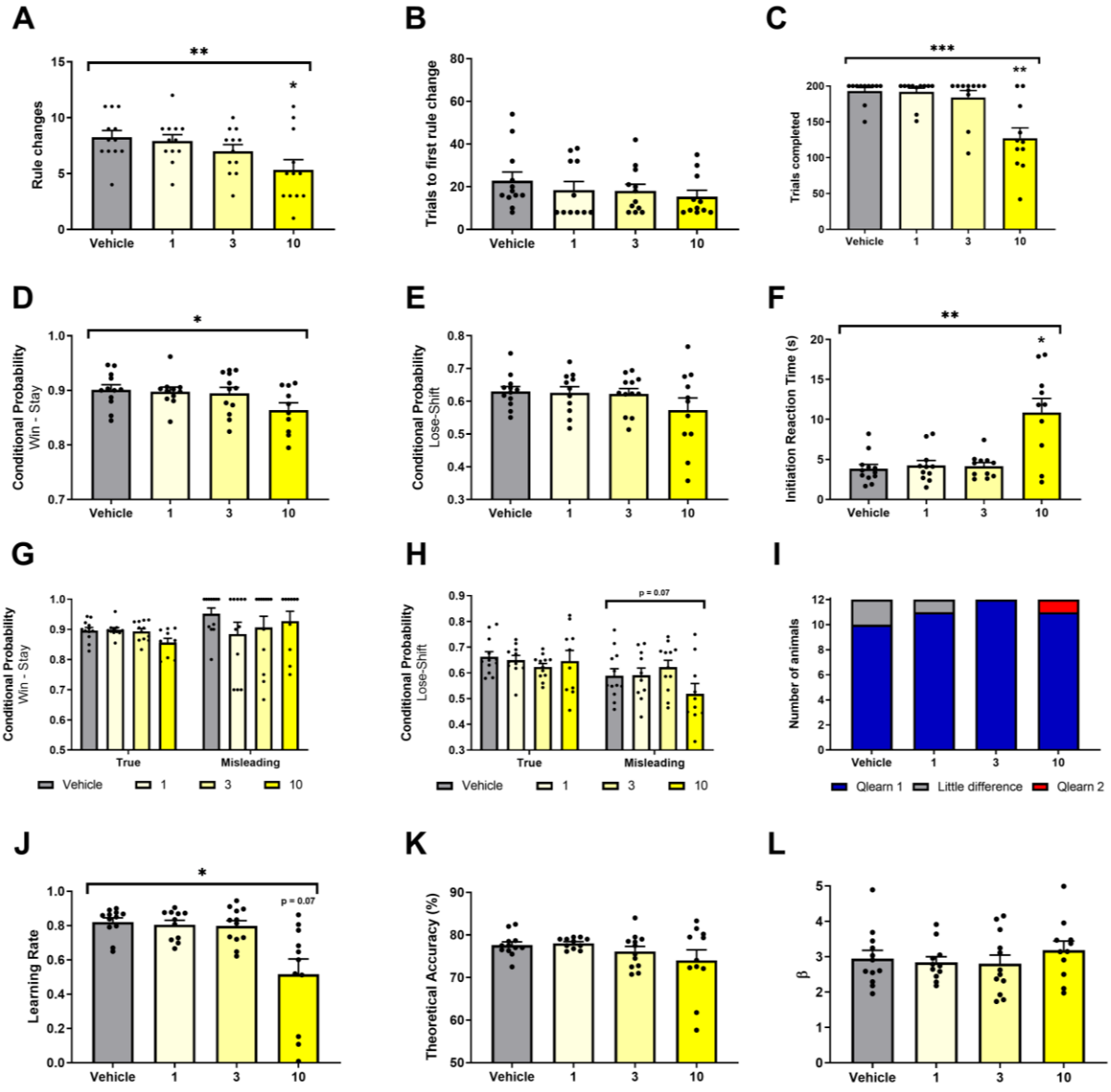


Figure 3.13 Effects of ketamine upon reward learning and feedback sensitivity in the PRLT. **(A)** rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** ß, the inverse SoftMax temperature. N = 11 rats. All doses are shown in mg/kg i.p.

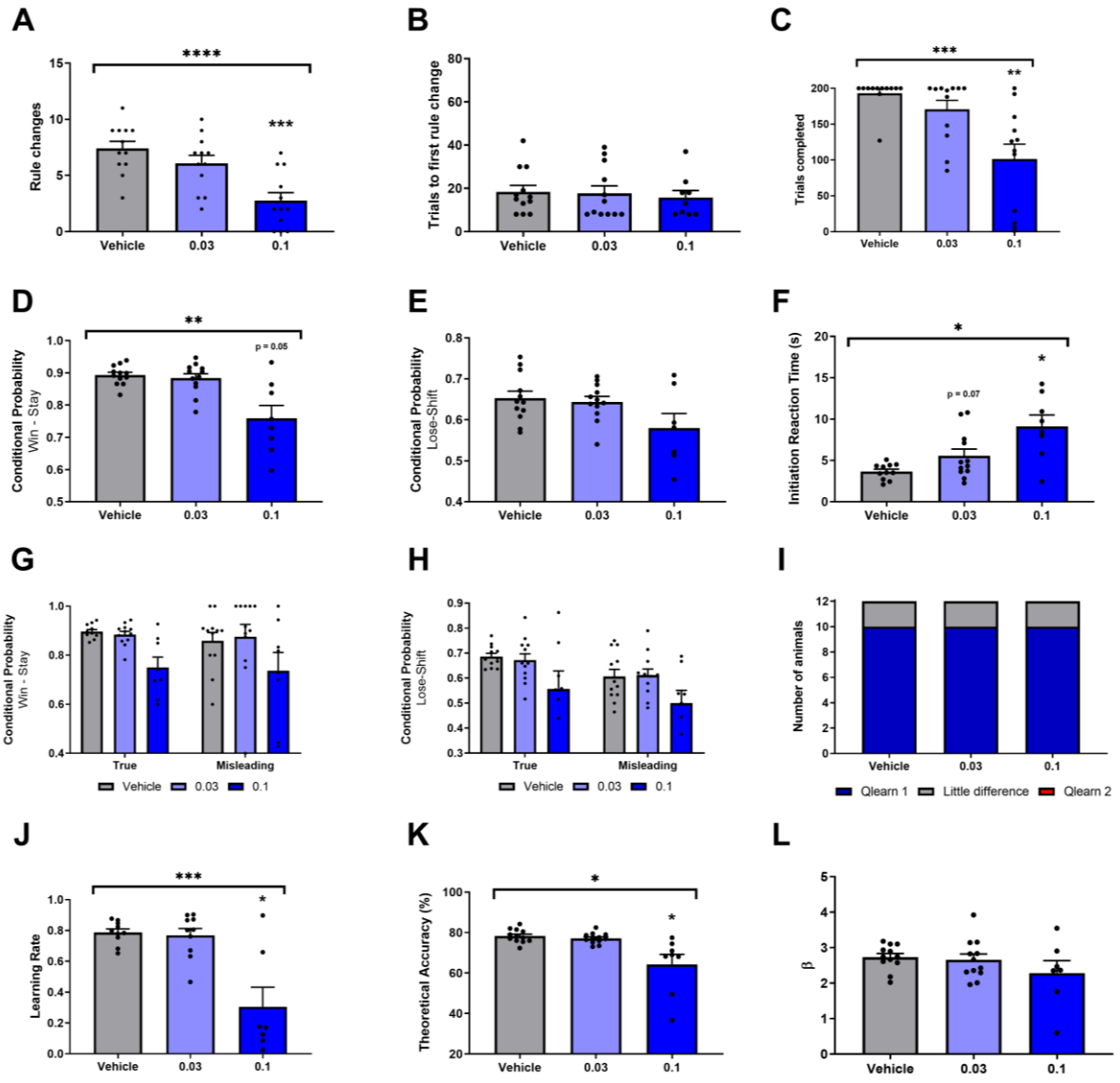


Figure 3.14 Effects of scopolamine upon reward learning and feedback sensitivity in the PRLT. (A) rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** β, the inverse SoftMax temperature. N = 12 rats, 4 rats excluded from analysis at 0.1 mg/kg for completing ≤ 50 trials. All doses are shown in mg/kg i.p.

3.4.6 No effects of pro-depressant pharmacological manipulations upon animals in the PRLT

Following the observations that acute antidepressant compounds can modulate behaviour in the PRLT animals were next tested with the pro-depressant compounds corticosterone and tetrabenazine which act both act through distinct pathways to cause depressive risk (Hinchcliffe et al., 2017; Stuart et al., 2017).

Corticosterone trended towards modulating the trials required for animals to learn the first probabilistic rule within a session (Figure 3.15B, Friedman test, $x^2(3) = 6.66$, $p = 0.084$) with this being manifested by an effect at the 1 mg/kg dose only (Bonferroni corrected Wilcoxon Signed Ranks test, $Z = -2.524$, $p = 0.036$). There was also a trend for corticosterone treatment to modulate theoretical accuracy (Figure 3.15K, RM-ANOVA, $F_{3,33} = 2.41$, $p = 0.085$) however this did not appear to be in any clear direction. Tetrabenazine similarly did not show any clear effects upon behaviour apart from a trend towards treatment decreasing theoretical accuracy (Figure 3.16K, RM-ANOVA, $F_{1.74, 19.1} = 3.55$, $p = 0.054$).

3.4.7 Loss of sensitivity in the PRLT with extensive repeated testing of animals

The previously described sertraline, tetrabenazine and corticosterone studies did not show results consistent with the hypothesises that sertraline would act similarly to citalopram as a SSRI and that the other two drugs would impair reward learning. Due to these being drugs being administered at the end of the experimental protocol after repeated testing of animals had already been conducted one possibility for these lack of effects could be that the PRLT loses sensitivity over time due to overtraining. Data from baseline sessions was analysed from the start of training to the end of all drug studies. Over repeated testing, rule changes (Figure 3.17A) increased over time with animals performing roughly 40% more rule changes in sessions 103-106 compared to sessions 16-18 (Figure 3.17B, Wilcoxon signed ranks test, $W_{12} = 58$, $p = 0.0068$). When win-stay and lose-shift behaviour (Figure 3.17C) were analysed it became apparent that the relative probability of both of these behaviour types increased with repeated testing (win-stay: Figure 3.17D, 8% increase, paired t-test, $t_{11} = 5.01$, $p = 0.0004$; lose-shift: Figure 3.17E, 12% increase, paired t-test, $t_{11} = 3.45$, $p = 0.0054$).

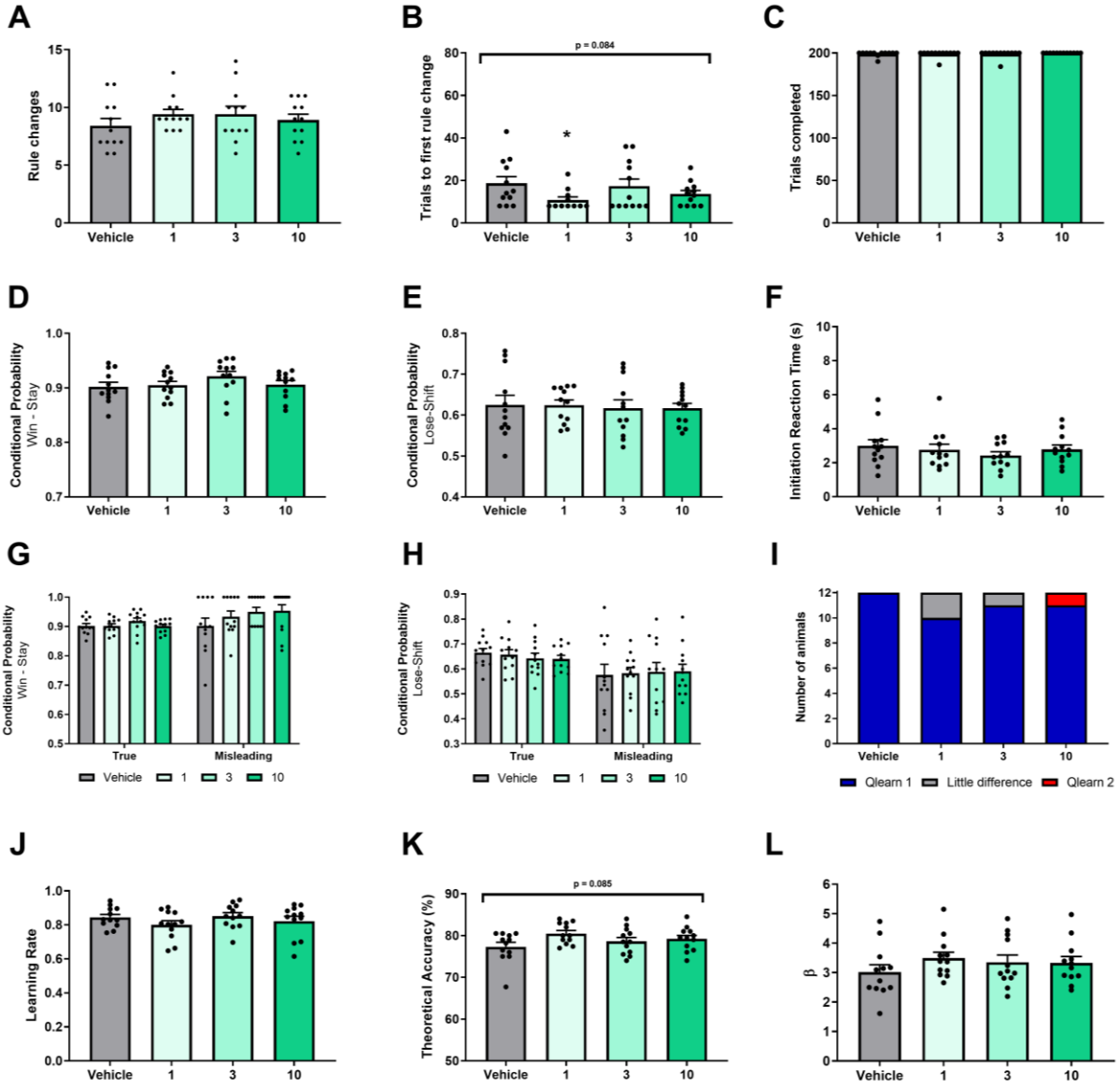


Figure 3.15 Effects of corticosterone upon reward learning and feedback sensitivity in the PRLT. (A) rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** ß, the inverse SoftMax temperature. N = 12 rats. All doses are shown in mg/kg s.c.

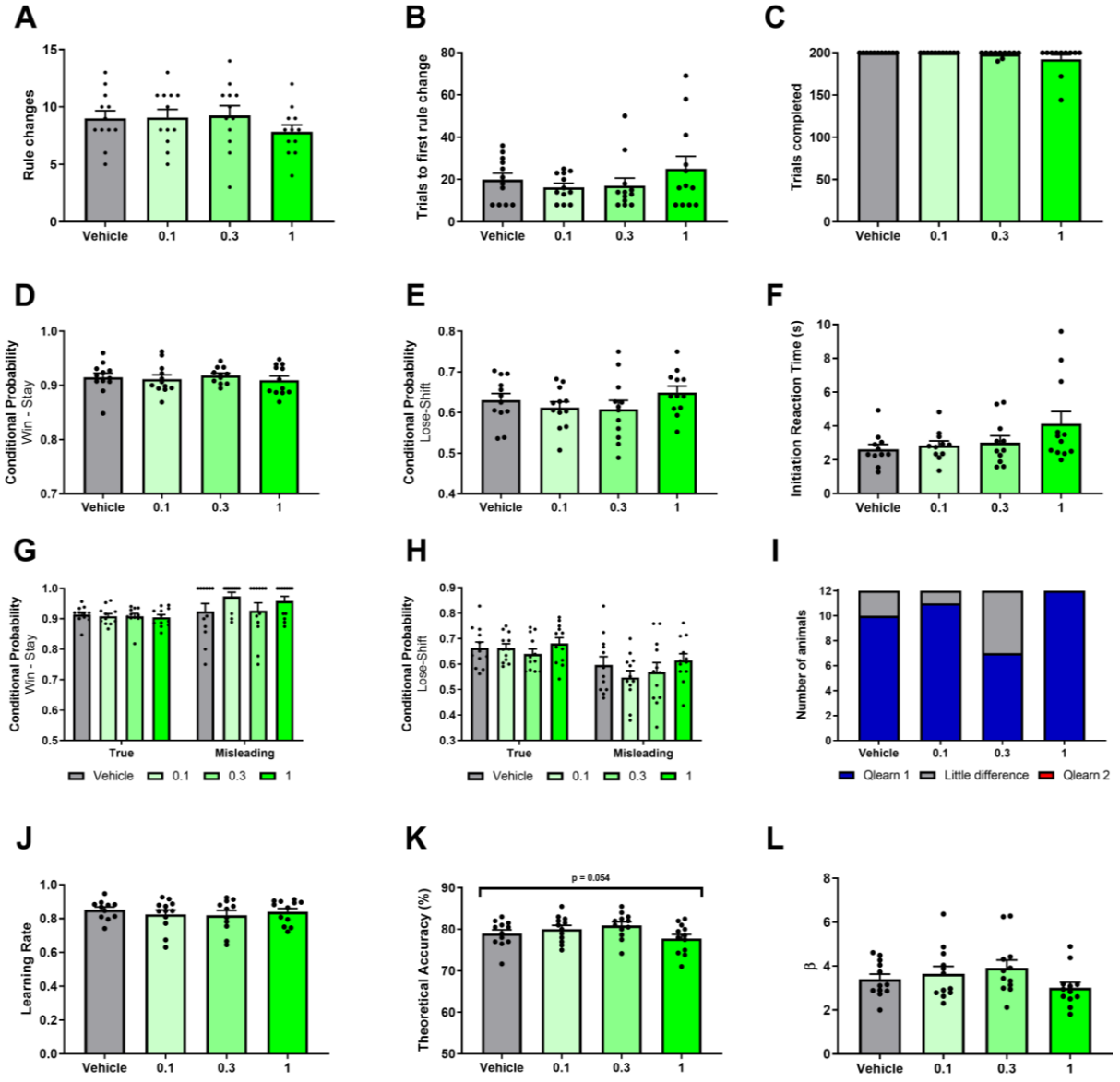


Figure 3.16 Effects of tetrabenazine upon reward learning and feedback sensitivity in the PRLT. **(A)** rule changes, **(B)** trials to first rule change, **(C)** trials completed, **(D)** win-stay probability, **(E)** lose-shift probability, **(F)** initiation reaction time, **(G)** true vs misleading win-stay probability, **(H)** true vs misleading lose-shift probability, **(I)** better fitting model of Qlearn1 and Qlearn2 by animal, **(J)** Qlearn1 learning rate, **(K)** theoretical accuracy and **(L)** β, the inverse SoftMax temperature. N = 12 rats. All doses are shown in mg/kg i.p.

Theoretical accuracy (Figure 3.17F) was also affected by repeated testing with animals displaying a roughly 8% rise in theoretical accuracy after the end of drug studies compared to before (Figure 3.17G, paired t-test, $t_{11} = 6.72$, $p < 0.0001$).

Due to these increases in rule changes and win-stay / lose-shift probabilities it was hypothesised that there might be a ceiling effect in the PRLT following repeated testing of animals. Animals were therefore administered with a single 3mg/kg dose of citalopram, the dose which was found previously to have the biggest effect, in order to assess if the PRLT had lost sensitivity over time due to a ceiling effect. Only the parameters for which a significant main effect of treatment in the original citalopram dose response study were analysed and were compared to data in that study re-analysed as a single dose study. Whereas previously citalopram treatment increased rule changes (Figure 3.18A), win-stay probability (Figure 3.18C) and Qlearn1 ß (Figure 3.18E) while decreasing the trials to first rule change (Figure 3.18B) after re-administration of citalopram only a trend towards animals having a higher Qlearn1 ß parameter value emerged (Figure 3.18E, Wilcoxon signed ranks test, $W_{11} = 42$, $p = 0.067$). It should be noted that although there was a main effect of citalopram treatment in the original study for initiation reaction time (Figure 3.18D) this was not significant when re-analysed as a single dose drug study and again the repeat 3mg/kg study had no effect upon this measure.

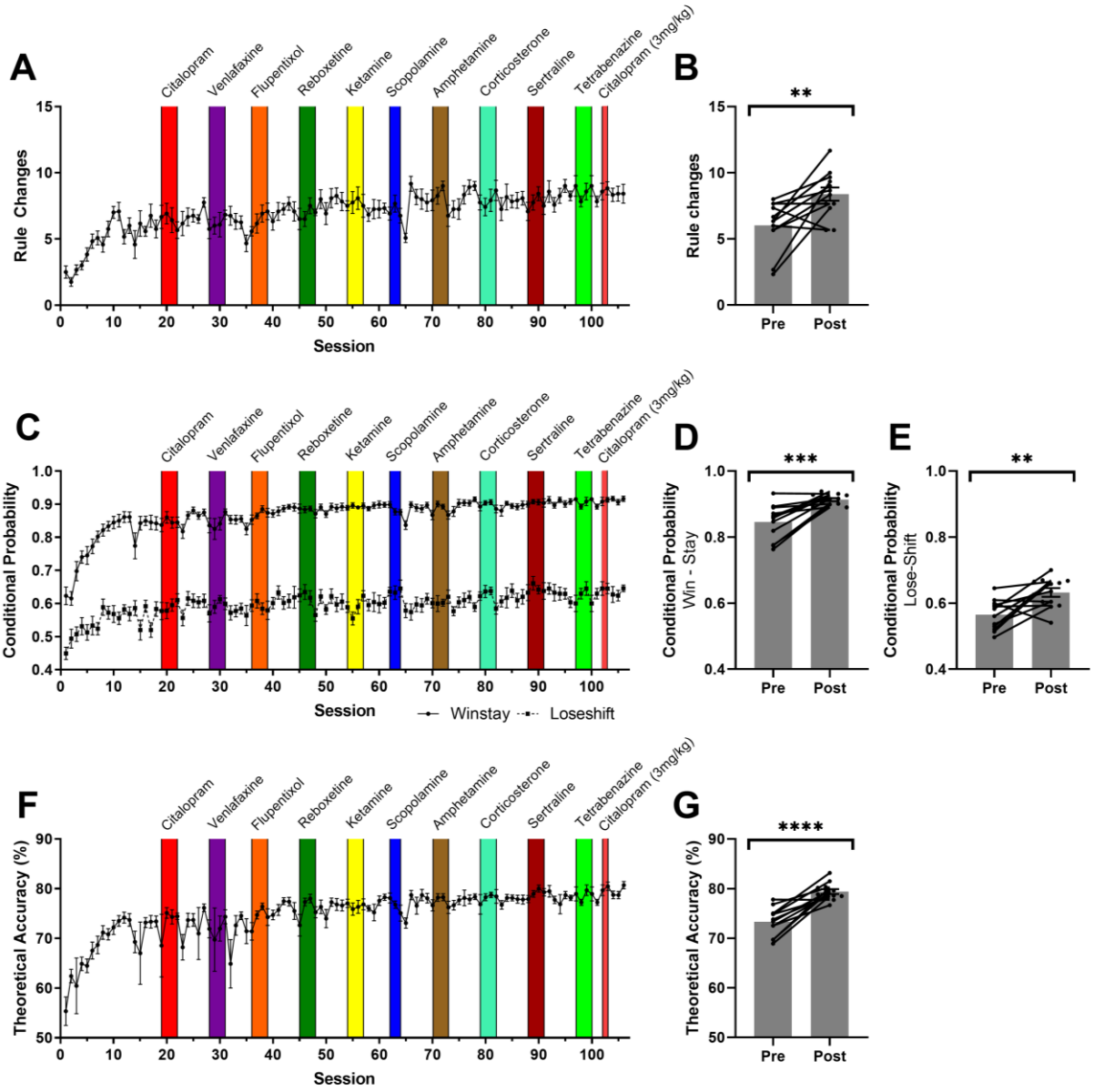


Figure 3.17. Baseline changes in key PRLT parameters with repeated testing. (A) Rule changes from all baseline sessions animals completed showing the baseline sessions for each drug study as a shaded zone over the relevant sessions. **(B)** Comparison between rule changes before the start of drug study experiments (sessions 16-18 (pre)) and after all experiments had concluded (sessions 103-106 (post)). **(C)** Win-stay (solid lines and circular markers, top) and lose-shift (dashed lines and square markers, bottom) probabilities across all baseline sessions. **(D)** Win-stay comparison of pre and post drug study experiments. **(E)** Lose-shift comparison of pre and post drug study experiments. **(F)** Qlearn1 theoretical accuracy across all baseline sessions animals completed. **(G)** Theoretical accuracy before and after animals completed acute pharmacology studies. N = 12 rats

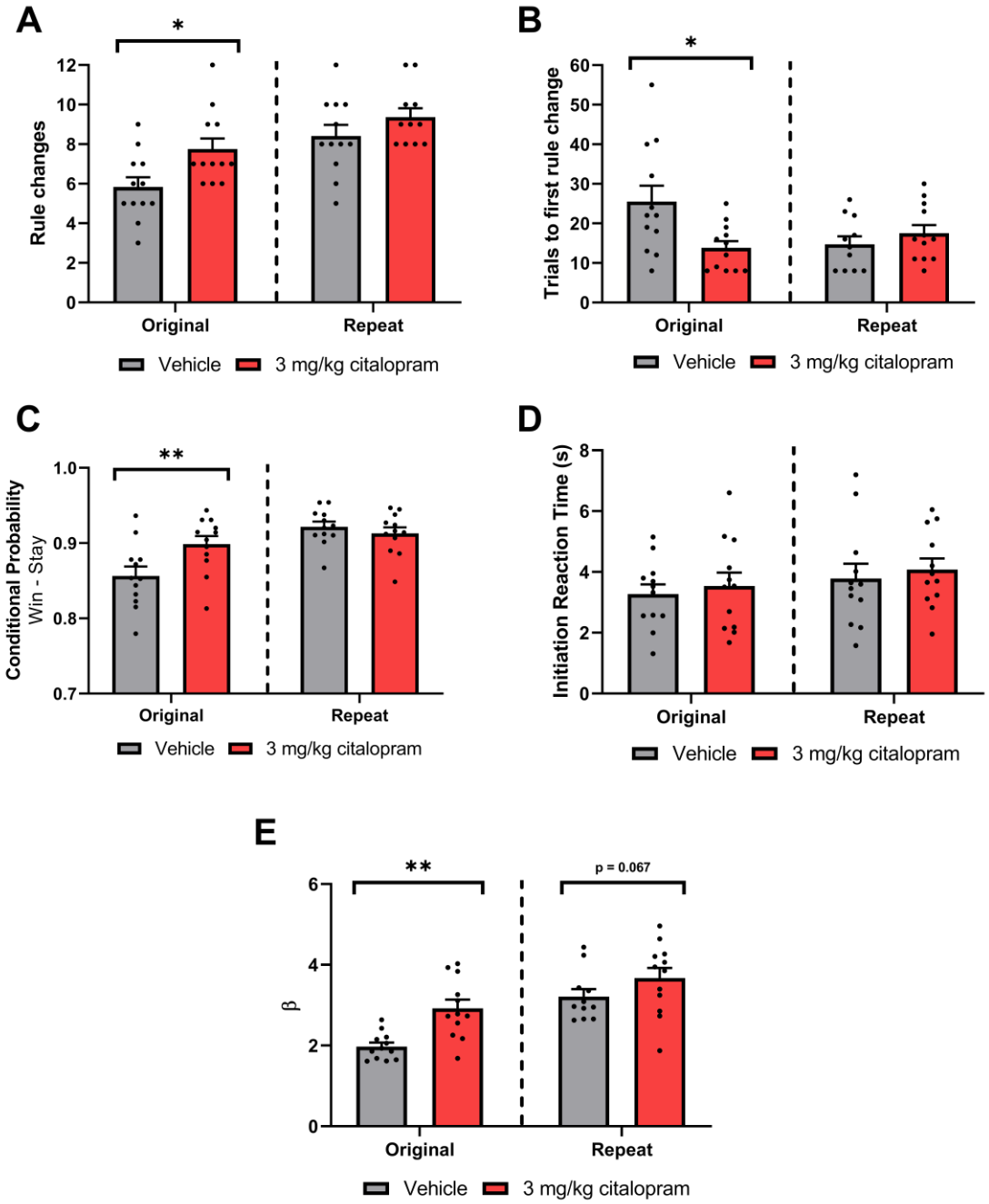


Figure 3.18. Loss of citalopram sensitivity in the PRLT after repeated testing. Comparison between the 3mg/kg dose of the original full citalopram dose response study and a 3mg/kg repeat dose following repeated testing in the PRLT. **(A)** Rule changes, **(B)** trials to first rule change, **(C)** win-stay probability, **(D)** initiation reaction time and **(E)** Qlearn1 ß. All original data has been re-analysed as a single dose drug study for comparison; rule changes: Wilcoxon signed ranks test, $W_{12} = 51$, $p = 0.046$; trials to first rule change: paired t-test, $t_{11} = 2.47$, $p = 0.03$; win-stay probability: paired t-test, $t_{11} = 3.3$, $p = 0.008$; beta: paired t-test, $t_{11} = 4.2$, $p = 0.002$. N = 12 rats

3.5 Discussion

3.5.1 Dopaminergic manipulations

Antagonism of dopaminergic neurotransmission through inhibition of D_1, D_2 and D_3 receptors by flupentixol led to decreased rule change performance coupled with decreased positive feedback sensitivity, motivation and within the computational model decreased overall learning rate, theoretical accuracy and stimulus choice determinism (see table 3.3 for an overview of all pharmacological treatments tested). As previously discussed, inhibition of the NAc shell impairs both reversal learning and PFS (Dalton et al., 2014). A recent study investigated bidirectional modulation of D_1 and D_2 receptors in different striatal regions within the PRLT (Verharen et al., 2019). Interestingly decreases in reversal performance were only seen following administration of the D_2 agonist quinpirole (also seen in Alsiö et al., 2019) while the D_1 antagonist SCH23390 decreased the overall number of trials completed. Through striatal infusions they also found that NFS is modulated by D_2 receptors while D_1 receptors modulate PFS.

Amphetamine, a dopamine, noradrenaline and 5-HT releasing agent hypothesised to act through agonism of the TAAR1 receptor (Miller, 2011), also decreased rule change performance, PFS, learning rate and theoretical accuracy in the PRLT in a similar manner to flupentixol. Additionally, amphetamine also decreased the trials required to learn the first rule in addition to NFS while also increasing motivation. A previous study has investigated increasing DAergic neurotransmission through knockdown of DAT which resulted in increased reversal performance (Milienne-Petiot et al., 2017), however a common problem with genetic knockdown models is adaptation (Barbaric et al., 2007) meaning that DAT function may have been being carried out by other compensatory transporters. Interestingly when humans who were chronic users of amphetamine were assessed in the PRLT this did not result in any performance changes whereas cocaine users, another dopamine elevating drug of abuse, were more likely to perseverate following reversal (Ersche et al., 2008). Within the rodent response bias probabilistic reward task conversely amphetamine has the opposite effects to that seen in the present study and actually increases reward learning as evidenced by a higher response bias towards the more highly rewarded stimulus compared to vehicle (Der-Avakian et al., 2013). Increased motivation following amphetamine administration is consistent with observations that amphetamine enhances the salience of reward (Glick, 1971; Wyvell and Berridge, 2000).

Group	Drug	Order	Rule changes	Trials to first rule change	Win-stay probability	Lose-shift probability	Initiation reaction time	Learning rate	Theoretical accuracy	β
			RL	RL	Feedback sensitivity	Feedback sensitivity	Motivation (lower = better)	Qlearn RL	Qlearn RL	Choice variability (lower = better)
Dopaminergic manipulation	Flupentixol	3	↓	-	↓	-	↑	↓	↓	↓
	Amphetamine	7	↓	↓	↓	↓	↓	↓	↓	-
Conventional antidepressant	Citalopram	1	↑#1	↓	↑	-	↑	-	-	↑
	Citalopram (repeat)	11	-	-	-	-	-	-	-	↑#2
	Venlafaxine	2	-	-	-	-	-	-	-	-
	Reboxetine	4	↓	-	↓	-	↑	-	↓	↓
	Sertraline	9	-	-	-	-	-	-	-	↑
Rapid acting antidepressant	Ketamine	5	↓	-	↓	-	↑	↓	-	-
	Scopolamine	6	↓	-	↓	-	↑	↓	↓	-
Prodepressant manipulation	Corticosterone	8	-	-	-	-	-	-	-	-
	Tetrabenazine	10	-	-	-	-	-	-	↓#2	-

Table 3.3 Overview: The effects of pharmacological treatment on key parameters in the PRLT. Text below each parameter shows the main construct this variable is believed to measure. #1: trend in vehicle + first two doses only, #2: overall trend only.

However, any increase in reward learning seen following amphetamine administration in the RBPRT is likely due to either D_1 receptor activation or other monoaminergic transmission due to the evidence that pramipexole, a D_2/D_3 agonist, impaired reward learning with rats failing to develop a response bias to the more highly rewarded stimulus when treated acutely (Der-Avakian et al., 2013).

Perhaps the most interesting observation following amphetamine treatment was that animals changed their reward learning strategy on the 1 mg/kg dose to better fit the dual learning rate Qlearn2 model as opposed to the single learning rate Qlearn1 model. This would suggest that animals under amphetamine are learning differently from positive and negative feedback whereas normally they learn equally. However it should be noted that at this dose animals had a markedly decreased learning rate which might be related to amphetamines bulk noradrenaline release impairing the ability of noradrenaline to correct learning rate adjustments under uncertainty, such as when the task rule switch (Sales et al., 2019). This could combine with amphetamine's bulk dopamine release to cause learning rate impairments. While dopamine is known to be crucial for reward learning and communicating the learning rate, broad increases or decreases in neurotransmission are likely to be detrimental due to the phasic nature of dopamine signalling in the basal ganglia (Schultz, 2016). If the ability of phasic dopamine to signal reward prediction errors is interrupted through pharmacological manipulation, then could be a reason for decreased animal performance when dopaminergic neurotransmission is bidirectionally modulated.

3.5.2 Conventional antidepressants

Of the conventional antidepressants tested only citalopram had effects in the PRLT consistent with its clinical role as an antidepressant. Under citalopram treatment animals did not show any changes in rule change performance but showed increased PFS and determinism of stimulus selection alongside requiring fewer trials to learn the first rule in a session. These results differ to Bari et al., 2010 who reported that acute 5mg/kg citalopram administration decreased NFS and increased rule changes but are congruent with data from Drozd et al., 2018 who found no effect of escitalopram treatment upon any outcome measure. One possibility for these divergent effects it the level of animal training when the experiment was carried out with animals in the current study and those used in Drozd et al., 2018 performing around three times more baseline rule changes compared to animals studied by Bari et al., 2010. Within the Q-learn model, citalopram was found to

increase choice determinism as measured by the ß parameter; an interesting finding in the context of citalopram acutely increasing anxiety (Urban et al., 2016). This suggests that under citalopram treatment animals were better able to form and execute a strategy to successfully complete the task. Within humans, both citalopram and escitalopram have been found to increase misleading negative feedback sensitivity alongside errors made while reaching criterion for the first reversal (Chamberlain et al., 2006; Skandali et al., 2018). However, tryptophan supplementation, known to increase synaptic 5-HT concentration, has been found to have no effect upon behaviour in humans within the PRLT (Kanen et al., 2020; Thirkettle et al., 2019).

In further agreement with Drozd et al., 2018 no effect of venlafaxine treatment was observed upon any behavioural or Q-learning measure. No effect of sertraline treatment was also observed and although this drug has not been previously tested in the PRLT it has been found to have similar clinical efficacy to citalopram (Ekselius et al., 1997) alongside similar serotonin transporter (SERT) binding kinetics. One potentially interesting difference between the drugs is the relative increase in dopamine transporter (DAT) binding in sertraline versus citalopram (SERT:DAT K_D ratio in citalopram is 2400 and 86 in sertraline, Tatsumi et al., 1997). The more likely suggestion for the difference in response seen following citalopram and sertraline treatment is due to decreased task sensitivity over time (discussed in section 3.5.6).

Reboxetine decreased reward learning performance while concurrently decreasing positive feedback sensitivity. Animals also exhibited a decreased choice variability as inferred from the ß parameter. In rhesus monkeys noradrenaline has been found to support choice variability with clonidine, an α adrenoreceptor agonist which leads to decreased noradrenaline release due to these receptors being pre-synaptic, being found to cause decreased choice variability in a sequential cost/benefit decision making task (Jahn et al., 2018). Within a fixed-reward reversal learning task desipramine, a noradrenaline biased tricyclic antidepressant, was found to increase reversal learning performance in rats (Seu and Jentsch, 2009). Within the PRLT decreased choice variability could lead to perseveration upon rule switching while too high a choice variability would impair the ability of animals to consistently respond to a stimulus for long enough to reverse (Delgado et al., 2011). This suggests that increased noradrenaline is detrimental to performance where rules are uncertain but can be beneficial where reward contingencies are deterministic.

Atomoxetine, a noradrenergic reuptake inhibitor, has also been tested within the human PRLT. However, no effects upon behaviour were observed (Chamberlain et al., 2006).

Within the affective bias task, an assay assessing how learning and memory are modulated by affective biases, citalopram, venlafaxine, reboxetine and sertraline have all been found to positively bias the valuation of reward during new learning over multiple days (Refsgaard et al., 2016; Stuart et al., 2013). In contrast to this within the judgement bias task, an assay involving interpretation of an ambiguous cue for reward, conventional antidepressants require chronic dosing over weeks to positively bias cue interpretation (Hales et al., 2017). Amalgamating these findings with those of the present study suggests that conventional antidepressants do not alter absolute reward learning ability with overnight integration of memories likely required for their effect.

3.5.3 Rapid-acting antidepressants

Ketamine and scopolamine both impaired reward learning, PFS and motivation in addition to learning rate within the Q-learning model. Scopolamine additionally impaired animals' reward learning compared to a model predicted perfect strategy. The fact that at higher doses both drugs are known to have sedative effects is likely to underly the motivational impairments seen in this study. Ketamine has also been observed to impair reward learning in other studies where administration to both humans and rats reduced reward anticipatory responses to food or reward in the ventral stratum (Francois et al., 2016). A similar effect of scopolamine upon impairing reward learning has also been observed by Pelsőczi and Lévay, 2017 where a 0.17 mg/kg dose in mice was able to increase error rate upon reversal in a non-probabilistic reversal learning task. This impairment in reward learning performance also maps well onto the learning rate impairments observed in the present study. However there appear to be translational differences between rodents and man. Ketamine administration in humans within a PRLT that additionally contained a risk-based element has been found to lead to a decreased ability to perform an optimal reward strategy (similar to theoretical accuracy) but with no concurrent changes in learning rate (Vinckier et al., 2016). Interestingly studies in humans have observed that reward sensitivity negatively correlates with learning rate in addition to MDD patients having decreased reward sensitivity but no change in learning rate (Huys et al., 2013). This implies that increasing both learning rate and reward sensitivity is not possible for antidepressant compounds to achieve. Although motivation and reward learning are dissociable within the PRLT (Roberts

et al., 2019) it seem unlikely that the decreased learning rate and PFS seen following ketamine treatment in this study is due to a specific effect on reward learning as opposed to general impairments in cognitive function.

A different mechanism for ketamine's effects was suggested by Rychlik et al., 2017 who observed that ketamine treatment in the PRLT selectively attenuated animals sensitivity to misleading negative feedback. This interaction was likewise observed in the present study. Nevertheless, with the cognitive impairments seen following ketamine treatment in this study it is difficult to interpret the significance of this finding in light of overall antidepressant efficacy. More consistent effects of ketamine's function have been observed within the affective bias test and judgement bias tasks where robust effects upon ambiguous cue interpretation and retrieval of memory biases have been observed at doses as low as 1 mg/kg (Hales et al., 2017; Stuart et al., 2015). These data suggest that ketamine is able to modify affective biases during memory retrieval and positively bias interpretation of ambiguous cues at doses where no effects upon reward learning were observed in the PRLT. Scopolamine appears to impair reward learning, however more research is needed into its mechanism of antidepressant action.

3.5.4 Pro-depressant manipulations

Administration of corticosterone and tetrabenazine had little effect upon behaviour in the PRLT with the exception of trends for CORT to decrease the trials required to learn the first rule change and a trend towards altered theoretical accuracy. A trend towards decreased theoretical accuracy was also observed following TBZ treatment. While no effects upon reversal performance were found following acute treatment in this study, chronic dosing has been found to impair performance in mice (Dieterich et al., 2019). Chronic 5 mg/kg CORT for 4 weeks was found to selectively impair reversal performance with no effect on feedback sensitivity. Chronic CORT administration has most commonly been used as an animal model of depression (Gourley and Taylor, 2009). However, effects of acute CORT administration have been observed in the affective bias task where CORT negatively biases the learning and memory of reward (Stuart et al., 2013). Within humans acute stress, known to raise endogenous CORT release, has also been found to impair reward processing with participants completing the RBPRT less able to bias their responding towards the more highly rewarded stimulus while under the threat of shock (Bogdan et al., 2010, 2011; Bogdan and Pizzagalli, 2006). Interestingly the effects of acute stress upon probabilistic reward learning

appear to be task specific with no effect of stress upon the PSST (Berghorst et al., 2013) observed in addition to a more simplistic probabilistic learning task (Zhang et al., 2020). Interestingly stress and CORT have not just been found to have negative effects upon reward learning. Stress has both been suggested to increase reward salience (Porcelli and Delgado, 2017) in addition to enhancing learning about cues predicting positive outcomes while decreasing sensitivity to recent feedback (Lighthall et al., 2013).

In comparison to the extensive investigations into the effects of CORT and stress upon reward learning, little to no work has been carried out upon tetrabenazine. Interestingly at the same 1mg/kg dose tested here TBZ has been observed to be much more selective towards depleting DA (roughly 50-75% of DA depletion in the striatum) as opposed to 5-HT (Yohn et al., 2015). However, its observed effects are completely different from the broad spectrum dopaminergic antagonist flupentixol suggesting that relatively little dopaminergic neurotransmission is required to support reward learning in the PRLT. In the affective bias task TBZ administration was able to negatively bias new learning and memory (Stuart et al., 2017). Unlike the current study, other investigations have also reported impairments in motivation following TBZ treatment in the effort for reward task (Contreras-Mora et al., 2018; Griesius et al., 2020).

Integrating these findings together suggests that acute negative manipulation of affective state through either corticosterone or tetrabenazine administration, while able to affect other forms of reward processing, is not able to modulate reward learning and feedback sensitivity in the PRLT.

3.5.5 Loss of task sensitivity over time

Within this study animals were repeatedly tested in the PRLT with over 100 sessions having been completed by the end of acute pharmacology experiments. Over the course of repeated testing rule change performance increased by roughly 40% in addition to positive feedback sensitivity, negative feedback sensitivity and theoretical accuracy which all increased by roughly 10%. These changes in baseline performance likely underly the inability of the single acute 3 mg/kg citalopram dose to have any effects upon behaviour in the PRLT with the exception of trending towards increasing choice variability, as seen in the original citalopram study. Feedback sensitivity has previously been described to be stable over 10

sessions within the PRLT (Noworyta-Sokolowska et al., 2019), however no previous studies have assessed animals over such a length of time as in the present study. The fact that animals were easily able to overcome reversal of the starting image suggests that animals had not transitioned from goal directed to habitual instrumental learning over the course of the study (Dezfouli and Balleine, 2012). It could also be possible that long term changes in task performance were pharmacologically mediated. Ketamine has been found to cause to changes in long term potentiation in the mesolimbic circuit that persist for at least 7 days (Yao et al., 2018).

3.5.6 Qlearn model baseline fitting

Behavioural data outputs correlated well with model derived outputs throughout the training process in the PRLT suggesting that the model is successfully recapitulating behaviour. This type of model has been well utilised in PRLT tasks previously, however previous studies found a dual learning rate model, where animals learnt at differing rates from positive and negative feedback, fitted better in contrast to the current study where a single learning rate model consistently fit better (Alsiö et al., 2019; Noworyta-Sokolowska et al., 2019). When a similar model has been applied to human probabilistic learning tasks a dual rate model was also better fitting (Grogan et al., 2017). Differences in training and animal performance strategy might underly differences in better fitting model between studies and laboratories. Animals always performed poorer than predicted by theoretical accuracy implying that animals did not perform optimally either due to non-optimal computation by the animals or due to other factors which were not considered by the Q-learning model. Other models have been suggested to better recapitulate behaviour such as those employing fictitious updating of the non-chosen option (Noworyta-Sokolowska et al., 2019) or including an element of choice stickiness (Alsiö et al., 2019).

3.5.7 Differences between human and rodent behaviour in the PRLT

Although a key strength of the PRLT is its translation and wide degree of use in both humans and animals, there are still important differences as to how the task is completed between species. In addition to the previously mentioned differences in model fitting between species, the task structure differs between species. In the human PRLT only one reversal opportunity is provided after a fixed period of 40 trials (Lawrence et al., 1999) while the rodent task allows reversal as many times as available within session length constraints after

the meeting of criteria (Bari et al., 2010). Additionally, there are differences in baseline feedback sensitivity between species. Rats in this study commonly switched to the opposite stimulus following misleading negative feedback (p(lose-switch) ≈ 0.65) while humans rarely display this behaviour (p(lose-switch) = 0.05, Skandali et al., 2018). Additionally rodents in the present study were unable to discriminate between true and misleading positive feedback while humans are extremely successful at this (win-stay following misleading positive feedback: p(win-stay) = 0.01 and true positive feedback: p(win-stay) = 0.86, Skandali et al., 2018). These findings in addition to the differential model fitting results and divergent task structures mean that interpretation of findings need to be nuanced when translating between animals and man in the PRLT.

3.5.8 Summary

These data suggest that performance in the PRLT is not acutely affected by affective state with the individual pharmacology of compounds tested guiding their effects upon reward learning and feedback sensitivity. The ability of bidirectional dopaminergic manipulation to impair task performance suggests that intact phasic dopamine signalling is crucial for mediating probabilistic reversal learning. Additionally, the ability of citalopram to improve reward learning and positive feedback sensitivity while other antidepressant drugs caused general impairments suggests that the task is potentially sensitive to serotonergic neurotransmission manipulations. These results additionally provide support that motivation and reward learning are dissociable constructs within the PRLT due citalopram impairing motivation but improving reward learning ability. Furthermore, these results also caution against extensive repeated testing of animals within the PRLT with the finding that baseline measures change to a degree which renders animals insensitive to a previously effective manipulation. Finally, the lack of effect upon animals when dosed with either corticosterone or tetrabenazine suggests that reward learning is not affected by acutely induced negative affective states, rather requiring a longer-term exposure to have effects. These results therefore suggest that the PRLT could be used to measure reward learning changes in animals with a history of ELS provided that the experiments were designed carefully to be temporally limited and interpretation was cautious with regards to the neural substrates underlying any potential changes in reward learning.

Chapter 4

Electrophysiological investigation of hippocampus CA1 in maternally separated rats

4.1 Introduction

The hippocampus is a key brain region for mediating the effects of early life stress upon behaviour due its slow developmental trajectory and high expression of glucocorticoid receptors (Tottenham and Sheridan, 2009). One of the key ways that ELS may alter hippocampal output is through modulation of long term potentiation (LTP) induction rules with LTP being believed to underly learning and memory within the hippocampal formation (Bliss and Collingridge, 1993; Morris et al., 1986). A previous meta-analysis has suggested that the effects of ELS upon CA1 LTP are model specific with maternal separation leading to increased LTP relative to controls whereas the offspring of low licking-grooming mothers show decreased LTP (Derks et al., 2017). However it should be noted that other studies have been published that were not included in this review concluding that LTP was lower in maternally separated animals compared to controls (Cao et al., 2014; Heydari et al., 2019; Sousa et al., 2014).

However, a major drawback of previous work is that it has largely failed to consider the important factors of animal sex, hippocampal region and input pathway. Male compared to female animals have been found to have greater LTP at TA-CA1 synapses following high frequency stimulation (Qi et al., 2016) and female rats have been found to have differing LTP induction ability depending upon oestrous cycle phase (Warren et al., 1995). Additionally oestrogen receptors have been found to be important in LTP induction in females while androgen receptors are involved in LTD induction in males (Tozzi et al., 2019; Wei et al., 2018).

As previously discussed, the dorsal and ventral regions of the hippocampus possess divergent responses to LTP induction protocols. Using high frequency stimulation, theta burst stimulation and theta pulse stimulation the DH has been reported to show increased LTP relative to the VH (Babiec et al., 2017; Kouvaros and Papatheodoropoulos, 2016; Papatheodoropoulos and Kostopoulos, 2000). Using these stimulation techniques it has been suggested that regional differences in LTP are due to a lower SK-type K^+ channel expression in the DH than VH, higher TBS induced $GABA_B$ activity in the DH and increased synaptic facilitation in the DH than VH (Babiec et al., 2017; Kouvaros and Papatheodoropoulos, 2016). However, utilising theta burst pairing Malik and Johnston, 2017 reported a higher threshold for LTP induction in DH neurones with this due to increased potassium channel regulation of dendritic plateau potentials.

Additionally, both the role of CA1 input pathway and the interaction between SC and TA pathways is underappreciated in studies focussing on hippocampal dynamics in ELS. As previously discussed these two pathways have a complex network of interactions with SC synapses able to gate TA generated plateau potentials (Jarsky et al., 2005; Nicholson et al., 2006). These plateau potentials have been found to play an important role in LTP formation with whisker stimulation evoked LTP being found to be dependent upon plateau potentials *in-vivo* (Gambino et al., 2014). TA bursting has also been found to be able to reduce LTP at SC synapses in addition to being able to modulate the formation of SC evoked CA1 action potentials (Remondes and Schuman, 2002). This ability of the TA pathway to reduce SC LTP has also been found to be effective at synapses where LTP has been previously stabilised (Izumi and Zorumski, 2019).

The majority of previous ELS studies used the high frequency stimulation (HFS) LTP induction protocol involving 100Hz stimulation of neurones for up to a second. This does not correspond with physiological conditions where CA1 pyramidal neurones typically only fire 3-4 spikes in a burst for 30-40ms at a time (Albensi et al., 2007; Grover et al., 2009). Induction protocols based upon theta rhythm stimulation (such as theta burst stimulation (TBS)) have been suggested to be much more physiologically relevant. These activity patterns have been recorded from CA1 during active exploration (Grastyán et al., 1959; Winson, 1972) and there is evidence that during learning CA1 pyramidal neurones fire in short bursts that lock with the theta frequency (Hill, 1978; Otto et al., 1991; Ranck, 1973). Previous evidence has shown that differing forms of LTP have divergent induction mechanisms with differences in cellular Ca^{2+} store utilisation (Raymond and Redman, 2002), GABA modulation (Albensi et al., 2007) and intracellular induction mechanisms (Zhu et al., 2015). HFS induced LTP has been found to require adenosine A2 receptor activation and protein kinase A while TBS induced LTP is dependent on calpain-1 and ERK activation (Zhu et al., 2015).

In order to consider hippocampal LTP and circuit dynamics following ELS in a rigorous manner it is critical to consider all of these previously discussed factors which likely interact in complex relationships to lead to behavioural changes in behaving rodents and humans. However, before considering LTP it is important to understand the effect of ELS upon NMDA receptor function with this receptor being a key gate for LTP induction (Collingridge et al., 1983). Previous reports have suggested a decreased NMDAR relative to AMPAR function following ELS (Pillai et al., 2018) in addition to decreased GluN2B subunit expression (Lesuis

et al., 2019; Pickering et al., 2006; Roceri et al., 2002). However apart from this single AMPAR/NMDAR experiment there is little published functional data relating to NMDARs and again little appreciation has been given to the factors of hippocampal region, sex and input pathway. NMDA receptors are also crucial in the formation and maintenance of plateau potentials (Schiller et al., 2000; Suzuki et al., 2008). These plateau potentials are vital in the process of feature selectivity whereby coincident SC and TA input to CA1 pyramidal neurones drives place cell formation (Bittner et al., 2015).

In this study it was aimed to investigate NMDA receptor function and LTP in animals bred in a maternal separation protocol. However, first it was necessary to validate successful application of an ELS phenotype utilising the novelty supressed feeding test (NSFT), measurements of paraventricular nucleus cFos and CORT in response to restraint stress in addition to dentate gyrus neurogenesis. Previous work has identified that ELS animals show increased anxiety in the NSFT, exaggerated restraint stress CORT and decreased DG neurogenesis (Mirescu et al., 2004; Stuart et al., 2019). Following this, ELS animals were assessed for changes in relative AMPAR and NMDAR function before miniature excitatory post synaptic current (miniEPSC) experiments were conducted to localise any AMPAR/NMDAR ratio changes to either AMPAR or NMDAR function. LTP was assessed using theta burst stimulation with potential plateau potentials during the induction phase being recorded. Finally basal neurotransmission was assessed through measurements of both standard properties such as paired pulse ratio and input resistance in addition to impedance and action potential characteristics.

Experiments were also piloted investigating methods to measure and detect changes in plateau potential induction between animals in a different cohort of wild type animals. This was then aimed to feed into future experiments investigating plateau potentials in ELS animals. Due to evidence that carbachol can enhance dendritic excitability through the involvement of SK channels the effects of the muscarinic agonist carbachol and the SK channel antagonist apamin were assessed (Buchanan et al., 2010). DAPV was also utilised to assess the NMDAR dependence of observed events.

By understanding how early life stress influences hippocampal CA1 circuit dynamics this may allow a greater understanding into the links between ELS, reward learning deficits and the development of psychiatric disease. Further understanding into these effects may also allow

the identification of novel targets that may either be beneficial in treating depression directly or reducing the risk to those who have suffered high levels of stress in childhood from developing mental health conditions.

4.2 Chapter Aims

- Validate successful induction of a ELS phenotype using the MS180 model of ELS mirroring that seen in previous literature in maternally separated rats using both behavioural and histological analyses.

- Assess NMDA receptor function in MS180 rats using whole-cell patch clamp electrophysiology

- Compare control and ELS animals in their ability to express LTP following theta-burst stimulation in CA1 pyramidal neurones

- Examine basal neurotransmission in MS180 animals and matched controls

- Develop a method of generating plateau potentials in ex-vivo slices using electrical stimulation and calculate the stimulation threshold needed to generate these events.

This work was completed between 2019 and 2020.

4.3 Methods

4.3.1 Study Design

MS180 animals were created in breeding cycles of which cycle 1 and cycle 3 were successful (see Figure 4.1 for an overview of the entire study). Cycle 1 animals were split into two cohorts following weaning: an electrophysiology and a validation cohort. The validation cohort completed the novelty supressed feeding test, sucrose preference and restraint stress corticosterone experiments before being terminally used in the restraint stress cFos and BrdU neurogenesis experiment. The electrophysiology cohort from cycle 1 were used for AMPA/NMDA ratio and miniEPSC experiments. Animals from cycle 3 were used to provide additional power in the NSFT experiment before some animals were used to supplement AMPA/NMDA and miniEPSC datasets. The majority of cycle 3 animals were however used for LTP, impedance and spike dynamics experiments. During all experiments following animal weaning the experimenter was blind to animal treatment and all efforts were made to counterbalance for sex and animal condition in experiments. In electrophysiology studies further distinction was made between studying both hippocampal region and CA1 input pathway with this described in detail later. Four other breeding cycles were also initiated with 2 incidences of dams failing to get pregnant, one incidence of litters being rejected by the dam and one incident of animals having to be culled due to the Covid-19 crisis.

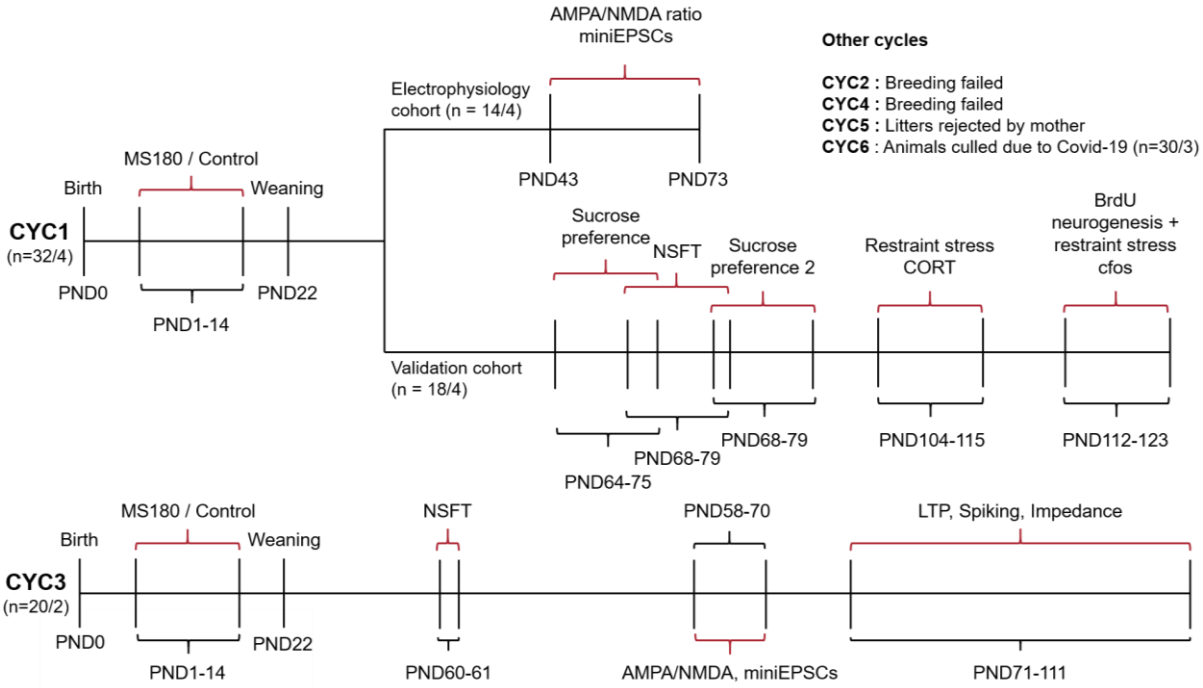


Figure 4.1 Study overview. Animals were generated in breeding cycles with two cycles being successful and used for experiments. n = 32/4 refers to 32 animals from 4 litters.

4.3.2 Animals

A total of 19 hooded long-Evans rats and their 82 offspring were used in ELS experiments with rats being derived from an in-house breeding colony. For breeding animals were housed in standard lighting conditions (12:12h cycle, lights off at 19:00) with offspring used for electrophysiology remaining in these conditions. Animals used for validation were transferred to reverse lighting conditions (12:12h cycle, lights on at 20:30). Animals always had free access to food and water and were provided with wooden chew blocks and red houses as enrichment in temperature and humidity controlled conditions. Sample size was estimated from previous studies (Stuart et al., 2019). For plateau potential pilot experiments a total of 13 male Wistar rats were used weighing between 275g and 300g and housed in standard lighting identically to all other electrophysiology animals. All experiments were undertaken in accordance with local institutional guidelines, the UK Animals (Scientific procedures) Act of 1986 and the European Community Council Directive of 24 November 1986 (86/609/EEC).

4.3.3 Maternal separation procedure

Maternal separation procedures were completed as described by Mirescu et al., 2004. Male and female rats were either pair housed or housed as trios (1 male and 2 females) for 2 weeks or until females were visibly pregnant. Pregnant animals were then singly housed and monitored daily for signs of birth. Animals were born on PND0 before having litter sizes adjusted to 8 pups (cycle 1) or 10 pups (cycle 2 onwards) on PND1 with equal numbers of male and female animals preferred. Litters were either randomised to control or MS180 conditions on PND1. MS180 pups were subject to daily separations of 180 minutes from PND1 to PND14 away from the dam and were placed into an incubator held at 32°C during this period. Separation always occurred between 13:00 and 16:00 with pups remaining as a litter in a container containing sawdust and bedding from the home cage. Control litters were left completely alone with no interventions. All cage cleaning halted during the 14-day experimental period. Following PND14 all animals were returned to standard husbandry with cage cleaning resuming and animals were weaned at PND22 into groups of 2-3 by sex and by litter. At this point animals from the cycle 1 validation cohort were transferred to reverse lighting while animals destined for electrophysiology were kept under standard lighting.

4.3.4 Model validation experiments

4.3.4.1 Novelty supressed feeding test

Experiments were performed as described by Stuart et al., 2019 upon animals from both cohorts during animals' active phase. Animals were food deprived for 24 hours before being placed into a 70cm diameter arena containing a 10cm food bowl filled with standard chow placed into the centre of the arena. Latency to both approach the bowl and eat from it were recorded and animals were recorded with a Logitech C920 webcam. Up to 15 minutes was allowed for animals to eat from the bowl before they were removed from the arena and classed as failing to eat. Tracking was performed utilising Noldus Ethovision XT software to generate the additional parameters of percentage time moving and average velocity. The area was additionally subdivided into an inner and outer zone (see Figure 4.3A) with this being used to calculate percentage time in the inner zone.

4.3.4.2 Sucrose preference test

Animals were first habituated to 1% sucrose in tap water with this being provided via two sipper sacks (Edstrom-Avidity Science, USA) for two days before animals were provided with standard drinking water from sipper sacks for a day. Animals were then water restricted for 4 hours and individually housed in test cages 90 minutes before testing. Animals were then provided with one sipper sack containing 1% sucrose and another containing standard drinking water. Consumption was measured at 30 min, 60 min and 120 min after the start of testing with bottle positions swapped at these timepoints. Following the final timepoint animals returned to their home cages and sucrose preference was calculated as:

$$Sucrose\ preference\ (\%) = \frac{Sucrose\ consumed\ (g)}{Sucrose + water\ consumption\ (g)} \times 100 \qquad \text{Eq 4.1}$$

Due to poor levels of fluid consumption upon initial testing the sucrose preference test was repeated a week later with only repeated data shown.

4.3.4.3 Restraint stress corticosterone

Animals' corticosterone response to restraint stress was assessed within-subject through tail vein blood sampling. Animals first had their cage heated on a warming mat in their holding room for 10 minutes before being moved to a procedure room and their tail being directly heated with a warming mat 1 minute before baseline sample acquisition. A baseline sample

of approximately 250µl was collected into tube containing 50µl of ice-cold EDTA before animals were placed into a restraint stress tube (Harvard Apparatus, USA) for 20 minutes. Just prior to the end of the 20-minute period animals had their tail heated again before another tail vein blood sample was collected. The experiment took place over two days with animals being allocated to each day such that the study was counterbalanced for condition and sex but the experimenters remained blind to condition. All blood samples were stored on ice before being centrifuged at 8000g for 10 minutes and the plasma being aspirated off before being frozen at -20°C prior to further analysis. Plasma CORT concentrations were assessed using previously described methods (George et al., 2017) with final concentrations being adjusted for the blood volume collected.

4.3.4.4 cFos and BrdU Immunohistochemistry

Animals were dosed with 50mg/kg BrdU (Sigma, USA) in 0.7% saline i.p every 2 hours four times and 22.5 hours following the final injection were subject to 20 minutes of restraint stress. 24 hours following the final BrdU injection animals were deeply anaesthetised with pentobarbital (Merial, UK) before being transcardiac perfused with first ice cold phosphate buffer (PB, 28mM NaH_2PO_4, 72mM Na_2HPO_4) and then ice cold 4% paraformaldehyde (PFA) in PB. Following adequate fixation brains were removed and then placed overnight into 4% PFA in PB. Brains were then transferred into a 25% sucrose solution until they sunk and were then snap frozen in optimal cutting temperature compound (Scigen, USA) using dry ice. Brains were cut into 40µm thick sections using a freezing microtome (Reichert, Austria) before being frozen in cryoprotectant (30% sucrose, 30% ethylene glycol, 50% PB in H_2O) prior to use in immunohistochemistry experiments.

Hippocampal sections (-1.92mm ≤ bregma ≤ -6.48mm) were used for neurogenesis experiments while sections containing PVN (-1.56mm ≤ bregma ≤ 1.92mm) were used for restraint stress cFos experiments. All sections were first washed with buffer (see table 4.1, PBS: 1.45mM KH_2PO_4, 8.1mM Na_2HPO_4, 136.5mM NaCl, 2.68mM KCl, pH 7.2; TBS: 100mM Tris, 155mM NaCl, pH 7.4) 4 times for 10 minutes while being subject to gentle agitation. At this point sections in the BrdU experiment completed an additional step. This step consisted of a 30m incubation in 2M HCl at 37°C then neutralisation by two 5-minute washes with 0.1M NaB_4O_7 followed by another wash phase with buffer (3x5 min). Sections from both experiments were then blocked for non-specific binding for 90 minutes in 2% bovine serum albumin and 3% serum in buffer. Primary antibody was then applied overnight at room

temperature within 3% serum in buffer. Following another wash phase (3 x 5 min in buffer) sections were then incubated in secondary antibody (diluted in 3% serum in buffer) for 2 hours before another wash (3 x 5 min in buffer). Sections were then incubated in a 1:1000 dilution of DAPI for 2 minutes before again being washed (3 x 5m) and mounted on distilled water using vectashield (Vectorlabs, US) fluorescent mounting medium. Images were captured at 10x magnification using a Leica DMI6000 widefield microscope with DFC365FX camera using LASX acquisition software. Cell counting was conducted manually utilising ImageJ. Due to observed ice damage in PVN sections this was quantified on a 10 point scale and used as a random factor in analysis; there was no correlation between ice damage and cFos count (data not shown). For the BrdU experiment sections were counted as being dorsal (bregma ≥ - 3.44mm), intermediate (-3.44mm ≥ bregma ≥ -5.08mm) or ventral (bregma ≤ -5.08mm).

		cFos	**BrdU**
Region of Interest		PVN	Hippocampus
Bregma		-1.56mm to 1.92mm	-1.92mm to -6.48mm
Buffer		PBS	TBS
Serum		Goat	Donkey
Primary	Dilution	1:4000	1:100
	Antibody	Rabbit α-cFos	Mouse α-BrdU
	Ab number	ABE457	B44
	Lot number(s)	2935662, 2967893	9172603
Secondary	Dilution	1:200	1:500
	Antibody	AF594 goat α-rabbit	AF488 donkey α-mouse
	Ab number	A11037	A21202
	Lot number(s)	1851471	1696430
Exposures	DAPI	55ms @ 5x gain	100ms @ 4x gain
	594nm	5s @ 6x gain	-
	488nm	-	150ms @ 5x gain

Table 4.1 Details of immunohistochemistry for restraint stress cFos and BrdU neurogenesis experiments. Abbreviations: Ab: antibody, AF: Alexa fluor.

4.3.5 Electrophysiology experiments

4.3.5.1 Slice preparation

Transverse hippocampal slices were prepared from rats following terminal anaesthesia under isoflurane and decapitation. Following removal of the brain hippocampi were dissected in ice cold cutting solution (see table 4.2) bubbled with 95% O_2, 5% CO_2 before being sliced into 400µm transverse slices using a Leica LS1200 vibratome. Brain slices were immediately transferred into aCSF (see table 4.2), again bubbled with 95% O_2 and 5% CO_2, and incubated at 35°C for 30 minutes then a further 30 minutes at room temperature prior to the start of patch clamp experiments. Dorsal and ventral slices were classified as those coming from the extreme 1/3 of the hippocampal axis. Additional glycine or D-serine (NMDAR co-agonists) were not added to solutions due to evidence that D-serine is released by astrocytes (Henneberger et al., 2010) and that in adult rats D-serine is the key NMDAR co-agonist in hippocampal CA1 (Le Bail et al., 2015; Shleper et al., 2005).

Reagent	Concentration (mM)	
	Cutting solution	aCSF
Sucrose	205	-
NaCl	-	124
Glucose	10	10
$NaHCO_3$	26	24
KCl	2.5	3
NaH_2PO_4	1.25	1.25
$CaCl_2$	0.5	2.5
$MgCl_2$	5	1.3

Table 4.2. Composition of solutions used for electrophysiological experiments

4.3.5.2 Whole cell patch clamp recordings

Prior to transfer into a submerged slice chamber for whole-cell patch clamp experiments all slices had CA3 manually removed. Slices were submerged in a constant flow (≈2.5ml/min) of aCSF and held at 32°C. Slices were stimulated (protocols described later) by tungsten

bipolar electrodes (Microprobes for Life Science, USA) and visualised utilising differential interference contrast microscopy using an Olympus BX51WI upright microscope. Patch pipettes with resistance 2-9MΩ were pulled using a Sutter P-97 from borosilicate glass (Harvard apparatus, USA) before being filled with internal solution (see table 4.3). Recordings were made utilising a MultiClamp 700A amplifier (Axon instruments, US) coupled to a CED Micro 1401 digitiser. For AMPAR/NMDAR, miniEPSC and LTP experiments data were captured using Signal version 5 (CED, UK), filtered at 2.4kHz and digitised at 10kHz with 2x gain unless otherwise stated. For impedance and spike dynamic measurements data were captured in Spike version 5 (CED, UK) with 2x gain and digitised at 20kHz. For all experiments series resistance (R_{ser}) and input resistance (R_{in}) was monitored through injection of a 20pA square pulse lasting 500ms with cells being excluded if their R_{ser} increased above 35MΩ. In all experiments junction potential was not corrected for and cells were perfused with 50μM picrotoxin (PTX).

Reagent	Concentration (mM)	
	$KMeSO_3$ internal	**$CsMeSO_3$ internal**
$KMeSO_3$	120	-
$CsMeSO_3$	-	117
HEPES	10	10
EGTA	0.2	0.3
Mg-ATP	4	2
Na-GTP	0.3	0.3
NaCl	8	9
KCl	10	-
TEA	-	10
QX-314	-	1

Table 4.3 Internal solutions used for whole-cell patch clamp experiments. Both internal solutions were adjusted to pH7.4 with an osmolarity of 290-300mOsm.

4.3.5.3 AMPAR/NMDAR ratio measurement

Cells were held at -70mV in voltage clamp (V_{clamp}) utilising the caesium based internal for 10 minutes before the start of recording in order to ensure that LTP would not contaminate recordings. Each pathway was then stimulated in a paired pulse protocol whereby two stimulations separated by 100ms were delivered to generate excitatory post synaptic

currents (EPSCs) of approximately 100pA amplitude. Each pathway was stimulated every 10s sequentially such that the SC pathway was stimulated followed 5s later by the TA pathway and then after another 5s the SC pathway was stimulated again. Stimulation was delivered at -70mV for 5 minutes to isolate the AMPA mediated component of the EPSC before cells were held at +40mV to allow NMDAR activity. Following being held at +40mV cells were then returned to -70mV and if greater than a 50% change in EPSC amplitude or R_{ser} was observed then the cell was excluded from analysis. From these recordings an AMPA / NMDA ratio was calculated as:

$$AMPA/NMDA\ ratio\ = \frac{-70mV\ EPSC\ amplitude}{+40mV\ amplitude\ 50ms\ post\ stimulation} \qquad \text{Eq 4.2}$$

A paired pulse ratio was also calculated from traces as the ratio between the amplitudes of the first and second EPSC in the paired pulse stimulation protocol.

4.3.5.4 miniEPSC recordings

Neurones were held at -70mV in V_{clamp} while exposed to 500nM tetrodotoxin (TTX). Recordings were made at 20x gain with the caesium based internal for 5 minutes in order to record miniEPSC events.

Recorded traces were analysed for miniEPSC events utilising WinEDR v3.9 (Strathclyde university, UK) utilising a detection template (amplitude: -3pA, tau rise: 0.1ms, tau decay 3ms, dead time: 15ms, rising edge window: 2ms). Detected events were then filtered (10ms ≤ duration ≤ 1000ms, 4ms ≤ tau rise ≤ 1000ms, 0 $pAms^{-1}$ ≤ area ≤ 1000 $pAms^{-1}$) before remaining events were manually screened for inclusion. Output measures per cell were either calculated from per event averages (interval, t90% and duration) or from the average miniEPSC trace of each cell (amplitude, area and tau rise). Average interval was described as being the average inter-miniEPSC interval while amplitude was the maximum depolarisation achieved. Area was the integral of the miniEPSC with respect to time and tRise was the 10-90% rise time of each event. t90% described as the time to decay to 90% of the baseline from the miniEPSC peak and finally duration pertained to the overall length of the event. In addition to per-cell averages cumulative distribution plots were also made for each condition containing the first 40 events per cell binned by condition (e.g. MS180 vs control). It has been reported that not adding polyvalent cations such as spermine to the internal solution results in a ramp up in miniEPSC amplitude in the first 10 minutes after

achieving a whole cell configuration (Rozov et al., 2012). However, this effect was not observed in the present work when miniEPSC amplitude was compared with event time following the start of the experiment (data not shown, linear regression, $R^2 = 9.0 \times 10^{-4}$, $F_{1,20955} = 18.9$, $p < 0.0001$, slope = -7.1×10^{-4}).

4.3.5.5 LTP experiments

CA1 pyramidal neurones were held at -70mV in V_{clamp} and stimulated through bipolar electrodes placed in the slm (TA pathway), orthodromic sr and antidromic sr to stimulate the SC pathway therefore forming a control and test SC pathway. Cells were stimulated in a paired pulse stimulation protocol to produce a ≈100pA EPSC followed by another stimulation 50ms later in each pathway. Pathways were stimulated sequentially every 5 seconds. For the length of the experiment cells were recorded using the $KMeSO_4$ internal. A 5 minute baseline period was recorded before LTP was induced utilising a theta burst stimulation (TBS) protocol (Buchanan and Mellor, 2007) no more than 10 minutes after a whole cell configuration was achieved in order to avoid the washout of LTP. In order to induce LTP, cells were switched to current clamp (I_{clamp}) with current injected to maintain the cell at -55mV before three trains of TBS were applied to the test pathways with a 10s interval. The TBS trains consisted of 10 bursts with an inter-burst frequency of 5Hz and each burst containing 5 stimulations at a frequency of 100Hz. Following LTP induction cells were returned to -70mV in V_{clamp} and again stimulated sequentially for another 30 minutes. Responses were normalised in each pathway to the EPSC amplitude during the baseline period and STP was taken to be the response 0-5 min post TBS while LTP was taken to be the period 25-30m post TBS. Cells were excluded from analysis if the series resistance or control pathway amplitude increased by over 50% during a recording.

Action potentials during TBS induction were counted as any membrane voltage change over 0mV before spikes were removed from traces. Due to the hyperpolarisation following the first theta burst, a baseline was interpolated for each 2s theta burst train consisting of three segments. The area of each theta burst was calculated as the integral of V_m with respect to the fitted baseline while the area of the decay phase of each burst was calculated to be the integral of each burst with respect to the fitted baseline following the maximal Vm deflection.

PPR and R_{in} were measured as previously described in section 4.3.5.3. Following the second EPSC a prominent afterhyperpolarisation (AHP) was observed from which an area was calculated as the integral of V_m with respect to baseline.

4.3.5.6 Impedance measurements

CA1 pyramidal neurones were held in I_{clamp} with input current adjusted to maintain V_m at -65mV. As described by Domanski et al., 2019 a sinusoidal current of 40pA amplitude increasing from 0.2Hz to 20Hz over 20s was applied to cells with the resulting membrane voltage response recorded. Both the input and output waveforms were transformed utilising a fast Fourier transform (FFT) and complex impedance was calculated as:

$$Impedance = \frac{FFT\ (output\ voltage\ waveform)}{FFT\ (input\ current\ waveform)} \qquad \text{Eq 4.3}$$

Impedance is a complex number and for all analysis the absolute value was utilised. The phase shift between input and output waves was also calculated as the ratio of the real and imaginary components of complex impedance:

$$Phase\ shift = arctan\left(\frac{Impedance\ (imaginary)}{Impedance\ (real)}\right) \qquad \text{Eq 4.4}$$

Impedance was filtered utilising a Savitsky-Golay filter (Savitzky and Golay, 1964) before data were fit between 0.5 and 10Hz with a double exponential to determine both the maximum impedance and the frequency at which this occurred.

4.3.5.7 Spike dynamics assessment

Cells were held at approximately -50mV in I_{clamp} mode with the same sinusoidal injection current as described in section 4.4.5.5 in order to generate action potentials. Output traces were differentiated with respect to time to identify action potentials where the depolarisation rate exceeded 200 mV/ms. Action potentials from each cell were averaged to create an average trace for each cell from which output measures were calculated. Maximum depolarisation and repolarisation speeds were calculated as the maximum and minimum of the first derivative of membrane voltage with respect to time. Halfwidth was calculated as the time taken for the action potential to pass from 50% maximum depolarisation to 50% maximum repolarisation. Action potential threshold was calculated as the membrane voltage at the point where the depolarisation rate exceeded $20Vs^{-1}$ (Kasten

et al., 2007). Finally, action potential height was taken as the difference between baseline and maximum depolarisation.

4.3.5.8 Plateau potential pilot experiments

Cells were patched in CA1 using the $KMeSO_4$ internal as previously described but with the addition of 1mM QX-314 within the internal to prevent action potentials. Cells were first held in V_{clamp} at -70mV for 10 minutes to wash out LTP before being transferred to I_{clamp} (again at -70mV) where they were stimulated sequentially every 15 seconds in either SC, TA or SC and TA pathways combined. Stimulation consisted of one single stimulation followed 400ms later by 5 stimulations at 100Hz (see Figure 4.2). Experiments took place in the presence of the $GABA_B$ antagonist GCP55845 (1µM, Hellobio, UK). Stimulation intensity was initially adjusted to evoke responses of approximately 1mV before stimulation was successively increased until robust plateau potentials were observed in all pathways. Stimulation was then turned off and either 50µM DAPV, 100nM apamin or 10µM carbachol (all Hellobio, UK) were washed on to the slice for 10 minutes before another stimulation response was conducted in the same cell. Throughout the experiment input current was adjusted to maintain the cell at -70mV ± 0.5mV.

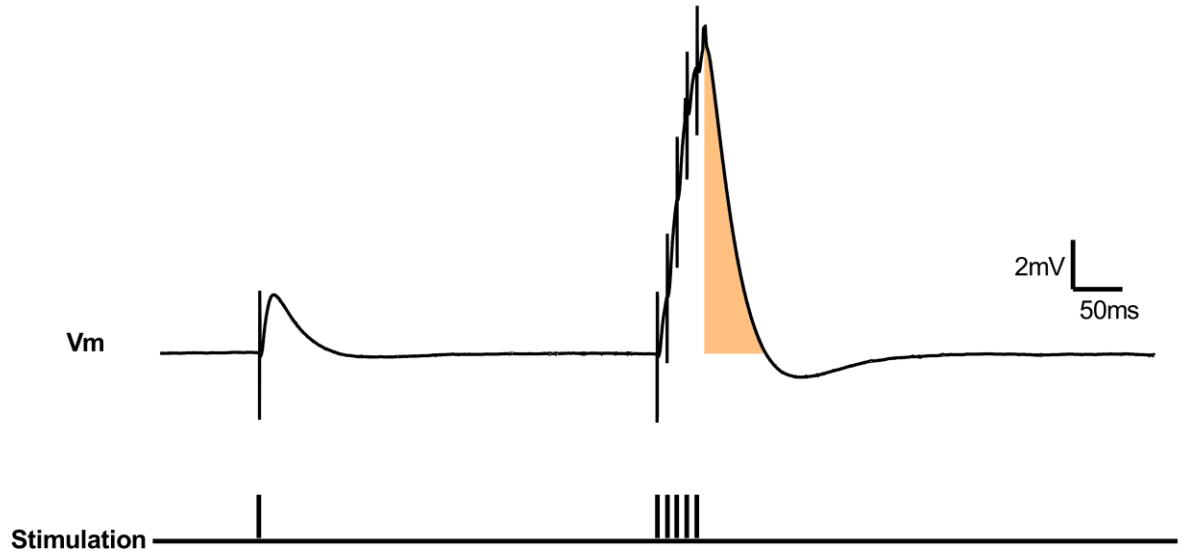


Figure 4.2 Example plateau potential stimulation protocol. EPSP slope was taken from the single EPSP while AUC (highlighted orange) was taken from the decay phase of the compound EPSP.

In order to calculate the plateau potential threshold, individual traces from each experiment were first smoothed using a Savitsky-Golay filter (Savitzky and Golay, 1964) before the EPSP slope of the single EPSP and the area under the curve (AUC) during the decay phase of the compound EPSP was calculated (AUC Max to baseline). For each condition (i.e. stimulation pathway + drug combination) a graph was constructed of single EPSP slope vs compound decay AUC and a 2-component piecewise linear function was fitted whereby a first line was fit with y-intercept y_0 and slope b_1. Once the breakpoint x_b was exceeded then a second line with slope b_2 was fit:

$$\begin{cases} y = y_0 + b_1 x & if\ x < x_b \\ y = y_0 + b_2 x & if\ x \geq x_b \end{cases} \qquad \text{Eq4.5}$$

In order to calculate the breakpoint a range of x_b values were fitted using the lsqcurvefit function in MATLAB with the fit generating the lowest normalised residuals being taken as the final breakpoint value. AUC changes associated with drug treatment were assessed through binning of plateau traces by EPSP slope into three bins. Due to the differing size of EPSPs between SC and TA pathways it was necessary to have differing cut-offs for these pathways (see table 4.4).

Bin	Pathway	
	SC and SC + TA	TA
1	$s < 0.4$	$s < 0.2$
2	$0.4 \leq s < 0.8$	$0.2 \leq s < 0.4$
3	$s \geq 0.8$	$s \geq 0.4$

Table 4.4 Binning of plateau potential AUC data by EPSP slope. S = single EPSP slope and all values are in $mVms^{-1}$.

4.3.6 Statistical Analysis

In conducting a complex study consisting of multiple nested factors it is necessary to account for these in the statistical analysis approach in order to avoid pseudoreplication (Lazic et al., 2020). Due to this a multi-level approach has been implemented considering the following multilevel structure:

$$Cell \in Animal \in Litter$$

Due to the extended time-period over which electrophysiology experiments occurred a random factor of age has also been included in the analysis. In order to achieve this a generalised linear mixed model (GLMM) was fitted using the glmmTMB package in R version 4.0 (Brooks et al., 2017; R Core Team, 2020). Models were first fit with all possible main factor combinations before being subject to stepwise removal of terms using Akaike information criterion (AIC) for model term deletion. Multilevel and other random effects were always included as a random intercept. Once the simplest model had been achieved, the point at which removing terms decreased the model fit, the final model was compared with a null model containing no fixed predictors using AIC calculated from the bbmle package (Bolker and R Development Core Team, 2020). Assuming that the model containing predictors was a better fit to data then the normality and homoscedasticity of residual assumptions were checked using both visual methods in addition to Shapiro-Wilk and Breusch-Pagan tests (Zeileis and Hothorn, 2002). Where a satisfactory model fit could not be made using a gaussian error family, efforts were made to both use altered link functions (e.g. log or sqrt) and altered error families (e.g. gamma). The R^2 value for each model was also always calculated to assess ultimate model fit (Barton, 2020). Estimated marginal means, the change in outcome where one factor is changed and all other remain constant, for the top level factors of condition, sex, region and pathway were calculated where appropriate using the ggeffects package (Ludecke, 2018). Where at least a trend towards an interaction was observed the this was investigated through fitting of the relevant simplified model where each factor was assessed in turn.

For data from PVN cFos and BrdU neurogenesis experiments each section was included in the analysis with an additional random factor of ice damage for the cFos experiment. For data from cycle 3 it was unnecessary to include litter as a factor as there was only one litter per condition.

miniEPSC cumulative distributions were compared using Kolmogorov-Smirnov tests (KS-tests) as has been previously reported for these kinds of experiments (Udakis et al., 2016). Data from plateau potential experiments were analysed utilising repeated measures two-way ANOVAs in SPSS (SPSS v24, IBM, US) with AUC data being analysed separately in each pathway due to the differing bin criteria. All graphs were constructed using either GraphPad Prism 8 or MATLAB with main effects indicated over the relevant data with a bar and stars. Main effects from the overall mixed model have been indicated on marginal means with the # symbol to emphasise that these are predicted marginal means. All data is shown as mean ±

SEM except for marginal means which are mean ± 95% confidence intervals (CI). #/* ≤ 0.05, ##/** < 0.01, ###/*** < 0.001, ####/**** < 0.0001.

4.4 Results

4.4.1 Model Validation

In order to validate that a phenotype mirroring that previously seen in the MS180 model (Stuart et al., 2019) had been generated, animals first completed the novelty supressed feeding test (NSFT). MS180 animals took longer to feed from the bowl than controls (Figure 4.3B, GLMM, $Z = -2.71$, $p = 0.007$) while showing no difference in time to approach the bowl (Figure 4.3C). There was additionally no difference between groups when average velocity (Figure 4.3D) and percentage time moving (Figure 4.3E) were analysed from tracking data. When the proportion of time animals spent in the inner zone of the arena compared to the outer zone was analysed a difference between groups emerged (Figure 4.3F, GLMM, $Z = -2.62$, $p = 0.009$) with MS180 animals spending less time in the inner zone compared to controls.

Animals additionally completed the sucrose preference test, a measure of reward sensitivity. There was no difference between groups in either sucrose preference (Figure 4.4A) or total fluid consumption over the course of the 2-hour test (Figure 4.4B). When both groups were assessed together, animals did however show a sucrose preference (Wilcoxon signed ranks test against hypothetical mean of 50%, $Z = 2.98$, $p = 0.003$).

Plasma corticosterone concentrations were assessed both in response to 20 minutes of restraint stress and at a basal level prior to slice preparation. In the restraint stress experiment both groups of animals exhibited a robust increase in CORT due to restraint (GLMM, $Z = 6.9$, $p < 0.0001$) while there was also a main effect of condition (Figure 4.5A, GLMM, $Z = 2.175$, $p = 0.03$) with MS180 animals having overall higher plasma CORT concentrations across both stress and baseline periods. Additionally, female animals exhibited higher overall levels of CORT (GLMM, $Z = -3.89$, $p < 0.0001$). However, there was no interaction between maternal separation and restraint stress either in the main analysis or when relative increase in CORT due to restraint was analysed (Figure 4.5B). When plasma CORT was analysed from animals during the slice preparation process there was interestingly a lower CORT concentration observed in MS180 animals relative to controls (Figure 4.5C, $Z = -2.80$, $p = 0.005$). As before females were observed to have higher CORT concentrations ($Z = -9.33$, $p < 0.0001$).

As another measure of stress responsiveness, activation of the PVN following restraint stress was assessed through cFos immunohistochemistry (Figure 4.6A). When the number of cFos$^+$ cells was assessed, a trend towards an interaction between condition and sex was observed (Figure 4.6B, GLMM, Z = 1.74, p = 0.083). When this was investigated it became apparent that male MS180 animals showed increased cFos expression compared to controls (GLMM, Z = 2.26, p = 0.024) while there was no difference in the female cohort. A main effect of sex was also observed overall with females showing higher cFos expression in response to restraint stress than males (GLMM, Z = -3.39, p = 0.0007). PVN area was also analysed as a measure of long-term PVN activation, however only sex influenced this measure with females having on average a larger PVN area (Figure 4.6C, GLMM, Z = -3.19, p = 0.001).

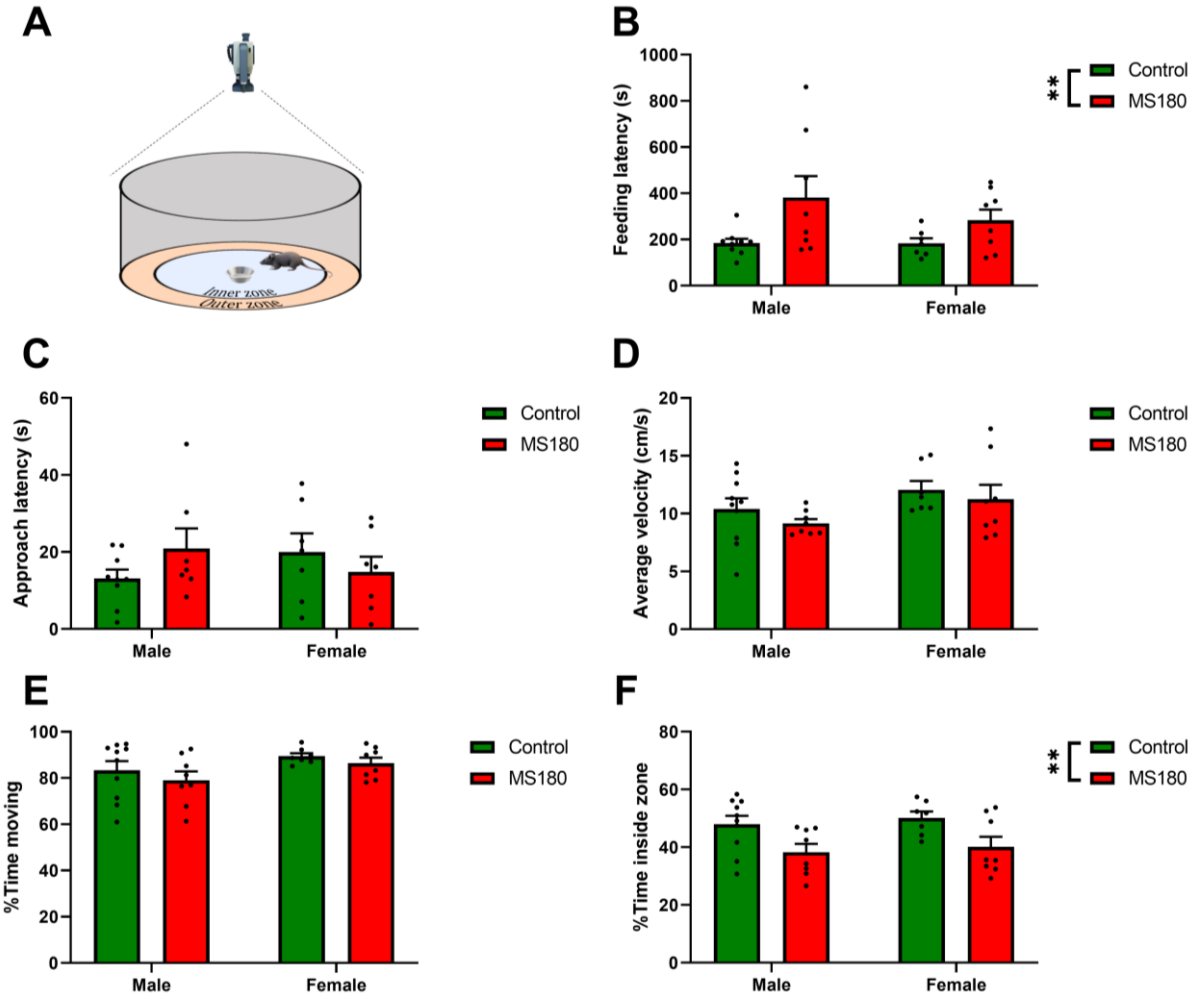


Figure 4.3 MS180 animals show increased anxiety in the novelty supressed feeding test. **(A)** Overview of the experimental setup and the zones defined for behavioural tracking. **(B)** Feeding latency and **(C)** Approach latency. **(D)** Average velocity, **(E)** percentage time that was movement and **(F)** percentage time that was spent in the inner zone throughout the session. N = 32 animals (16 control, 16 MS180).

Neurogenesis in the dentate gyrus was also examined as a biomarker of the ELS phenotype using BrdU immunohistochemistry. There was no difference between control and MS180 groups in the number of $BrdU^+$ cells in the SGZ of the DG (Figure 4.7B). There was additionally no effect of sex, although the total number of $BrdU^+$ cells was higher in the VH as opposed to the DH (GLMM, $Z = -6.92$, $p < 0.0001$).

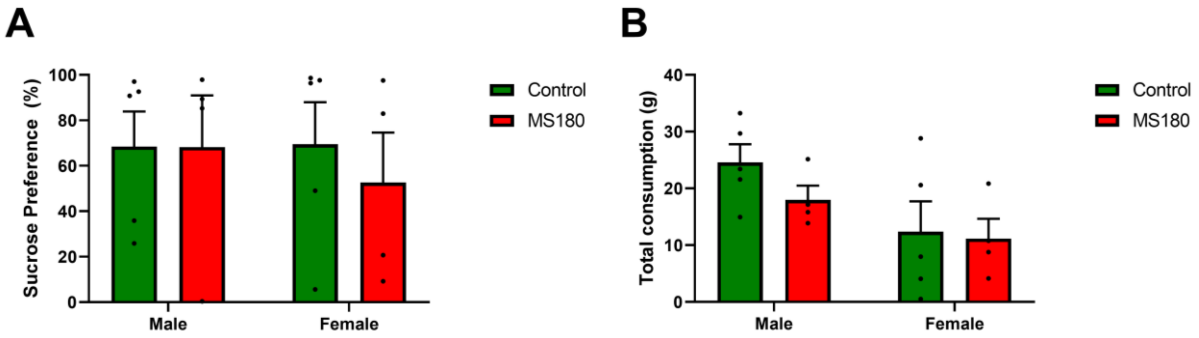


Figure 4.4 No change in sucrose preference between control and MS180 animals. (A) Sucrose preference and **(B)** total consumption over the 2-hour session. N = 18 animals (10 control, 8 MS180).

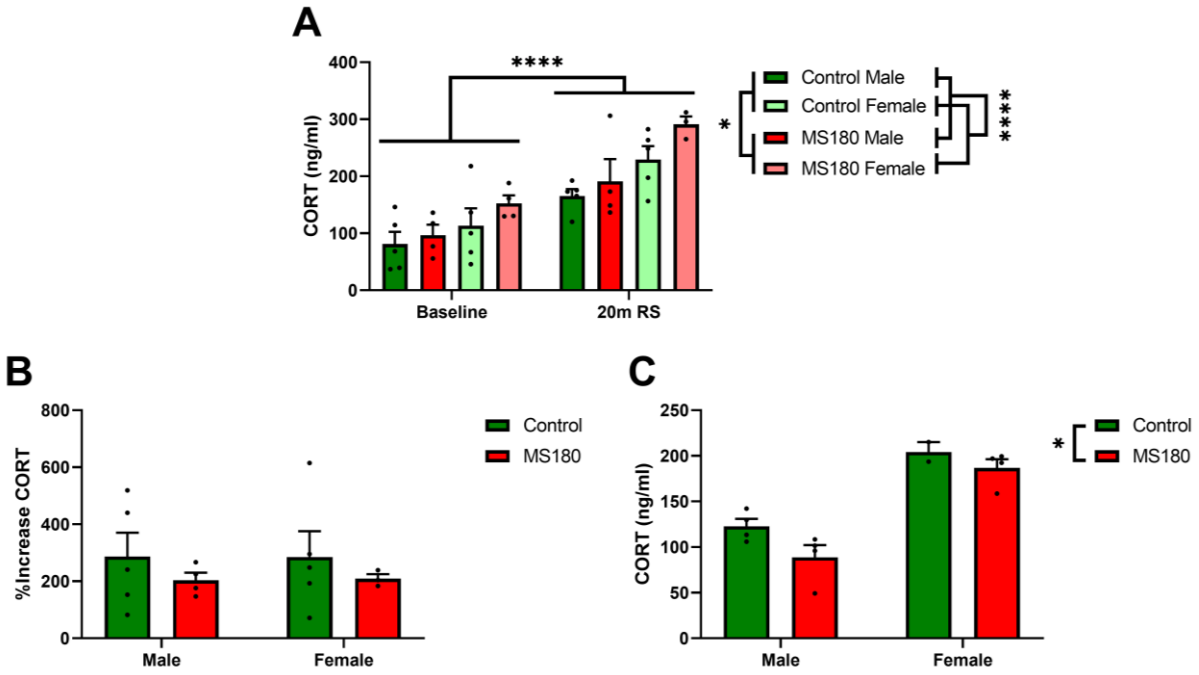


Figure 4.5 MS180 animals show altered CORT compared to controls. (A) Restraint stress corticosterone and **(B)** percentage increase in CORT as a result of restraint stress in validation cohort animals. **(C)** Basal CORT in electrophysiology animals from CYC1 prior to slice preparation. N = 18 animals (10 control and 8 MS180) for RS CORT and 14 animals (6 control and 8 MS180) for basal CORT.

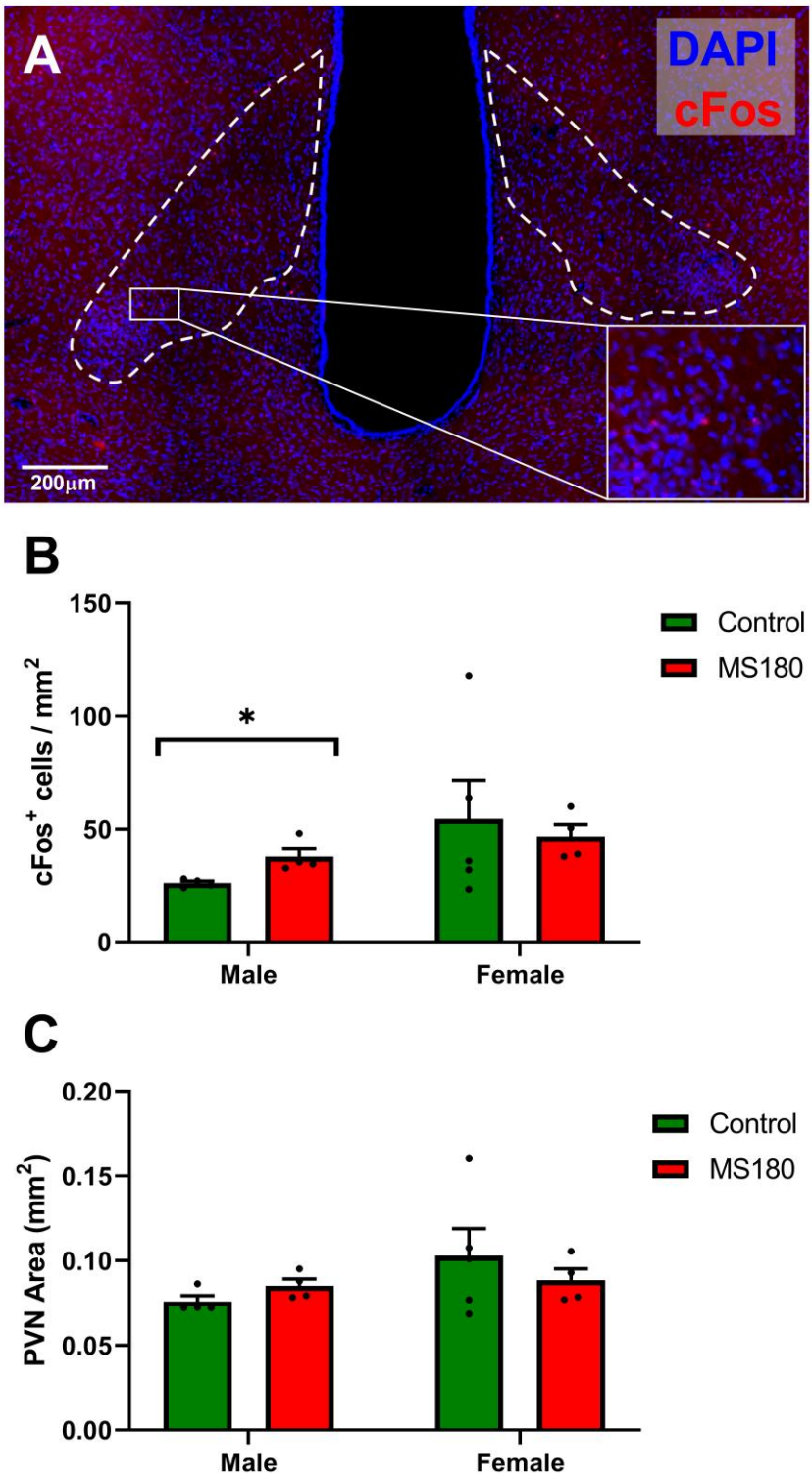


Figure 4.6 Restraint stress PVN cFos in MS180 and control animals. (A) Example photomicrograph of cFos staining in the paraventricular nucleus with inset showing DAPI$^+$/cFos$^+$ cells in the PVN. **(B)** Average cFos$^+$ cells normalised by area in the PVN and **(C)** average PVN area. N = 17 animals (9 control and 8 MS180).

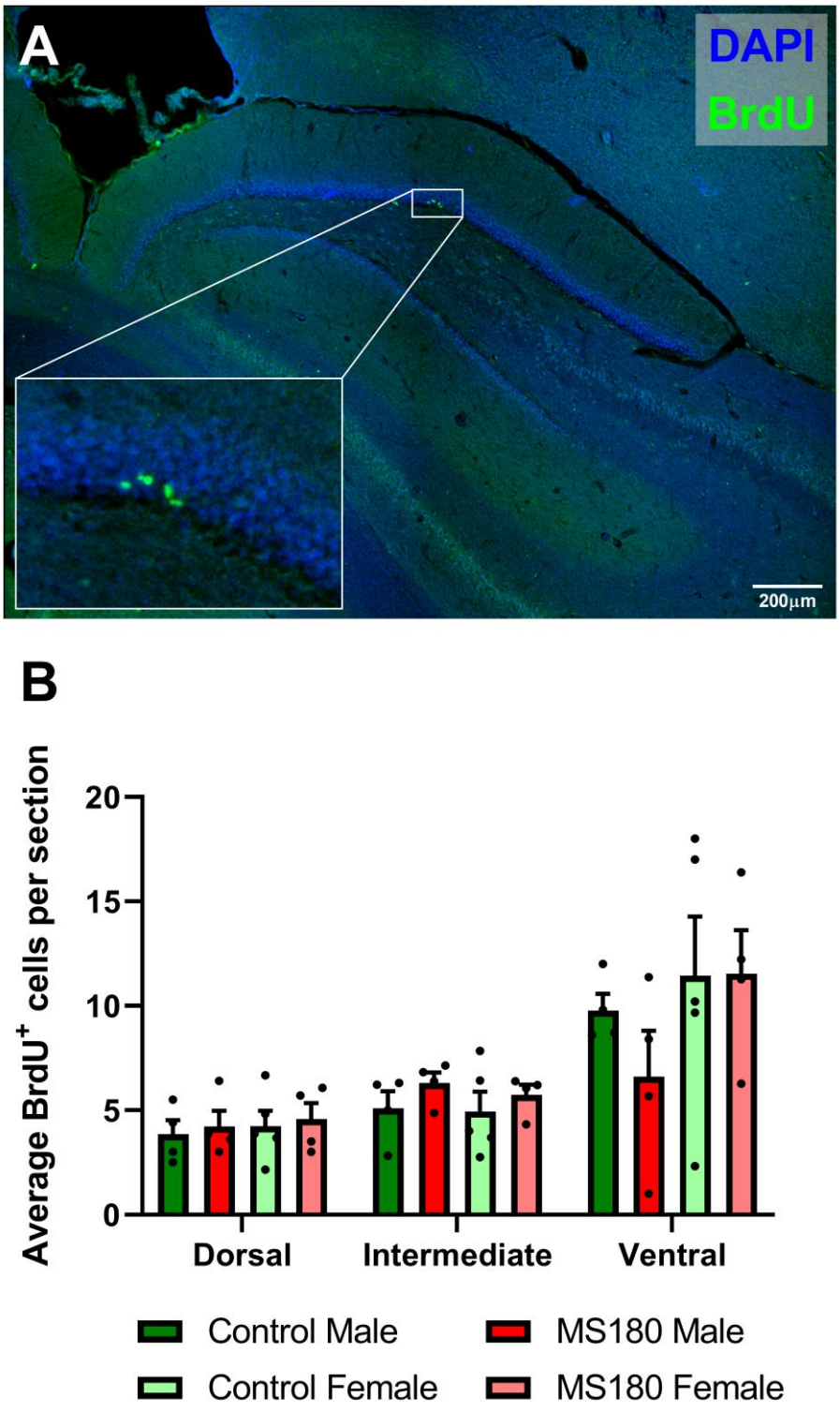


Figure 4.7 Dentate gyrus neurogenesis in MS180 and control animals. (A) Example photomicrograph of BrdU staining in the dentate gyrus with inset showing $BrdU^+$ cells in the SGZ. **(B)** BrdU positive cells in the subgranular zone subdivided by hippocampal region. N = 18 animals (10 control and 8 MS180).

4.4.2 MS180 animals show increased NMDAR function compared to controls

In order to assess the relative function of AMPAR and NMDAR mediated CA1 pyramidal cell transmission AMPA/NMDA ratios were assessed in the SC and TA pathways of CA1 in control and MS180 animals (see Figure 4.8A for recording setup). MS180 animals overall had a lower AMPA/NMDA ratio compared to controls (Figure 4.8B, H and I, GLMM, Z = -3.16, p = 0.0016) with an additional interaction between sex and condition (GLMM, Z = -2.81, p = 0.005). When this was further investigated a clear effect of condition in females but not males was apparent (females: GLMM, Z = -2.31, p = 0.021). An overall trend for the TA pathway to have a lower AMPA/NMDA ratio was also observed (Figure 4.8J, GLMM, Z = -1.802, p = 0.072) with this manifesting as an interaction between region and pathway (GLMM, Z = 2.13, p = 0.03) whereby the TA pathway had a lower ratio in the DH (GLMM, Z = -2.04, p = 0.041) but not VH. This additional pathway analysis also elucidated an interaction between sex and condition in the DH with male MS180 animals only having a reduced AMPA/NMDA ratio in the DH (GLMM, Z = -2.50, p = 0.013).

In order to isolate the effects of a changed AMPA/NMDA ratio to either AMPAR or NMDAR mediated transmission, miniEPSCs were recorded from CA1 pyramidal neurones (see Figure 4.9A for recording setup). miniEPSCs were analysed both as a cumulative distribution as is traditionally reported in addition to averaged events per cell in order to reduce any potential pseudoreplication. There was no difference in the amplitude cumulative distributions between control and MS180 animals (Figure 4.9D), however MS180 animals showed a decreased event frequency (Figure 4.9E, KS-test, F = 0.092, p < 0.0001) and increased event area (Figure 4.9F, KS-test, F = 0.085, p = 0.0004). There was no difference in cumulative distributions between control and MS180 groups in event duration (Figure 4.9G), rise kinetics (Figure 4.9H, TRise) or decay kinetics (Figure 4.9I, T90%).

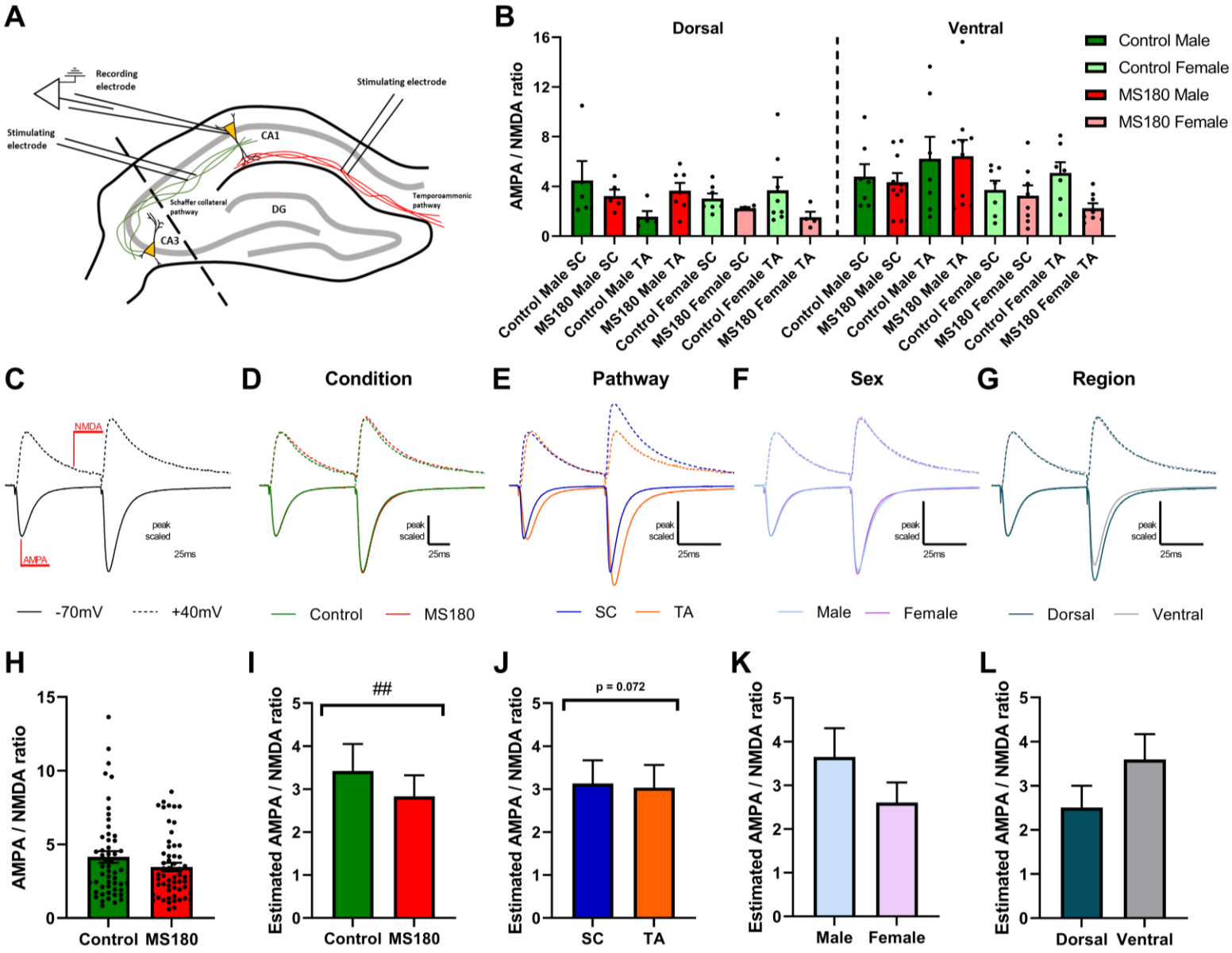
When data were analysed per cell, again there was no main effect of MS180 upon miniEPSC amplitude (Figure 4.10A) but additionally the effects of condition upon miniEPSC frequency (Figure 4.10B) and area (Figure 4.10C) disappeared. A trend for males to have a lower amplitude miniEPSC was observed however (GLMM, Z = -1.89, p = 0.060). When miniEPSC duration was analysed a trend towards an interaction between condition and sex was observed (Figure 4.10D, GLMM, Z = -1.74, p = 0.082) however upon further investigation there was not a main effect of condition in either sex. An interaction between condition and sex was also observed for miniEPSC rise kinetics (Figure 4.10E, Z = 1.978, p = 0.048)

which upon further investigation revealed a trend towards males but not females having a slower rise time (males: GLMM, Z = 1.73, p = 0.083). Additionally, cells from ventral CA1 also trended towards having a slower rise time than their counterparts from the DH (GLMM, Z = 1.91, p = 0.057). Finally, another interaction between MS180 and sex was observed in miniEPSC decay kinetics (Figure 4.10F, GLMM, Z = -3.28, p = 0.001) with the effect of MS180 in males being to speed up the decay kinetics (GLMM, Z = -2.87, p = 0.004) while in females MS180 trended towards slowing the decay (GLMM, Z = 1.72, p = 0.09).

4.4.3 LTP in MS180 and control animals

In order to ascertain if increased NMDAR function would translate into altered synaptic plasticity properties, theta burst stimulation induced LTP was studied in control and MS180 animals in both SC and TA pathways (see Figure 4.11A and B for overview). The stimulation protocol induced LTP in only the SC pathway (Figure 4.11E, F and G, GLMM, Z = 3.19, p = 0.0014) while no LTP was observed in the TA pathway. A trend towards an interaction between MS180 and sex was also observed (Figure 4.11H, GLMM, Z = -1.92, p = 0.055) with this manifesting as a trend towards decreased LTP in MS180 males (GLMM, Z = -1.84, p = 0.066) compared to controls with no corresponding changes observed in female animals. A main effect of sex was also observed (Figure 4.11I, GLMM, Z = 2.85, p = 0.004) whereby males exhibited higher LTP relative to females.

Figure 4.8 MS180 animals have a lower AMPA/NMDA ratio than controls. (A) Diagram of experimental setup with cells being recorded in CA1 and stimulated in SC and TA pathways. **(B)** AMPA/NMDA ratio. **(C)** Example trace showing where AMPAR and NMDAR mediated EPSC components were measured from. **(D-G)** Peak scaled average traces for the factors: condition, pathway, sex and region. Solid lines indicate recordings at -70mV while dotted lines indicate recordings from +40mV. **(H)** Example graph showing AMPA/NMDA ratio when all data is pooled by condition. **(I-L)** Estimated marginal means for condition, pathway, sex and region whereby the effect of changing only the factor of interest is examined. N = 53 cells (26 control and 27 MS180) from 37 animals (18 control, 19 MS180) and marginal means are shown as mean ± 95% CI.


A
Recording electrode
Stimulating electrode
Stimulating electrode
CA1
Schaffer collateral pathway
Temporoammonic pathway
DG
CA3
B
Dorsal
Ventral
AMPA / NMDA ratio
Control Male
Control Female
MS180 Male
MS180 Female
Control Male SC
MS180 Male SC
Control Male TA
MS180 Male TA
Control Female SC
MS180 Female SC
Control Female TA
MS180 Female TA
C
NMDA
AMPA
peak scaled
25ms
-70mV
+40mV
D
Condition
Control
MS180
E
Pathway
SC
TA
F
Sex
Male
Female
G
Region
Dorsal
Ventral
H
AMPA / NMDA ratio
Control
MS180
I
##
Estimated AMPA / NMDA ratio
J
p = 0.072
K
L

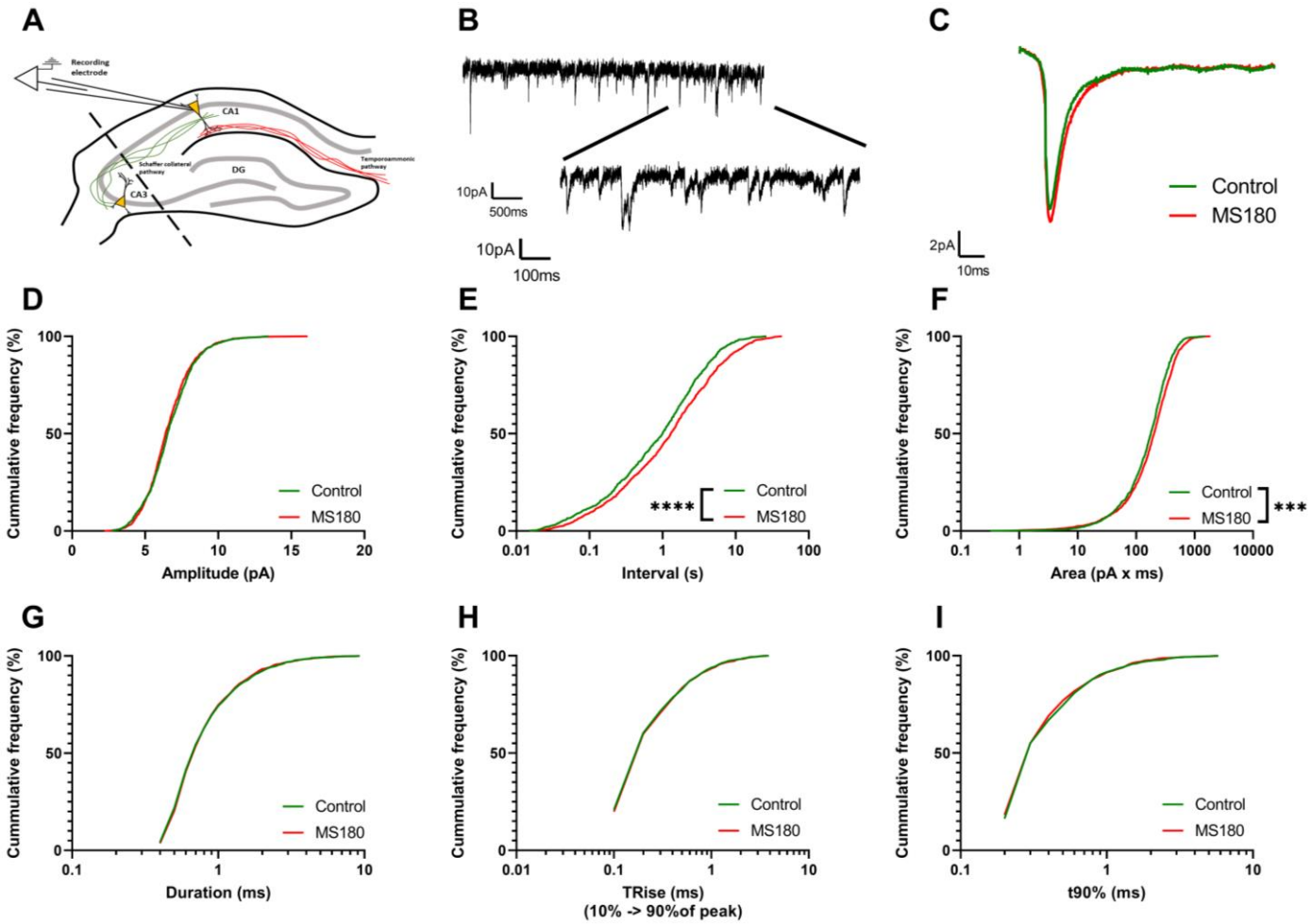


Figure 4.9 MS180 animals show reduced miniEPSC frequency and increased area in a cumulative distribution analysis. (A) Experimental setup diagram with cells recorded from CA1. **(B)** Example trace showing multiple miniEPSC events. **(C)** Average miniEPSC trace for control and MS180 animals. Cumulative distributions binned by condition for **(D)** Amplitude, **(E)** inter-event interval, **(F)** area, **(G)** event duration, **(H)** Trise, the time taken to rise from 10-90% of peak depolarisation and **(I)** t90%, the time taken to complete 90% of the decay.

However, there was a dissociation between STP and LTP with no change between the control pathway and either SC or TA pathways observed (Figure 4.12A). There was additionally no effect of MS180 or sex upon STP albeit there was an effect of hippocampal region with ventral cells exhibiting lower STP relative to dorsal cells (Figure 4.12B, GLMM, Z = -3.5, p = 0.0005). Additionally, a region by pathway interaction emerged (Figure 4.12C, GLMM, Region: SC Z = 2.17, p = 0.030, Region: TA, Z = 2.04, p = 0.041) whereby there was no effect of TBS in DH cells but a robust STP was observed in the SC pathway of VH cells (GLMM, Z = 2.56, p = 0.01). In order to assess that any potential group differences were not due to differences in stimulation intensity, baseline EPSC amplitudes were analysed for all cells (Figure 4.12D). TA EPSC amplitudes were lower than those from either SC pathway (GLMM, Z = -3.56, p = 0.0004) while EPSCs from ventral cells tended to be of a larger amplitude (GLMM, Z = 2.10, p = 0.045).

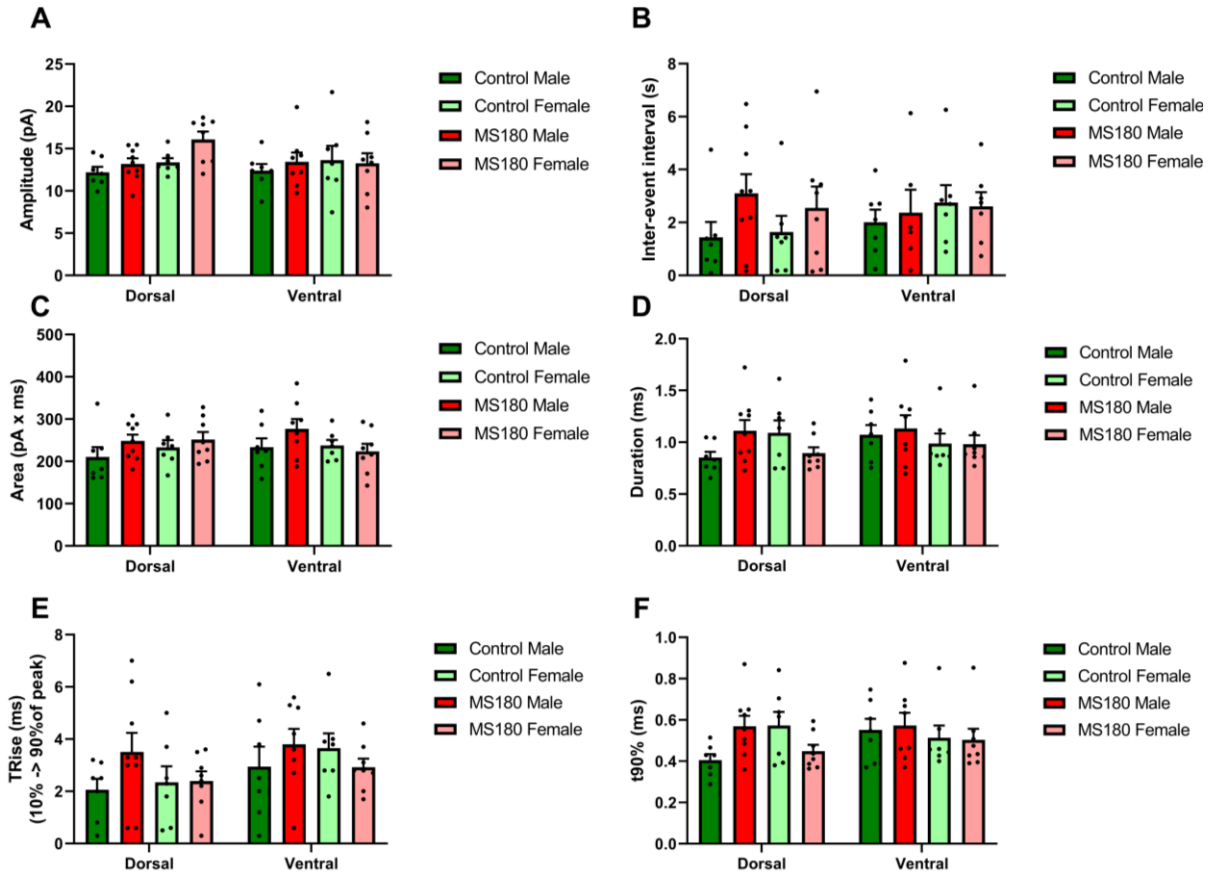


Figure 4.10 miniEPSC experiment analysis by cell reveals little difference between MS180 and control conditions. (A) Amplitude, **(B)** inter-event interval, **(C)** area, **(D)** event duration, **(E)** trise, the time taken to rise from 10-90% of peak depolarisation and **(F)** t90%, the time taken to complete 90% of the decay. N = 58 cells (28 control, 30 MS180) from 37 animals (17 control, 20 MS180).

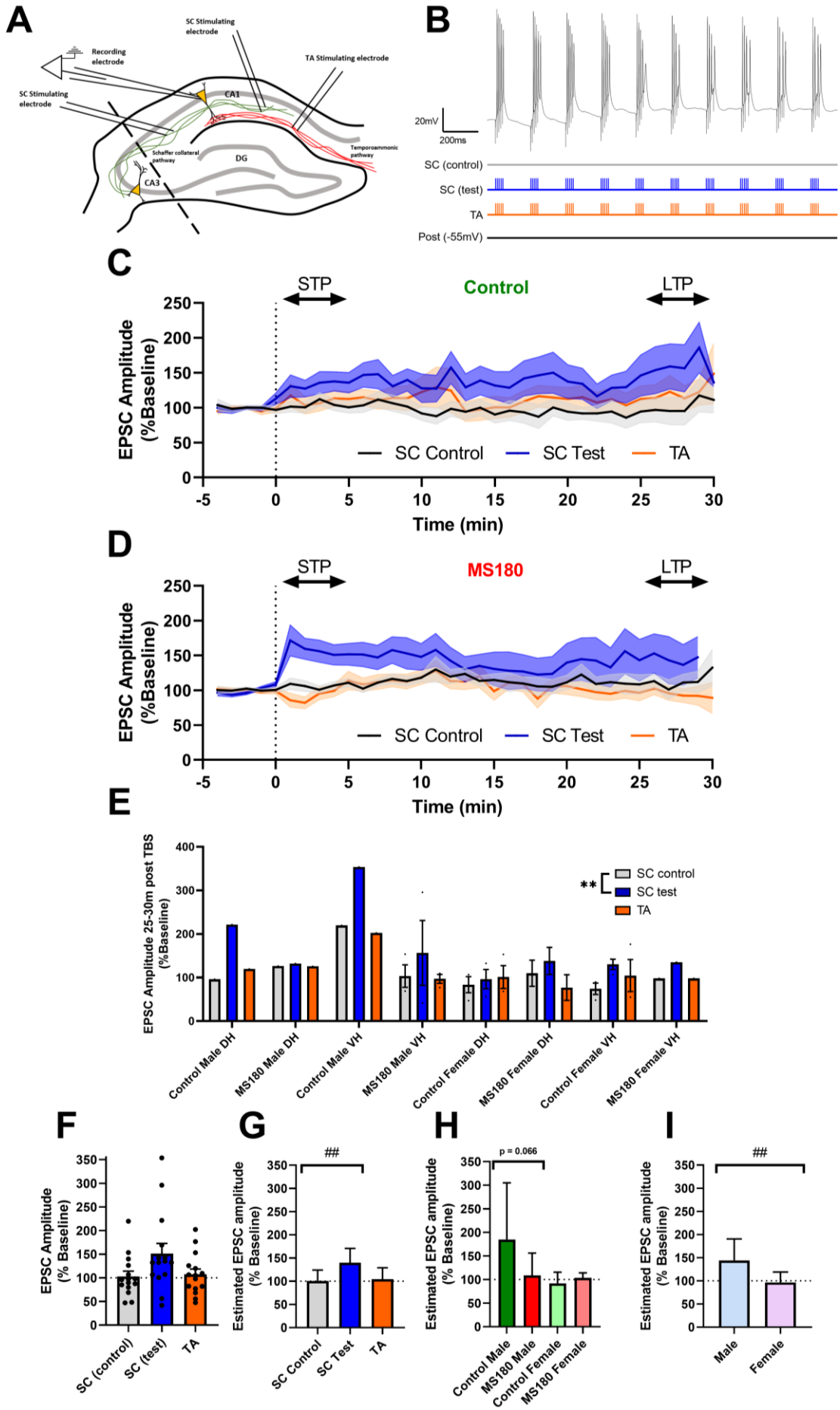

A
SC Stimulating electrode
Recording electrode
TA Stimulating electrode
SC Stimulating electrode
CA1
Schaffer collateral pathway
DG
Temporoammonic pathway
CA3
B
20mV
200ms
SC (control)
SC (test)
TA
Post (-55mV)
C
STP
Control
LTP
EPSC Amplitude (%Baseline)
SC Control
SC Test
TA
Time (min)
D
STP
MS180
LTP
EPSC Amplitude (%Baseline)
SC Control
SC Test
TA
Time (min)
E
EPSC Amplitude 25-30m post TBS (%Baseline)
**
SC control
SC test
TA
Control Male DH
MS180 Male DH
Control Male VH
MS180 Male VH
Control Female DH
MS180 Female DH
Control Female VH
MS180 Female VH
F
EPSC Amplitude (% Baseline)
SC (control)
SC (test)
TA
G
##
Estimated EPSC amplitude (% Baseline)
SC Control
SC Test
TA
H
p = 0.066
Estimated EPSC amplitude (% Baseline)
Control Male
MS180 Male
Control Female
MS180 Female
I
##
Estimated EPSC amplitude (% Baseline)
Male
Female

Figure 4.11 Long term potentiation in control and MS180 animals. (A) Diagram showing experimental setup with cells recorded in CA1 and two stimulation electrodes in sr alongside a single electrode in slm. **(B)** The theta burst stimulation LTP induction protocol showing an example trace resulting from it and a diagrammatic representation of the stimulation pattern. **(C, D)** Minute averages of normalised EPSC amplitude for control and MS180 animals respectively. LTP was induced at minute 0 and STP/LTP markers represent where these measurements were taken from. **(E)** LTP at 25-30m post TBS split by all factors. **(F)** Data pooled by pathway as opposed to estimated marginal means **(G, H and I)** from the LTP generalised linear mixed model of sex, pathway and condition respectively. N = 15 cells (8 control, 7 MS180) from 14 animals (8 control, 6 MS180) and marginal means are shown as mean ± 95% CI.

Data from LTP induction were analysed in order to understand if any potential changes in LTP between MS180 and control animals were due to differential excitability during the TBS protocol. There was no difference between conditions observed when the total number of action potentials were analysed (Figure 4.13B) although a sex by region interaction (GLMM, $Z = 2.079$, $p = 0.038$) and trend towards increased spike generation in the DH (GLMM, $Z = -1.73$, $p = 0.083$) were observed. Both TBS area and the area in the decay phase (see Figure 4.13A) were analysed as measures of total depolarisation and plateau potential generation during the TBS phase respectively. There was no effect of MS180 observed for either of these measures (Figure 4.13C and D) although ventral cells had a lower total area during the TBS than their dorsal counterparts (GLMM, $Z = -2.06$, $p = 0.040$).

4.4.4 Basal transmission is not significantly altered by early life stress

Basal neurotransmission parameters from the AMPA/NMDA and LTP experiments were analysed in order to understand the influence of ELS upon these measures. Due to these experiments being conducted using different internal solutions which block different conductances data were separately analysed each by internal: $CsMeSO_4$ or $KMeSO_4$.

When tested using the $CsMeSO_4$ internal there was no difference in facilitation between control and MS180 animals as measured by paired pulse ratio (Figure 4.14C).

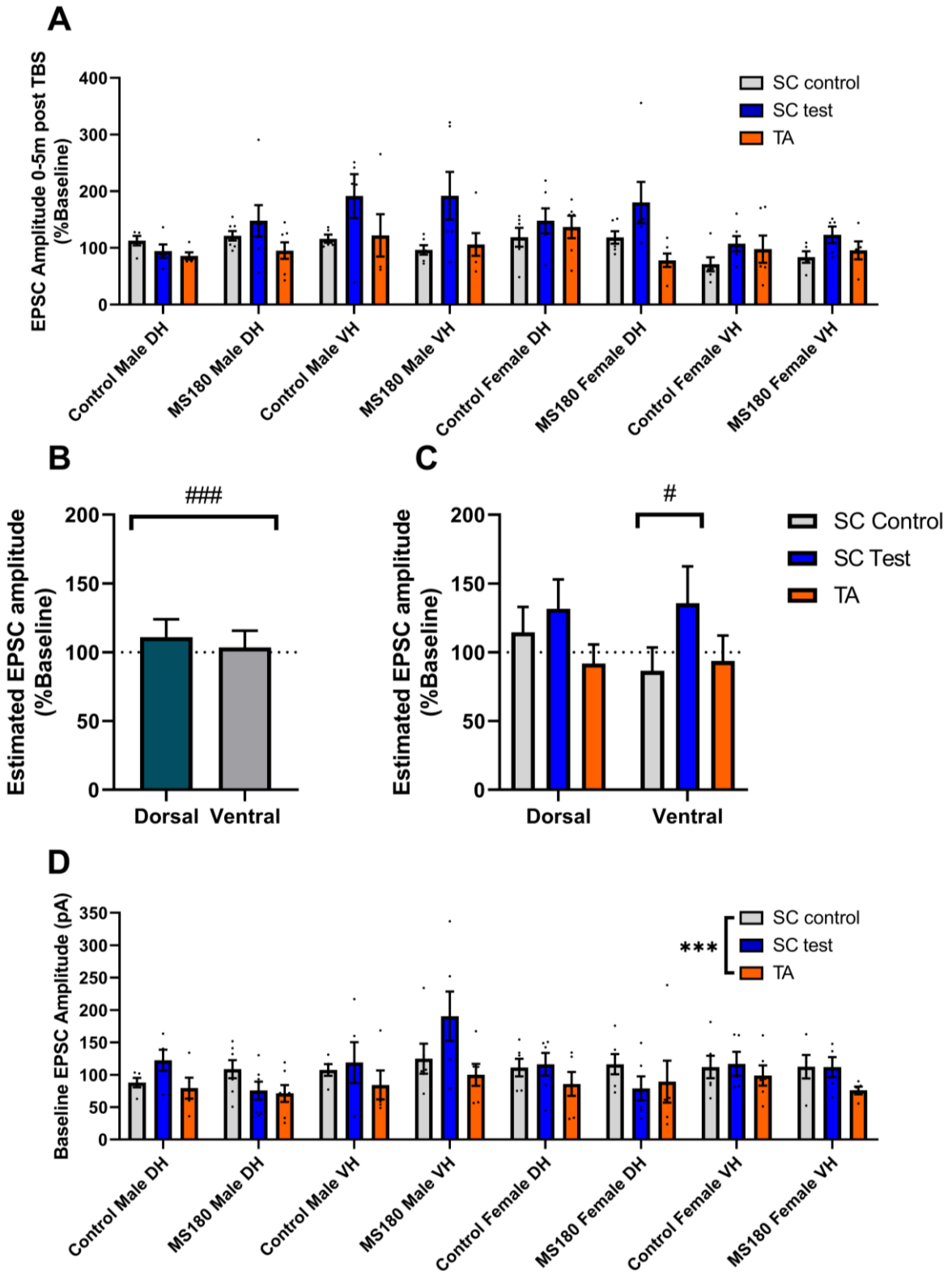


Figure 4.12 No effect of early life stress upon short term potentiation. (A) STP at 0-5m post LTP induction split by all factors. **(B)** Estimated marginal means from the STP model for region overall and then **(C)** split by pathway. **(D)** Average baseline amplitude before induction of LTP. N = 46 cells (22 control, 24 MS180) from 15 animals (8 control, 7 MS180) and estimated marginal means are shown as mean ± 95% CI.

There were however differences in pathway and region observed with the TA pathway (Figure 4.14D, GLMM, $Z = 4.5$, $p < 0.0001$) and dorsal region (Figure 4.14E, GLMM, $Z = -5.98$, $p < 0.0001$) showing increased facilitation compared to the SC pathway and ventral region respectively. Likewise there was no effect of ELS on input resistance (Figure 4.14F), however male animals were observed to exhibit an increased R_{in} compared to females (Figure 4.14, GLMM, $Z = 2.3$, $p = 0.021$).

Due to the $KMeSO_4$ internal not blocking K^+ conductances this meant that experiments in this internal additionally exhibited an afterhyperpolarisation (AHP) following stimulation (see Figure 4.15A) which was additionally analysed. Similarly to previous data there was no effect of MS180 upon R_{in} (Figure 4.15B), however compared to before there was no effect of sex observed. When the AHP was analysed a clear effect of pathway was apparent. TA inputs led to much a greater magnitude AHP than SC inputs (Figure 4.15C and D, GLMM, $Z = 3.80$, $p = 0.0001$) while ventral cells showed a lower AHP than dorsal cells (Figure 4.15E, GLMM, $Z = -2.232$, $p = 0.026$). Interestingly there was a trend towards a 3-way interaction between sex, the TA pathway and condition (GLMM, $Z = 1.81$, $p = 0.070$). When this was investigated further it was revealed that male MS180 animals had an increased AHP amplitude in the TA pathway (GLMM, $Z = 2.2$, $p = 0.027$) while no difference was observed between conditions for females.

Paired pulse ratio was also assessed for experiments using the $KMeSO_4$ internal where interestingly in contrast to the $CsMeSO_4$ results the TA pathway exhibited a lower PPR (Figure 4.15F and G, GLMM, $Z = -6.74$, $p < 0.0001$). A trend towards an interaction between condition and hippocampal region was also observed (GLMM, $Z = -1.71$, $p = 0.087$) however upon further investigation there was no effect of MS180 in either DH or VH regions individually.

Impedance is another property of neurones relating to how they both selectively amplify and transmit signals in response to an oscillatory input (see Figure 4.16A for examples). There was no effect of ELS nor any other condition upon either maximal impedance (Figure 4.16F) or the frequency at which neurones exhibited maximal impedance (Figure 4.16G). There also did not appear to be any difference between conditions or hippocampal regions in the phase-shift spectra (Figure 4.16D and E) calculated from the voltage oscillation resulting from the input current application.

As the key output from CA1 pyramidal neurones the spike dynamics of action potentials generated in response to oscillatory input were analysed in control and MS180 animals. There was no effect of maternal separation upon either maximal depolarisation (Figure 4.17D) or repolarisation rates (Figure 4.17E) in addition to halfwidth (Figure 4.17F). However, ventral cells were slower for both depolarisation and repolarisation rates with a trend towards having a longer halfwidth (GLMM, max depolarisation: Z = -2.156, p = 0.031, max repolarisation: Z = -2.36, p = 0.018, halfwidth: Z = -1.82, p = 0.069) compared to DH neurones. There was a trend towards MS180 cells having a more depolarised action potential threshold (Figure 4.17G, GLMM, Z = 1.74, p = 0.083).

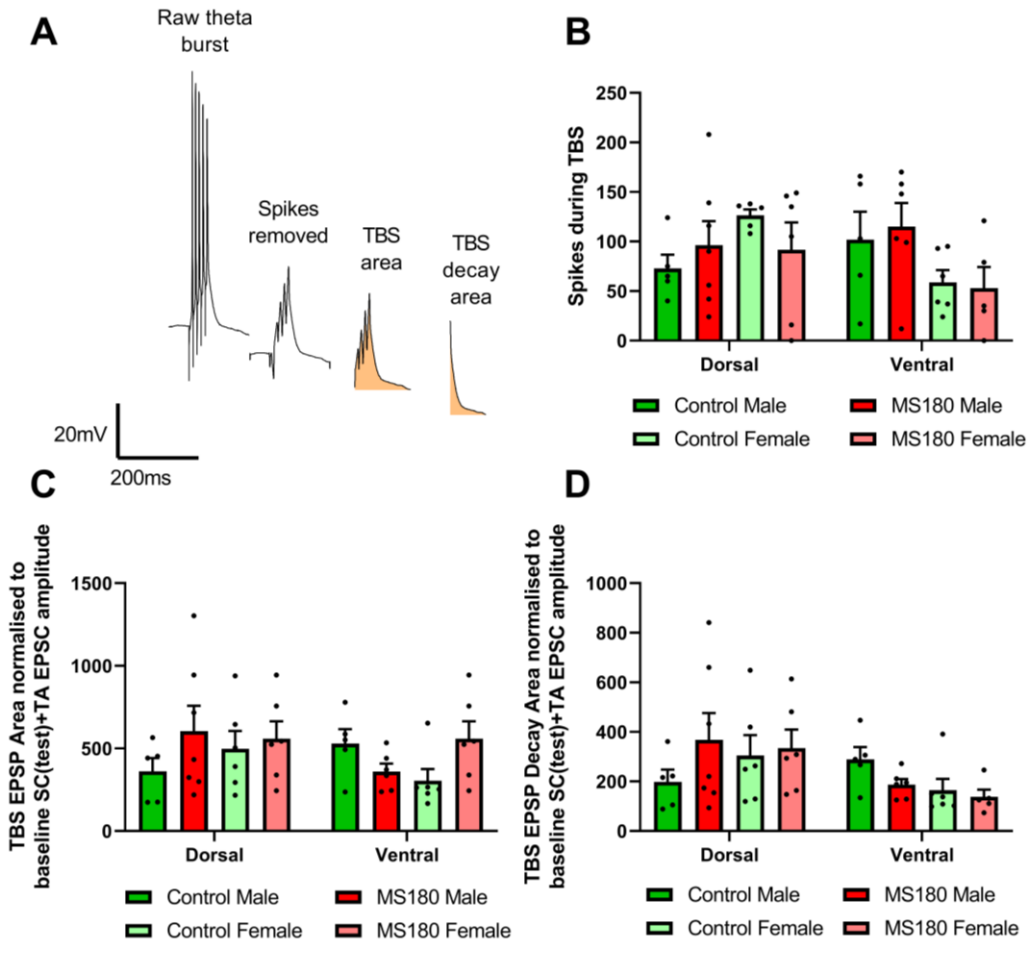


Figure 4.13 No effect of maternal separation upon theta burst parameters. (A) Example trace showing how output measures were created. **(B)** Total number of spikes counted during the theta bursts. **(C, D)** Total EPSP area and area from the decay phase respectively from the LTP induction phase. N = 46 cells (22 control, 24 MS180) from 15 animals (8 control, 7 MS180).

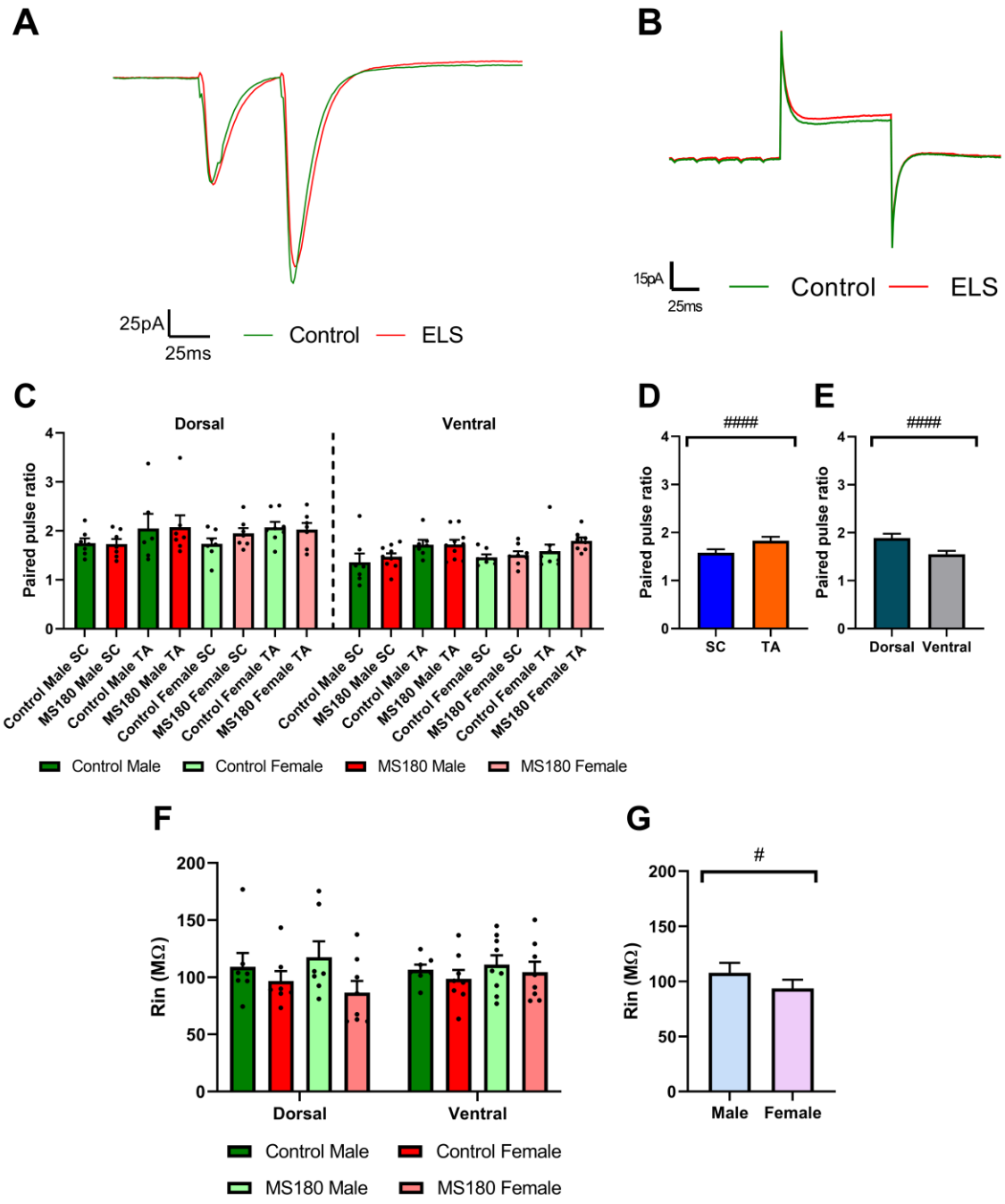


Figure 4.14 Basal transmission measures from the AMPA/NMDA experiment are no different between control and MS180 animals. (A and B) Example traces from control and MS180 animals showing paired pulse ratio calculation and the current step used to calculate input resistance. **(C)** Paired pulse ratio and **(D, E)** the estimated marginal means from the PPR analysis model. **(F)** Input resistance alongside **(G)** estimated marginal means for sex using the R_{in} analysis model. N = 60 cells (28 control, 32 MS180) from 38 animals (18 control, 20 MS180) and estimated marginal means are shown as mean ± 95% CI.

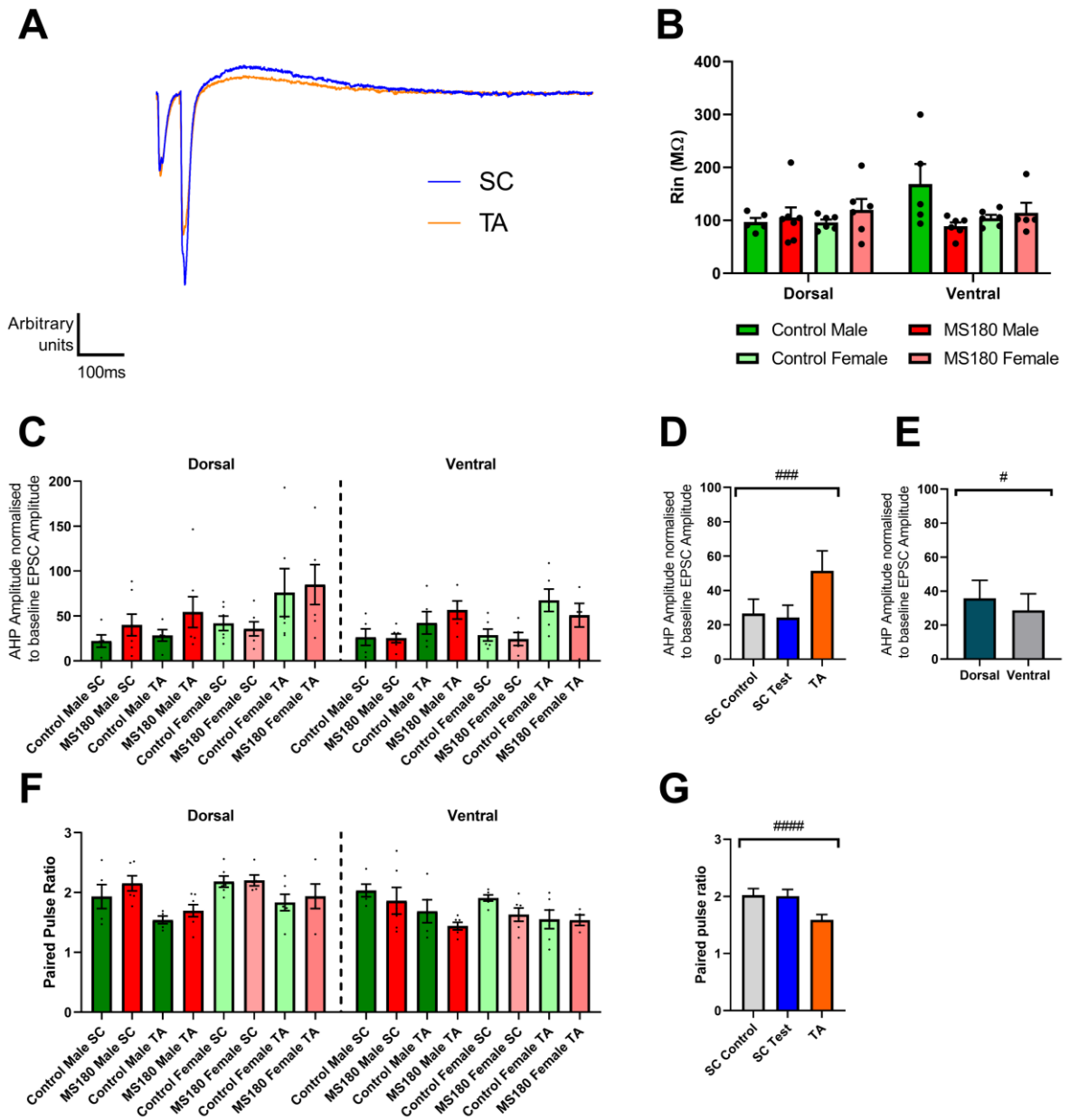


Figure 4.15 Basal transmission measures from the LTP experiment. **(A)** Example traces showing both paired pulse facilitation and a prominent afterhyperpolarisation. **(B)** Input resistance. **(C)** AHP amplitude normalised to baseline EPSC amplitude and the estimated marginal means from this analysis for **(D)** pathway and **(E)** hippocampal region. **(F)** Paired pulse ratio and the estimated marginal means for **(G)** pathway utilising the PPR analysis model. N = 46 cells (22 control, 24 MS180) from 15 animals (8 control, 7 MS180) and marginal means are shown as mean ± 95% CI.

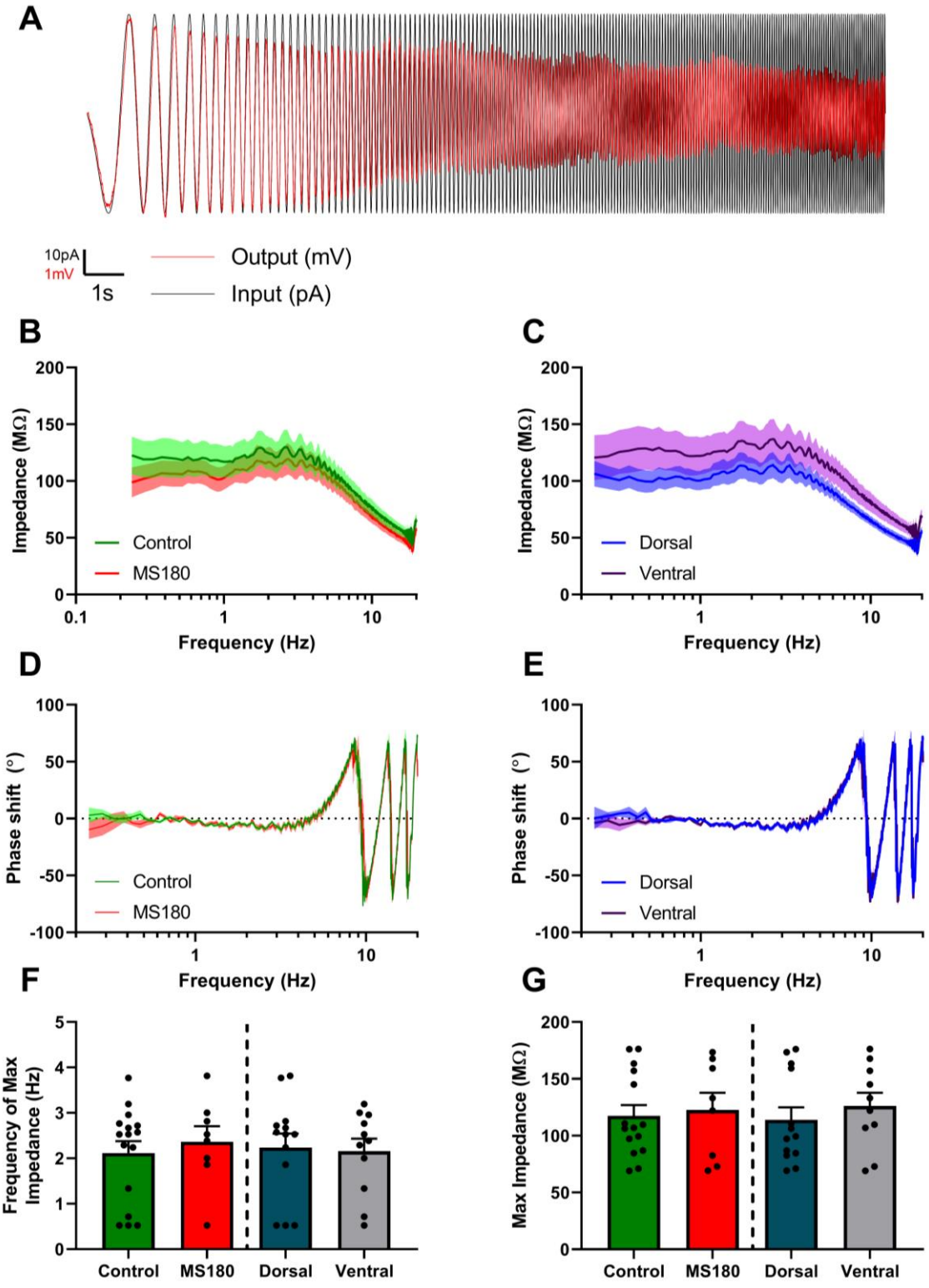


Figure 4.16 Impedance measurements in control and MS180 animals. (A) Example trace showing input current oscillation and its resulting output voltage trace. **(B, C)** Impedance spectra for condition and hippocampal region respectively. **(D, E)** Phase shift by frequency for condition and region respectively. **(F)** Frequency of max impedance and **(G)** maximal impedance. N = 23 cells (16 control, 8 MS180) from 12 animals (7 control, 5 MS180).

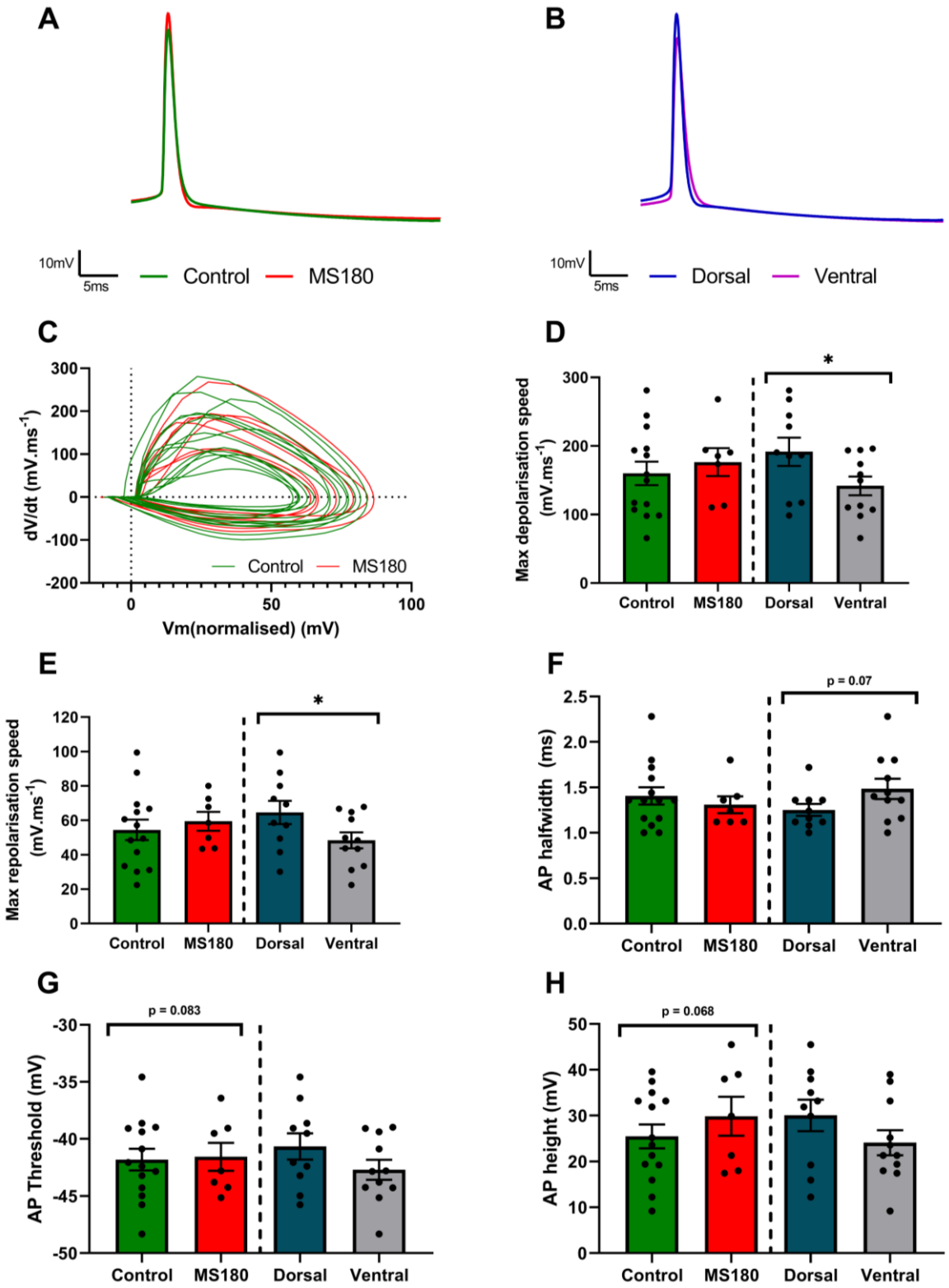


Figure 4.17 Spike dynamics analysis in MS180 and control animals. **(A, B)** Average traces for condition and hippocampal region respectively. **(C)** Phase plot for each cell split by condition. **(D)** Maximal depolarisation and **(E)** repolarisation speed. **(F)** Action potential halfwidth, **(G)** threshold and **(H)** maximal height. N = 21 cells (14 control, 7 MS180) from 9 animals (6 control, 3 MS180).

There was also an interaction between hippocampal aspect and condition (GLMM, Z = -2.10, p = 0.036) with this expressing as a trend towards MS180 animals having a more depolarised AP threshold in DH cells only (GLMM, Z = 1.76, p = 0.079). Finally, AP height was analysed with this revealing a trend towards MS180 animals having a more depolarised action potential (GLMM, Z = 1.83, p = 0.068) in addition to there being a condition by region interaction (Z = -2.02, p = 0.044). When this interaction was investigated it revealed a condition by sex interaction in VH only (GLMM, Z = -2.33, p = 0.020) itself manifesting as a trend towards a less depolarised AP height in DH females only (GLMM, Z = -1.86, p = 0.063).

4.4.5 Somatically recorded plateau potentials allow investigation of dendritic non-linear summation.

In order to investigate the effect of changes in NMDAR function upon dendritic integration via plateau potentials in MS180 animals it was necessary to pilot these experiments in wild type animals first. By stimulating cells with increasing intensity (see Figure 4.18A for overview) utilising the $KMeSO_4$ internal containing QX314 it was possible to generate robust plateau potentials in both SC and TA pathways in addition to during coincident stimulation (Figures 4.18B-D). Through fitting of the previously described two-component linear piecewise function it was possible to locate an intersection point that corresponded with the stimulation intensity threshold necessary to generate a plateau potential (Figures 4.18E-G). Upon application of the NMDAR antagonist DAPV plateau potentials were no longer able to form, even at extreme stimulation intensities (Figures 4.18H-J).

When the threshold for plateau potential generation was calculated it was apparent that the TA pathway required a lower level of stimulation to generate plateaus compared to the SC or SC + TA combined pathways (Figure 4.19M, Mixed-effects model, main effect of treatment: $F_{1.109,14.97} = 35.6$, $p < 0.0001$, Dunnett's multiple comparison test SC vs TA : $q_{12} = 5.58$, $p = 0.0002$). Apamin treatment did not influence the threshold at which plateau potentials were generated compared to baseline (Figure 4.19A-F and N). However, the muscarinic agonist carbachol decreased the stimulation level needed for plateaus to form (Figure 4.19G-L and O, Mixed-effects model, $F_{1,5} = 36.3$, $p = 0.0018$).

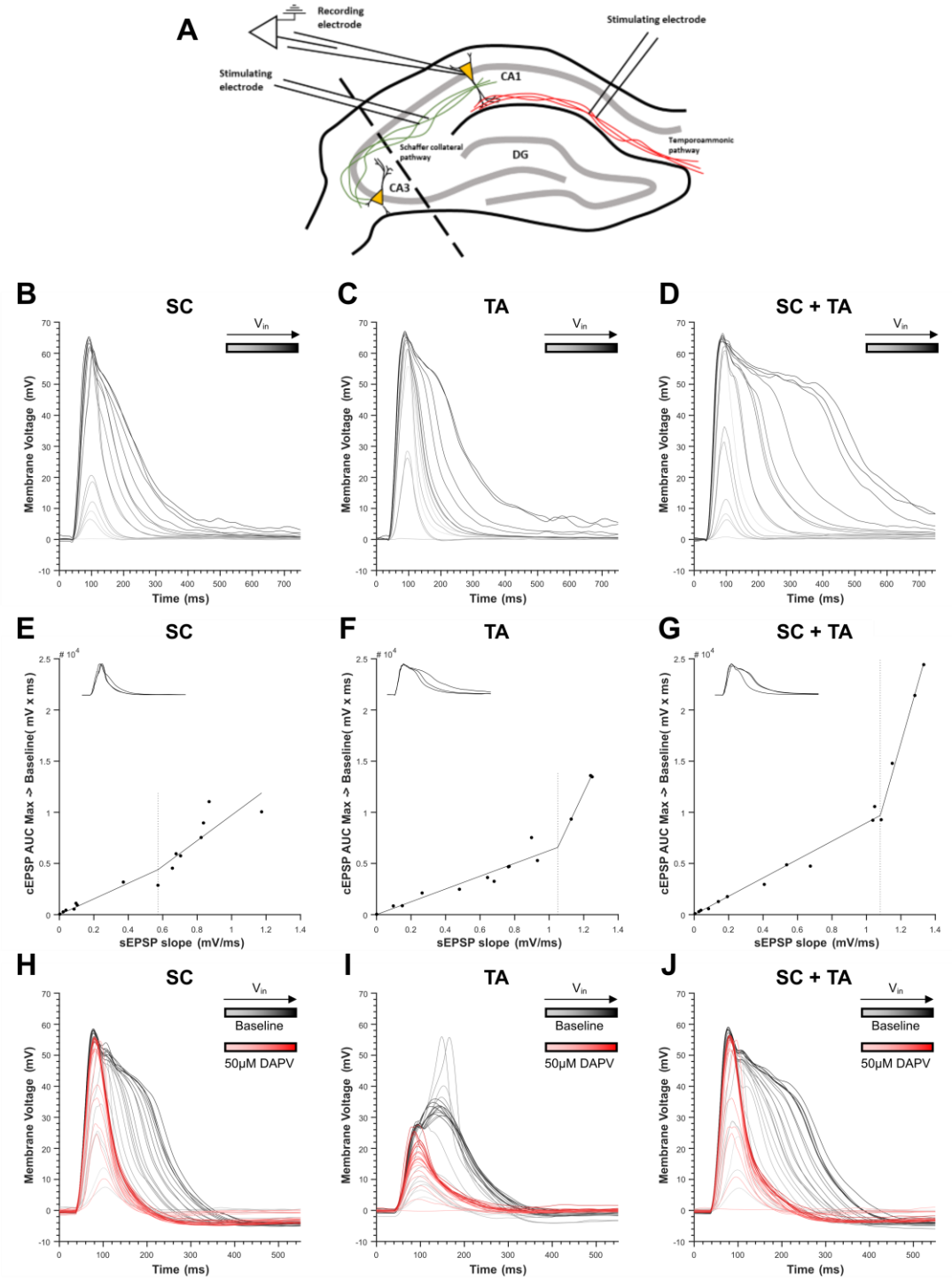


Figure 4.18 Plateau potentials can be recorded somatically from CA1 neurones and are NMDAR dependent. (A) Diagram showing experimental setup. Stimulation response traces for **(B)** SC, **(C),** TA and **(D)** coincident TA and SC stimulation. Intensity of trace indicates stimulation intensity. **(E - G)** Graphs showing calculation of plateau potential threshold from the relationship between single EPSP (sEPSP) slope and compound EPSP (cEPSP) decay AUC. Inset traces show the three nearest frames recorded to the calculated intersection point of the piecewise linear function. **(H - J)** Example traces showing the effects of 50μM DAPV upon plateau potential generation.

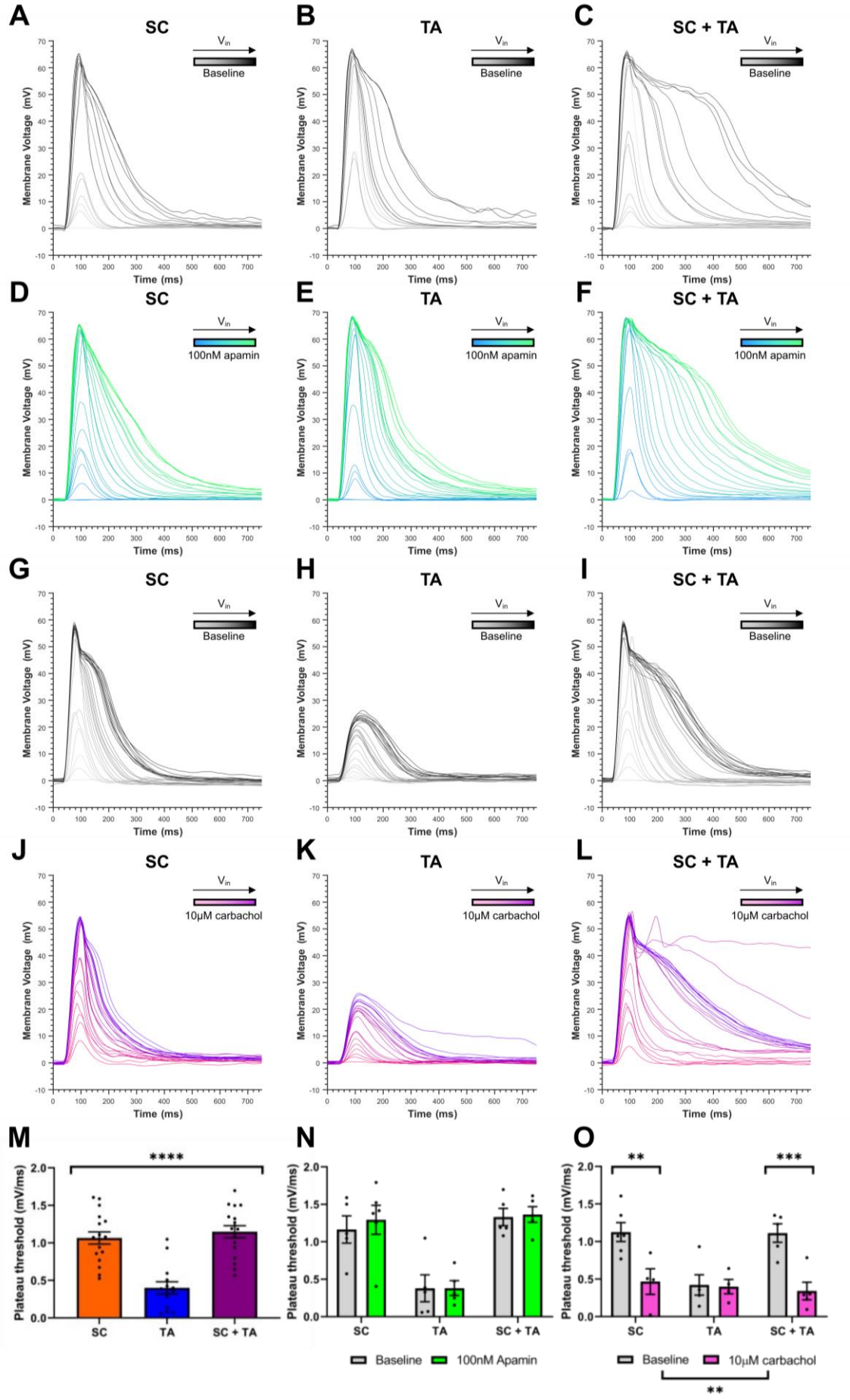

A
SC
B
TA
C
SC + TA
Baseline
D
SC
E
TA
F
SC + TA
100nM apamin
G
SC
H
TA
I
SC + TA
Baseline
J
SC
K
TA
L
SC + TA
10μM carbachol
Membrane Voltage (mV)
Time (ms)
M
N
O
Plateau threshold (mV/ms)
SC
TA
SC + TA
****
**
***
Baseline
100nM Apamin
10μM carbachol

Figure 4.19 10μM carbachol enhances plateau potential generation while apamin has no effects. (A – F) Example traces from a single cell showing the effects of application of 100nM apamin upon plateau potential generation. Each line shows a single trace with colour intensity representing stimulation intensity. **(G – L)** Example traces showing the effects of 10μM bath application of carbachol upon plateau potential generation. **(M)** Calculated plateau potential threshold between measured pathways (n = 20 cells from 12 animals), **(N)** no change in threshold after 100nM apamin application (n = 6 cells from 3 animals) and **(O)** decreased plateau potential threshold following application of 10μM carbachol (n = 6 cells from 5 animals).

There additionally was an interaction between pathway and drug treatment (Mixed-effect model, $F_{2,3}$ = 9.97, p = 0.049) which upon further investigation revealed an effect of carbachol in the SC (Sidak's multiple comparison test, t_8 = 4.95, p = 0.003) and SC + TA (Sidak's multiple comparison test, t_8 = 6.0, p = 0.001) combined pathways but not the TA pathway.

In order to understand the effect of drug treatment upon plateau potential size the average decay AUC for each cell was analysed in three strengths of input stimulation. Due to using differing binning criteria for each pathway these were analysed separately. Apamin (Figure 4.20A) did not have any effect upon plateau potential total depolarisation in any pathway whereas carbachol increased plateau potential decay AUC in all three pathway conditions (Figure 4.20B, 2-way RM-ANOVA, main effect of carbachol, SC: $F_{1,3.074}$ = 177.6, p = 0.0009, TA: $F_{1,8}$ = 69.8, p = 0.001, SC + TA : $F_{1,5}$ = 132.7, p < 0.0001) with this increase being uniform across EPSP slope bins. DAPV at an overall level decreased the decay AUC of the compound EPSP (Figure 4.20C, 2-way RM-ANOVA, SC: $F_{1,6}$ = 36.1, p = 0.009, TA: $F_{1,6}$ = 17.4, p = 0.001, SC + TA: $F_{1,6}$ = 58.7, p = 0.005). However, there were also interactions between single EPSP slope bin and compound EPSP AUC for the SC and SC + TA pathways (2-way ANOVA, SC: $F_{2,6}$ = 7.97, p = 0.02, SC + TA: $F_{2,6}$ = 5.138, p = 0.050). Further investigation revealed an effect of DAPV only in bin 3 for the SC + TA coincident stimulation (Sidak corrected t-test, t_4 = 4.392, p = 0.012) with trends towards an effect of DAPV in bin 2 for SC and SC + TA pathways (Sidak corrected t-test, SC: t_3 = 4.53, p = 0.06, SC + TA: t_3 = 4.62, p = 0.057).

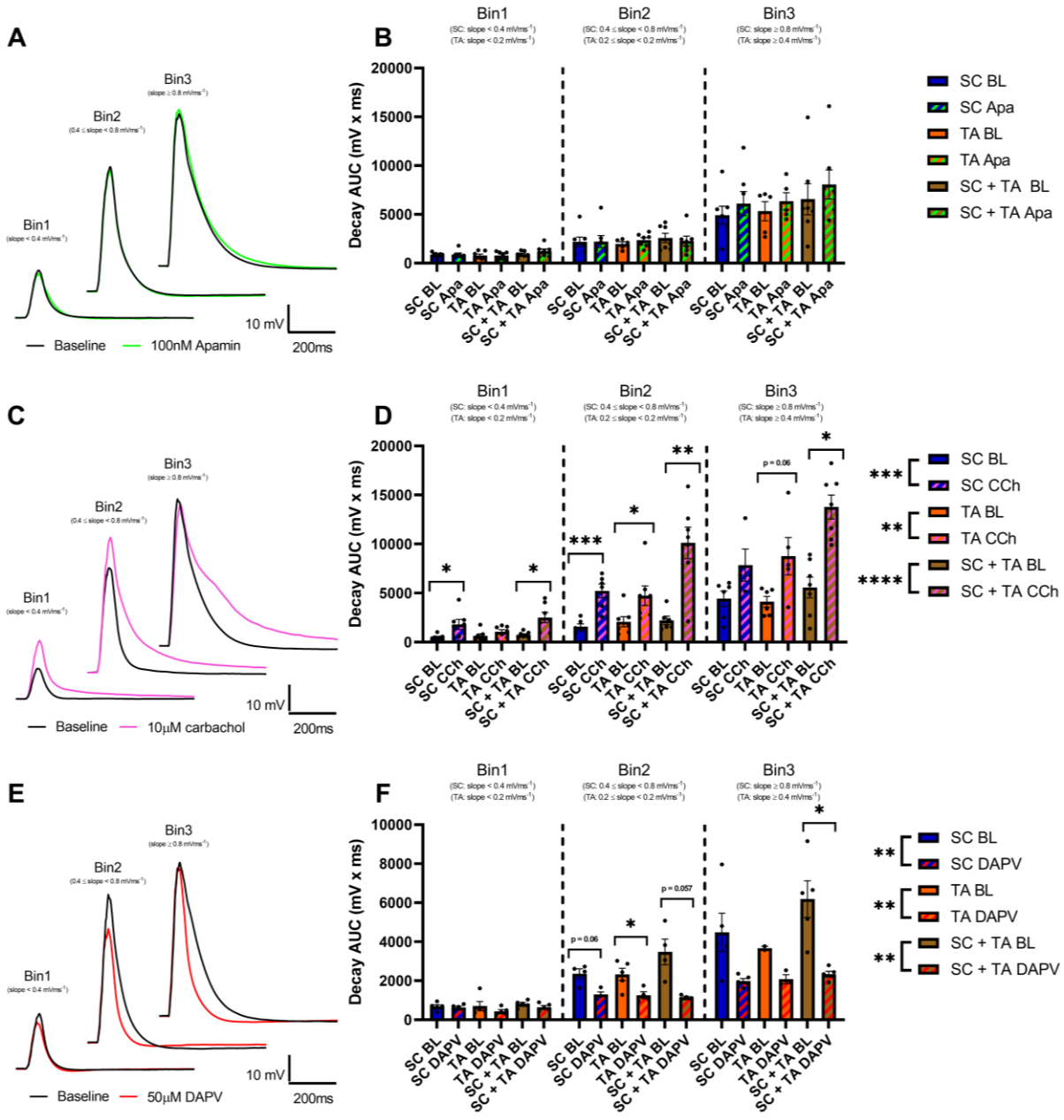


Figure 4.20 Carbachol increases plateau potential AUC while DAPV decreases plateau potentials. Compound EPSP decay AUC binned by single EPSP slope for **(B)** apamin (Apa, n = 6 cells from 3 animals), **(D)** carbachol (CCh, n = 6 cells from 5 animals) and **(F)** DAPV (n = 6 cells from 3 animals). Average traces for each bin for the SC pathway only are shown in **(A, C** and **E)** for apamin, carbachol and DAPV respectively.

4.5 Discussion

4.5.1 Model validation

Initially a battery of experiments were carried out to establish that animals displayed a phenotype consistent with that previously seen in ELS animals. MS180 animals displayed increased anxiety as evidenced by an increased latency to feed in addition to increased thigmotactic behaviour in the NSFT. This matches well both with previous data from the NSFT (Bonapersona et al., 2019; Stuart et al., 2019). The increase in proposed thigmotactic behaviour was interesting considering that a recent meta-analysis concluded that maternal separation did not lead to increased anxiety in the open field test (Wang et al., 2020). Interestingly the effects of maternal separation upon anxiety may be test specific with the same study reporting significant effects of MS upon behaviour in the elevated plus maze. In the present study there was also no difference between male and female rats in terms of anxiety which compares to reports that ELS leads to greater anxiety in male rather than female animals (Bonapersona et al., 2019). As reported previously (Shalev and Kafkafi, 2002; Stuart et al., 2019), MS180 rats in the present study did not show changes in sucrose preference indicating no changes in hedonic behaviour.

Although during the restraint stress CORT experiment a main effect of ELS was observed this was not consistent with previous studies suggesting that MS180 animals should have potentiated responses to stress but no changes at baseline (Aisa et al., 2007; Plotsky and Meaney, 1993; Stuart et al., 2019). However, as seen with humans following ELS (Bunea et al., 2017; Fogelman and Canli, 2018) there is considerable heterogeneity in the HPA axis consequences of maternal separation. Other studies have reported decreased ACTH secretion (Daniels et al., 2004; Marais et al., 2008) and decreased CORT release (Roman et al., 2006) following restraint stress in MS180 animals relative to controls. Interestingly it has been observed that maternal care and maternal separation act independently upon HPA axis outcomes with high levels of maternal care able to compensate for extended maternal separations (Macrì et al., 2008). Differences in maternal care being able to compensate for maternal separation may be the cause of much heterogeneity between studies with Long-Evans mothers reported to show an increased quality of maternal care relative to Wistar and Sprague-Dawley mothers (McIver and Jeffrey, 1967).

Lower basal CORT was observed in the second cohort of animals used for electrophysiology experiments; however the low sample size means that interpretation is difficult. CORT release is subject to tight circadian and ultradian control (Walker et al., 2010) and previous studies have reported lasting changes to circadian regulation following maternal separation in monkeys (Rawashdeh and Dubocovich, 2014; Reite et al., 1982). This may explain overall higher CORT in the restraint stress experiment but lower CORT before slice preparation.

Male MS180 rats appeared to exhibit increased cFos activation of neurones in the PVN following restraint stress in a result matching previous observations (Sanders and Anticevic, 2007). However for males to have higher restraint stress PVN cFos but not increased CORT suggests downstream changes in the HPA axis such as decreased ACTH release (Daniels et al., 2004; Marais et al., 2008) or altered CORT feedback regulation (van Oers et al., 1998). However, many of the PVN neurones release oxytocin as opposed to CRH (Nishioka et al., 1998) with it not being possible to know which population the increase in activation resulted from.

There was no difference between control and MS180 animals with respect to dentate gyrus neurogenesis utilising BrdU labelling. This contrasts with previous studies that reported robust decreases in proliferation (Mirescu et al., 2004; Stuart et al., 2019). One potential reason for differences between studies is animal age with animals in the discussed studies being between PND60 and PND70 while animals in the present study were aged PND112-123. A previous study investigating neurogenesis across the life course reported that proliferation as measured by Ki67 staining at PND120 is between 50 – 100% lower than at PND70 (Epp et al., 2009). This could mean that by PND123 neurogenesis had decreased to a degree where detecting further decreases would be challenging.

4.5.2 NMDAR function

Female MS180 animals showed a reduced AMPAR/NMDAR ratio while showing no changes in miniEPSC amplitude. Due to the fact that miniEPSCs recorded at -70mV are AMPA dependent (Kato et al., 2007) this indicates the MS180 females have increased NMDAR function. This is in contrast to Pillai et al., 2018 who reported decreased NMDA receptor function relative to AMPA. However this was carried out in male mice using the LNBM model which as previous discussed appears to have critical differences in phenotype compared to the MS180 model.

Brunson et al., 2005 also tried to measure NMDA function by assessing EPSC decay at membrane voltages between -80mV and 0mV using the LNBM model, albeit in rats. They did not observe any changes in NMDA function between ELS and control animals, however again only males were assessed. It should be noted that an alternative explanation for these results may be increased numbers of silent synapses in female MS180 animals (Kullmann, 1994). The lack of changes observed in the present study in AMPA receptor function are also in contrast to previous work which observed decreased GluR1 and GluR2 mRNA expression in rats that were maternally separated for 360 minutes per day (Pickering et al., 2006). Decreased miniEPSC frequency was also observed in MS180 animals in the current study which coupled with a lack of effects in other parameters suggests this change has a pre-synaptic origin (Choy et al., 2018). Causes for this decreased pre-synaptic function could include decreased release probability and decreased calcium entry into terminals. However, it should be noted that this change in miniEPSC frequency was not observed in the per-cell analysis suggesting that the overall effect size is small due to this analysis being less sensitive. The cumulative distribution analysis also highlighted increased event area in MS180 animals, but this was not coupled with any changes in amplitude or decay kinetics suggesting this effect may not be robust.

4.5.3 Long term potentiation

It is worth prefacing that data regarding LTP in MS180 animals is underpowered and therefore needs interpreting cautiously. There was a trend towards MS180 males showing lower LTP compared to their control counterparts. However this seemed to be mainly driven by a failure to generate LTP in the control female animals and the fact that there were only two control male cells included in the analysis. This was also potentially a driver of the observation that males showed higher LTP than females although this would match previous results from Qi et al., 2016 in the TA pathway. Interestingly it should be noted that in the present study LTP was assessed in naïve animals that had no other manipulations. Previous data from maternally deprived animals in the DG reported no differences between controls and ELS animals at baseline but greater LTP following stress exposure via CORT injection (Oomen et al., 2010). There was a general failure to induce LTP in the TA pathway in the current study which may be due to a lower baseline EPSC amplitude meaning that not enough depolarisation was generated during LTP induction to generate LTP. As previously discussed other studies have observed increased LTP following ELS (Derks et al., 2017) which matches that which would be predicted by the increased NMDA function seen in ELS females. The fact that there was no effect of hippocampal region upon LTP is interesting in itself and

matches with Kouvaros and Papatheodoropoulos, 2016 who reported no difference in LTP magnitude between regions but an increased ability for induction and stability of LTP in the DH as opposed to VH.

There was no effect of ELS observed when short term potentiation was analysed. STP is understood to be mediated by pre-synaptic and post-synaptic mechanisms as opposed to LTP which, under most conditions, is mediated by postsynaptic mechanisms through the NMDA receptor (Bliss and Collingridge, 2013; Lauri et al., 2007; Schulz and Fitzgibbons, 1997). Although LTP requires GluN2A/GluN2B containing NMDARs with ELS being suggested to reduce hippocampal GluN2B expression (Lesuis et al., 2019; Pickering et al., 2006; Roceri et al., 2002) it has been suggested that STP has two differing postsynaptic mechanisms involving GluN2A and GluN2B/GluN2D respectively (Volianskis et al., 2013). Interestingly, unlike the LTP data, there was not an effect of pathway overall but an interaction between pathway and hippocampal region with significant STP only being observed in the VH. This contradicts with the report from Papatheodoropoulos and Kostopoulos, 2000 that the VH has a lower ability to evoke STP than the DH. However it is more than likely that differences between that study and the present one stem from differences in induction protocol and the fact that the region:pathway interaction in the present study seemed to be driven by higher STP in dorsal relative to ventral control SC pathway.

There was also no effect of early life stress upon spiking or depolarisation during the theta burst LTP induction protocol. This suggests that control and MS180 cells were equally excitable in response to theta burst stimulation and that any putative differences in LTP could be due to later phase effects such as changed protein kinase C or A function in addition to a multitude of other potential factors (Derkach et al., 1999; Lüscher and Malenka, 2012; Wikström et al., 2003).

4.5.4 Basal transmission

No changes in any basal transmission parameter were observed between control and MS180 animals corroborating with conclusions from a recent systematic review (Derks et al., 2017). ELS animals showed no change in paired pulse ratio, a facilitatory effect dependent upon elevated presynaptic Ca^{2+}(Fioravante and Regehr, 2011). This suggests that the decreased miniEPSC frequency observed was not due to increased pre-synaptic intracellular calcium

but another mechanism increasing release probability. This conclusion is strengthened by observations that release probability and PPR are independent in SC-CA1 synapses (Manita et al., 2007). Interestingly the TA pathway had a higher PPR when measured using a $CsMeSO_4$ based internal solution, but the contrary was observed when measured using a $KMeSO_4$ internal. Goswamee and McQuiston, 2019 also report a higher PPR in SC while using a $KMeSO_4$ internal. In-vivo measurements using fEPSP recordings have suggested that the TA pathway has a higher PPR during high intensity stimulation, however at low intensities there is no difference between pathways (Aksoy-Aksel and Manahan-Vaughan, 2013). Other fEPSP experiments, albeit *ex-vivo* also found no difference between pathways (Speed and Dobrunz, 2009). Interestingly the fact that internal solution changed PPR indicates a postsynaptic locus for the difference between pathways. Cs blocks K^+ channels (Cameron et al., 2000) and therefore changes membrane resistance and additionally TA synapses are more distal than SC synapses. This means that it is logical for SC synapses to be relatively unaffected by changing internal solution while TA synapses suffer decreased PPR in the potassium based internal where more current leaks by the time it reaches the soma due to potassium conductances. Using the $CsMeSO_4$ internal, PPR in the current study was also depressed in VH relative to DH neurones in agreement with similar observations in mice (Milior et al., 2016). However, in contrast to the discussed study there was no difference between DH and VH neurones in input resistance and when PPR was assessed using the $KMeSO_4$ internal any differences between regions was not present.

The afterhyperpolarisation following paired pulse stimulation was no different between control and ELS animals (similarly to Brunson et al., 2005) although was markedly higher in the TA pathway and slightly depressed in ventral relative to dorsal cells. This AHP is likely caused by SK type Ca^{2+} activated K^+ channels (Robles Gómez et al., 2018; Stackman et al., 2002). It has been suggested that activation of AHPs following burst firing increases the threshold necessary for induction of LTP (Sah and Bekkers, 1996) therefore this increased AHP in the TA pathway may be one reason for a lack of LTP seen in this pathway in the present study. There also appeared to be a lower amplitude AHP in ventral as opposed to dorsal CA1 neurones. This is surprising considering reports from Babiec et al., 2017 suggesting increased SK channel activation at VH compared to DH synapses. One potential solution for this discrepancy is the contribution of other non-SK Ca^{2+} activated K^+ channels.

Increased theta oscillation power has been observed in ELS animals (Murthy et al., 2019; Sampath et al., 2014) with the fact that no differences between ELS and control animals in

impedance suggests this is not a compensatory mechanism for a lack of response to the rhythm. There was no difference in any factor in either maximal impedance or the frequency at which this occurred. Impedance can be thought as resistance in an oscillatory circuit where signals are amplified at certain resonant frequencies (Matsumura et al., 2018). Peak CA1 impedance is normally in the theta range (Fox, 1989), however it is also temperature dependent (Hu et al., 2002); a potential explanation for the low maximal impedance frequencies seen in this study.

Finally, there was no effect of ELS upon spiking characteristics except for trends toward ELS animals to have a more depolarised threshold and increase AP height. Ventral CA1 cells were consistently slower with lower depolarisation and repolarisation speeds in addition to a trend towards increased halfwidth. It should be noted that these experiments were heavily underpowered and additionally action potentials were elicited by oscillatory stimulation as opposed to the more traditional current step application. Interestingly previous studies have observed a more depolarised ap threshold in ventral as opposed to dorsal neurones but no difference in halfwidth or maximal depolarisation rate (Dougherty et al., 2012; Ordemann et al., 2019).

4.5.5 Plateau potentials

For the first time the threshold needed to generate plateau potentials was ascertained using electrical stimulation ex-vivo with carbachol reducing the stimulation needed to generate a plateau. In agreement with previous work suggesting that plateau potentials are caused by regenerative NMDAR activity (Oda et al., 2014; Shai et al., 2014; Suzuki et al., 2008; Takahashi and Magee, 2009), recorded plateau potentials were abolished upon application of the NMDAR antagonist DAPV. It is worth also noting an important role for voltage gated calcium channels too with L-type Ca^{2+} channels being found to mediate plateau potentials in the trigeminal nucleus (Lo and Erzurumlu, 2002). The threshold for plateau formation was also lower in the TA as opposed to SC pathway; this is perhaps a compensatory mechanism for the increased distance of TA synapses to the soma compared to SC with dendritic spikes being believed to be the main form of communication to the soma (Nicholson et al., 2006). However, it should be noted that the difficulty of fitting a threshold point with satisfactory quality was much higher in the TA than SC pathways. Although this approach allows for comparison between different cells a more accurate approach would be to use either focal uncaging or local application of glutamate.

Previous studies have observed a carbachol dependent plateau potential in CA1 neurones following somatic depolarisation; an effect partially dependent upon plasma membrane insertion of transient receptor potential 5 channels (Fraser and MacVicar, 1996; Tai et al., 2011). Another possible suggestion for carbachol's ability to enhance plateau potentials could be through the ability of M1 receptors to inhibit SK type K^+ channels which themselves inhibit NMDARs (Buchanan et al., 2010). However, the inability of the SK channel antagonist apamin to modify plateau potentials in this experiment suggests that this is unlikely to be the case. Interestingly Bock et al., 2019 observed that apamin decreased dendritic excitability as measured by dendritic spiking and somatic burst firing. Muscarinic receptors also modulate a range of other potassium channels such as M-channels and G protein-coupled inward-rectifier potassium channels meaning these could be a locus for carbachol's effects on plateau potentials (Brown et al., 1997; Pfaffinger et al., 1985; Seeger and Alzheimer, 2001). Finally carbachol dependent plateau potentials at high (50μM) concentrations have been found to be dependent on Ca^{2+} conductances (Blitzer et al., 1991; Kawasaki et al., 1999).

4.5.6 Summary

These data suggest that although the MS180 model did not produce fully congruent results with previous literature it was successful in generating an anxiety phenotype suggesting a believable foundation for investigating neural circuit changes. MS180 females showed increased NMDAR function but the functional consequences of this were difficult to ascertain with low sample size being an impediment in the LTP experiments. As previously reported, there were no changes in basal transmission because of MS180. These findings warrant further investigation to complete this detailed investigation into the hippocampal consequences of ELS taking into respect hippocampal aspect, sex and pathway in a way not previously done before. Finally, the pilot experiments regarding plateau potential generation successfully determined the validity of this approach. Although there were no differences in plateau potentials in MS180 animals in response to TBS, this is a crude measure at a single intensity only and further investigations are merited into the effects of ELS upon plateau potential threshold due to the evidence for potential changes in NMDA receptor function.

Chapter 5

Reward learning in individuals with a history of early life stress

Part of the work contained in this chapter has been published as a pre-print which is available both in Appendix I and at BioRxiv:

> Wilkinson MP, Mellor JR and Robinson ESJ. 2020. Investigation of reward learning and feedback sensitivity in non-clinical participants with a history of early life stress. BioRxiv 2020.11.13.380444

All the work contained in this chapter and all writing is my own. The co-authors had no role beyond the expected level of standard PhD supervision.

5.1 Introduction

Early life stress is one of the biggest known predisposing factors to the development of depression (Agid et al., 1999; Lemoult et al., 2019; McCauley et al., 1997; Sadowski et al., 1999). Reward learning deficits are believed to be crucial in the aetiology of MDD with depressed patients exhibiting deficits in probabilistic reward tasks (see section 1.2.3, Halahakoon et al., 2020; Pizzagalli et al., 2008; Taylor Tavares et al., 2008). Additionally, it has been observed that reward learning deficits emerge prior to the development of depression symptoms and are able to predict the risk of disease development (Bress et al., 2013; Vrieze et al., 2013). Two of the key tasks that have been used to probe reward processing in depression are the probabilistic reward task (PRT) and the probabilistic reversal learning task (PRLT). Depressed patients are unable to bias responding towards a more highly rewarded ambiguous cue in the PRT (Pizzagalli et al., 2008). In the PRLT MDD patients show poorer accuracy following reversal of a previously acquired probabilistic stimulus-reward association in addition to displaying increased sensitivity to misleading negative feedback (Murphy et al., 2003; Taylor Tavares et al., 2008). Although both the PRLT and PRT are used to measure reward processing they have been described to assess different constructs. A recent meta-analysis describing the PRT as measuring reward bias while suggesting the PRLT is more associated with reinforcement learning (Halahakoon et al., 2020) while also probing changes in feedback sensitivity. Due to the presence of reversals the PRLT also requires cognitive flexibility to complete the task successfully.

However, there are few studies looking into whether reward processing deficits are present in people who have experienced early life stress. Hanson et al., 2017 recruited adolescents with a history of physical abuse who then completed a probabilistic learning task where they showed lower associative learning compared to controls. Changes in reward learning have also been reported within another probabilistic reward task, the probabilistic stimulus selection task, by Pechtel and Pizzagalli, 2013. Women with a history of childhood sexual abuse (CSA) and a diagnosis of MDD showed decreased performance on trials requiring learning of previously rewarded information compared to MDD only and control groups. Although these studies provide valuable insights, they use different tasks to those previously used to study depressed populations making comparisons difficult. Additionally, studies are needed in currently healthy adults to understand if any reward processing changes are present prior to the development of mental health disorders.

In this study it was therefore hypothesised that ELS leads to reward processing deficits in an otherwise healthy adult population. Two groups of adult participants that reported no diagnosis of a mental health condition or Parkinson's disease were recruited and completed the early life stress questionnaire (ELSQ). A no ELS group was formed of participants who experienced no adverse childhood experiences (ACEs, see figure 1.4 for examples of ACEs in the ELSQ) while a high ELS group contained those with 3 or more ACEs. Participants completed the PRT and PRLT with PRLT data additionally being analysed using a Q-learning model to probe reward learning parameter changes between control and high ELS participants. Due to both evidence that stress impairs reward learning (Bogdan and Pizzagalli, 2006; Pizzagalli et al., 2007) and that people with a history of ELS are more sensitive to stress (Hammen et al., 2000; Mclaughlin et al., 2010) participants were also asked about stressors they encountered in their adult lives. This was used in exploratory analysis to investigate if life stress interacts with ELS to cause reward processing deficits. By understanding the links between ELS and reward processing deficits as a hypothesised intermediate phenotype in depression, this aims to provide insights that may lead into novel preventative strategies for people with a history of ELS.

5.2 Chapter Aims

- Assess reward learning and feedback sensitivity in healthy human adults that have had a history of early life stress using the probabilistic reward task and probabilistic reversal learning task.

- Compare the ability of the PRLT and PRT to detect changes in reward processing in an early life stress population

- Explore if stress in adulthood modulates the relationship between early life stress and reward processing.

This work was completed in 2020.

5.3 Methods

All procedures detailed were approved by the Faculty of Life Sciences and Faculty of Science Research Ethics Committee at the University of Bristol and the study protocol was pre-registered (www.osf.io/538yk). All participants provided full written consent for both the collection, analysis and publication of their data.

5.3.1 Participants

586 participants were recruited using the Prolific (www.prolific.co) online platform to complete a short online screening questionnaire (see Figure 5.1 for full study overview). These participants were 25 – 65 years of age, fluent in English, resident in the UK and had no mild cognitive impairments or dementia. Participants were then screened utilising the early life stress questionnaire (Cohen et al., 2006) while also being asked to self-report if they had a diagnosis of a mental health condition or Parkinson's disease. For completing the screening questionnaire participants were reimbursed at a rate of £6.00 per hour.

Participants who did not report a diagnosis of a mental health disorder or Parkinson's were then invited to take part in a second phase of the experiment online within a week of screening and were allocated into two groups. A no ELS group (n = 65) contained people scoring 0 on the ELSQ while a high ELS group (n = 64) consisted of those who scored ≥3. This was based upon data from Cohen et al., 2006 which suggested this would encompass the top tercile of the population. In this second phase of the experiment participants entered basic demographic information before completing the MacArthur Scale of Subjective Social Status (Adler et al., 2000), Beck's depression inventory II (BDI-II, Beck et al., 1996), the Snaith Hamilton pleasure scale (SHAPS, Snaith et al., 1995) and the Holmes and Rahe stress scale (Holmes and Rahe, 1967). The SHAPS was additionally scored using the SHAPS-C criteria to provide enhanced resolution (Ameli et al., 2014) while for the stress scale participants were asked if each event occurred in either their adult life or the last year to provide estimates of both lifetime stress and stress in the last year. Participants then completed the PRLT followed by the PLT. Participants were compensated at £6.00 per hour with them being able to earn an additional £2.00 for high performance on the behavioural tasks. For all stages of the experiment participants were instructed to use a desktop or laptop only and that they should be in a quiet place with minimal distractions. Sample size was estimated for a medium effect size (Cohen's d = 0.5) and 80% power for a t-test at 64 participants per group.

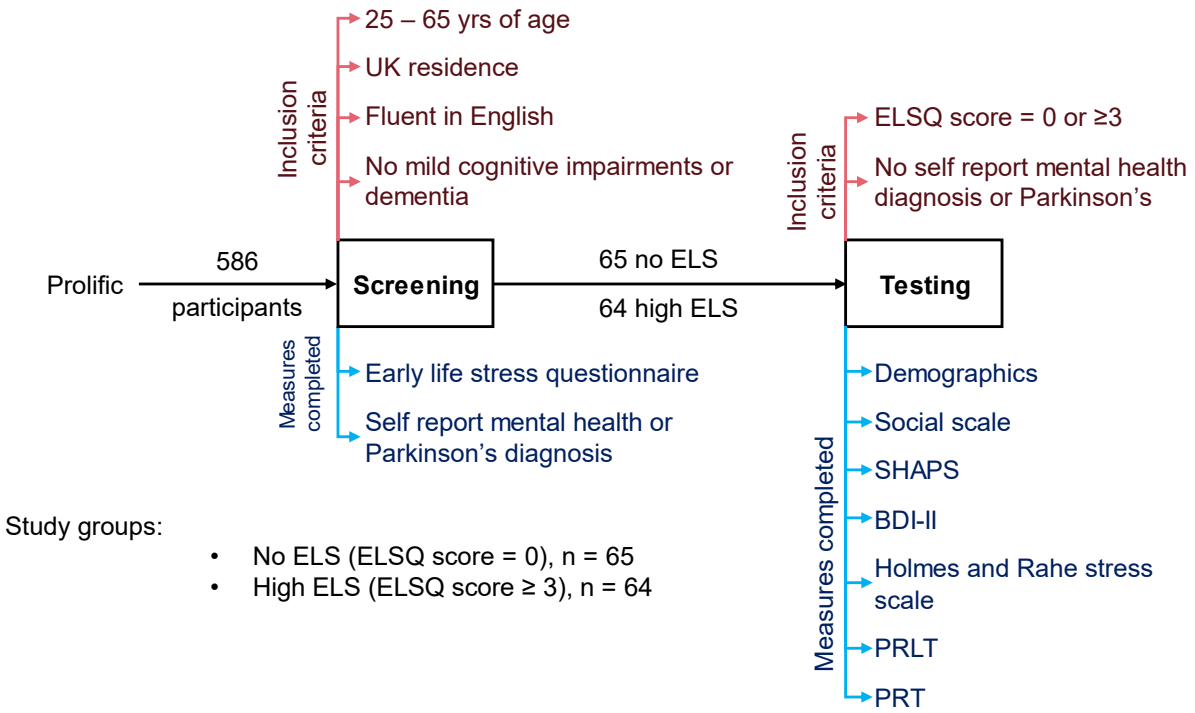


Figure 5.1 Study overview. Participants were screened by ELSQ score and then formed into two study groups: no ELS and high ELS.

5.3.2 Behavioural testing

Participants completed both the Probabilistic reward task (PRT, Pizzagalli et al., 2005) and the Probabilistic reversal learning task (PRLT, Cools et al., 2002; Waegeman et al., 2014) as measures of reward processing ability. To complete the tasks participants were required to download and install the Millisecond Inquisit web player (Millisecond, US) which ran both tasks using Millisecond Inquisit v6.2.1.

5.3.2.1 Probabilistic Reward Task

The PRT was conducted as previously described (see Figure 5.2, Pizzagalli et al., 2005, 2008; Vrieze et al., 2013) using the task from the Millisecond test library (Millisecond, 2020b). Participants were instructed to identify whether the mouth of a presented cartoon face was long or short to win points. Each trial consisted of a 500ms presentation of a fixation point followed by the presentation of the mouthless face for 500ms. The mouth was then rapidly presented for 100ms before participants had up to 1750ms to respond with their keyboard to select the mouth length they identified. Feedback was not provided on all trials but unknown to participants one mouth was rewarded with points three times more often than

the other (rich = 60%, lean = 20%). Participants completed three blocks of 100 trials lasting around 15 minutes in total. Response key and rich/lean stimuli assignments were counterbalanced across participants. Responses that were quicker than 150ms or slower than 1750ms were excluded from analysis. Additional responses that differed by more than 3 standard deviations from the mean following natural log transformation of latencies for each participant were excluded from analysis.

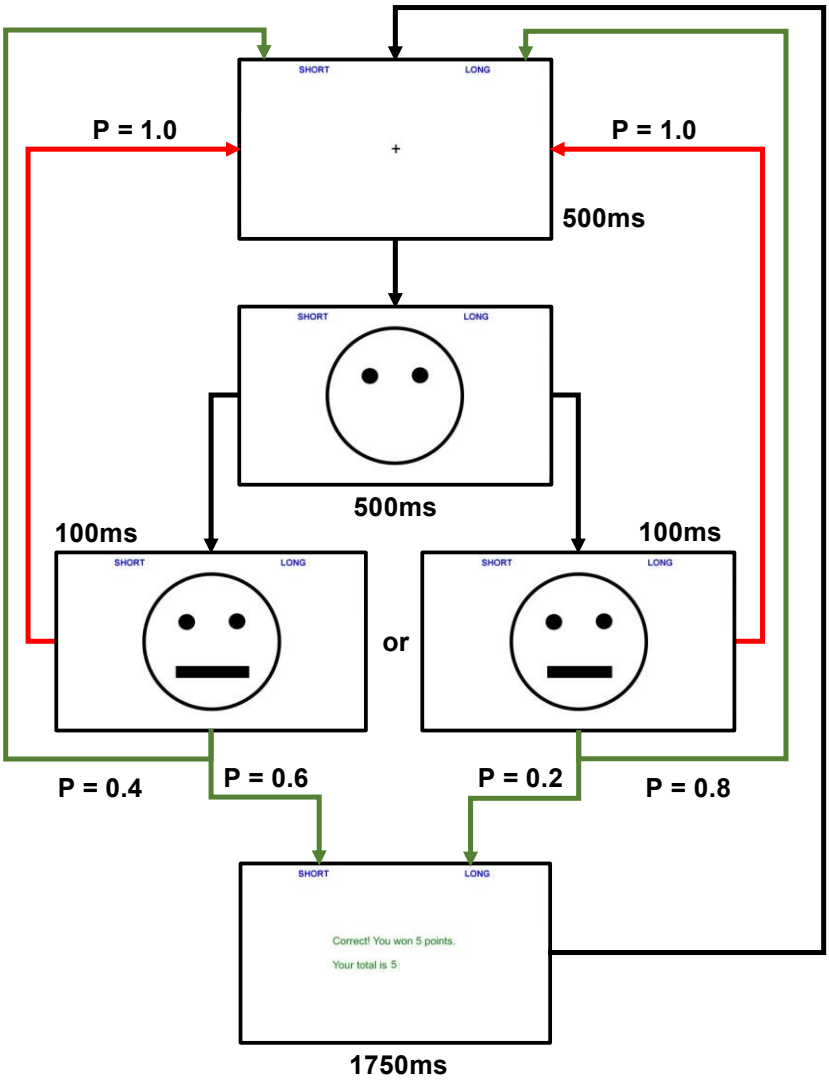


Figure 5.2 Overview of the probabilistic reward task. Participants had to identify whether the presented mouth was long or short with one mouth length rewarded three times more often than the other. In this example the long face forms the rich stimulus while the short face is the lean stimulus. Red arrows indicate routing because of incorrect identification of mouth length while green arrows indicate consequences of correctly identifying the face length. The probabilities associated with each route are shown next to the respective arrow while black arrows show automatic actions.

The unequal reward continencies between stimuli should lead participants to develop a response bias towards the more highly rewarded stimulus. This response bias is taken as a measure of reward learning and is described by logB:

$$log\ B\ = \frac{1}{2} log\left(\frac{Rich_{correct} \times Lean_{incorrect}}{Rich_{incorrect} \times Lean_{correct}}\right) \quad \text{Eq 5.1}$$

The ability of participants to discriminate correctly between the long and short mouths, a measure of task difficulty, was captured by the logD parameter:

$$log\ D\ = \frac{1}{2} log\left(\frac{Rich_{correct} \times Lean_{correct}}{Rich_{incorrect} \times Lean_{incorrect}}\right) \quad \text{Eq 5.2}$$

5.3.2.2 Probabilistic Reversal Learning task

The PRLT was conducted as previously described (see Figure 5.3, Cools et al., 2002; Waegeman et al., 2014) using the task from the Millisecond test library (Millisecond, 2020a). Participants were instructed to choose between a "lucky" and "unlucky" pattern to maximise points. These stimuli therefore formed rich and lean stimuli. Selection of the rich stimulus caused the participants to gain a point 80% of the time and lose a point 20% of the time with the lean stimulus having the opposite contingencies. If no stimulus was chosen within 2s then this was classed as incorrect and participants lost a point. After correct learning of the rich stimuli the contingencies reverse such that the rich stimuli becomes lean and vice versa. The reversal criteria was set randomly between 10 to 15 consecutive correct rich choices to stop participants counting to the criteria. Participants first completed a practise phase where they had to achieve the criterion for a single reversal before proceeding to the main task which was completed in three blocks each limited to 9 minutes. If participants did not pass the practice phase then their data were excluded from analysis. Output measures were calculated as previously described (see section 3.3.5) with data again being analysed using a Q-learning reinforcement learning model (see section 3.3.6). Additionally, data per phase (practice, acquisition of the first rule in block 1 and the following two reversals) was analysed consisting of participant accuracy, errors to criterion and win-stay / lose-shift probability.

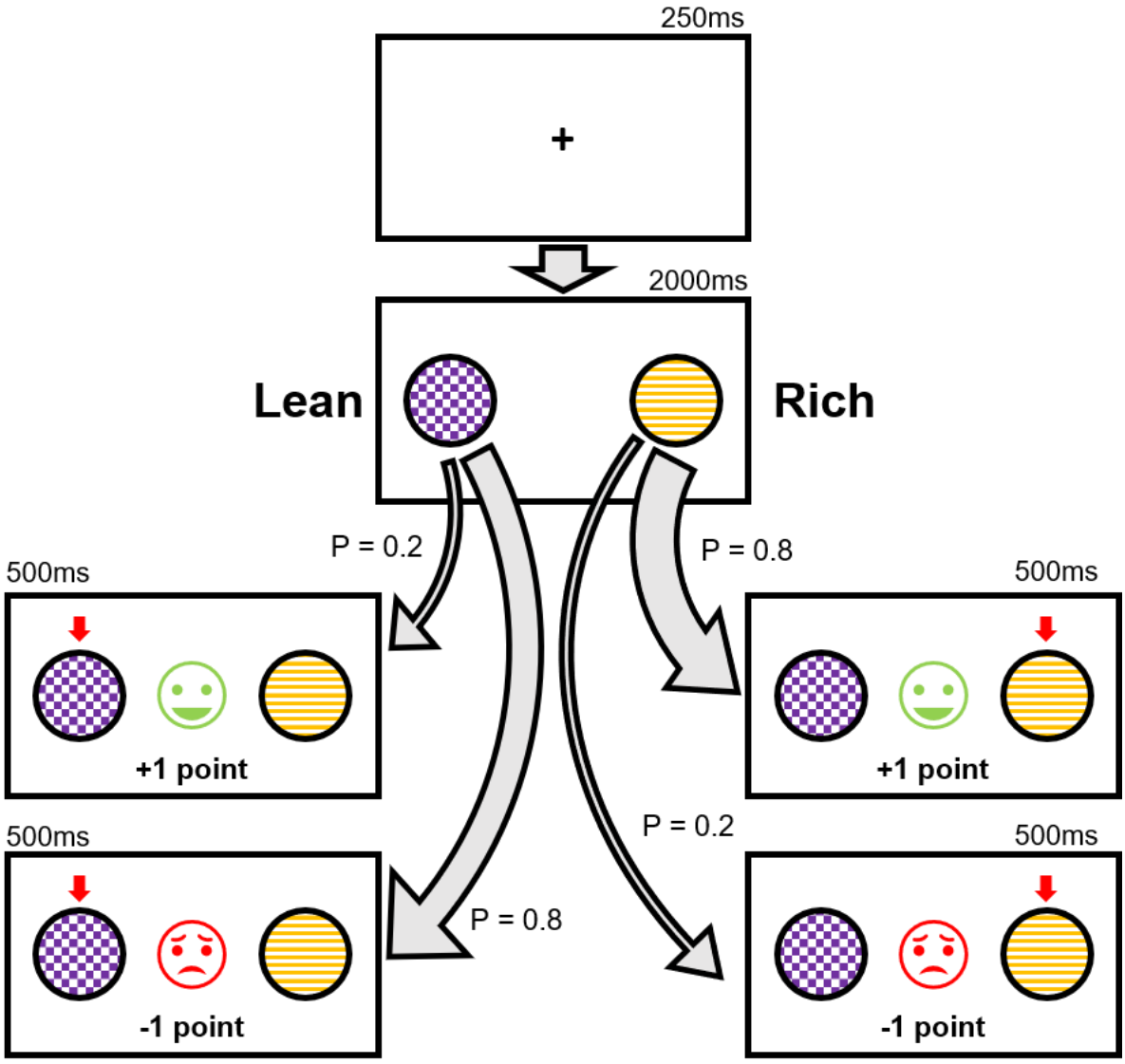


Figure 5.3 Probabilistic reversal learning task overview. Participants had to correctly choose stimuli associated with higher levels of reward. The relative probability of each outcome is shown as the thickness of each arrow and red arrows above a stimulus indicate a participant choice for this stimulus. In this example the purple checked pattern is the lean stimulus while the yellow striped pattern is the rich stimulus. Following meeting of the criteria contingencies switch so that the rich stimuli becomes lean and vice versa. If no stimulus was selected within 2000ms this was classified as an omission and participants lost one point.

5.3.3 Data Analysis

Demographic and self-report measures were compared between groups using either X^2, t-tests or Mann-Whitney U tests where appropriate. The primary analysis for each measure was a direct comparison between no and high ELS groups using either ANOVA where measures were by block or a t-test for overall measures. Where data were not normally distributed (assessed visually and using Shapiro-Wilk tests) then efforts were first made to transform data to normality and where this was not possible Mann-Whitney U tests were completed. Win-stay by block data were transformed using the bestNormalize package in R (Peterson and Cavanaugh, 2019). Where measures were split by a within subject factor such as block or feedback type these were analysed using repeated measures ANOVAs using either block or feedback type as the within subject factor. Where Mauchly's test identified a violation of the Sphericity assumption then this was rectified using the Huynh-Feldt correction.

Due to differences in social status, BDI-II score and SHAPS score between the no and high ELS groups, principal component analysis (PCA) was conducted to account for this as an analysis stage (see table 5.1 and table 5.2). Because only principal component 1 (PC1) differed between groups then this was used in ANCOVAs (analysis of covariance) to analyse whether parameter changes were due to ELS or due to changes in depression symptomology accounted for in the PC1 component.

Component	Explained variance (%)	No ELS	High ELS	Test statistic	P value
1	94.6	4.32 ± 0.24	5.65 ± 0.25	t_{127} = -3.86	**0.0002**
2	3.4	-0.19 ± 0.21	0.20 ± 0.24	t_{127} = -1.22	0.226
3	2.0	0.21 ± 0.15	-0.22 ± 0.18	t_{127} = 1.79	0.076

Table 5.1 Principal component analysis of social scale, SHAPS and BDI-II scores. The mean ± standard error is shown for each group with the relevant statistical comparison. Significant p values are shown in bold.

	Principal component		
	1	2	3
Social scale	-0.07	-0.40	0.91
BDI-II	0.98	-0.18	-0.003
SHAPS	0.17	0.90	0.40

Table 5.2 Principal component analysis component loadings.

In order to understand if stress and gender interacted with ELS to modify reward learning exploratory analysis was also undertaken using generalised linear mixed models (GLMMs) containing the factors: gender, ELS, lifetime stress, last year stress and age. Model generation and refinement were as previously described (see section 4.4.6) with an additional step of checking for the effects of PC1 following selection of the best model.

Statistical analysis was conducted in SPSS v26 (IBM, US), MATLAB 2018a (MathWorks, USA) and R 4.0 (R Core Team, 2020) with output graphics constructed in GraphPad Prism 8 (GraphPad, US). All data is shown as mean ± SE with a bar and stars showing a main effect of ELS in the primary analysis. * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

5.4 Results

5.4.1 Early life stress in the screening population

Early life stress was highly prevalent in the study population (Figure 5.4A) with only 21.0% of participants having no adverse childhood experiences (ACEs) and 44.4% of the population suffering three or more ACEs in their childhood. 16.0% of respondents self-reported a diagnosis of a mental health disorder or Parkinson's (Figure 5.4B) with this being associated with a higher ELSQ score (Figure 5.4C, Mann-Whitney, U = 15725, p < 0.0001).

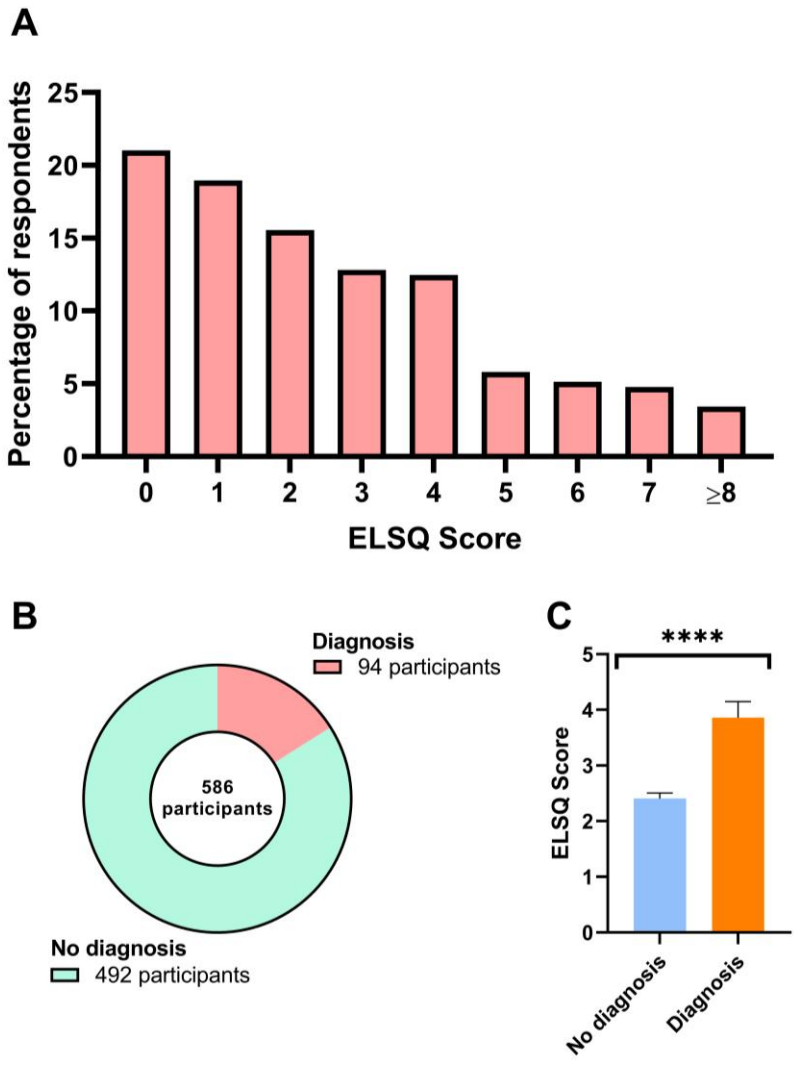


Figure 5.4 Early life stress in an online study population. (A) ELSQ scores in the study population. **(B)** The number of participants who self-reported a diagnosis of a mental health disorder or Parkinson's disease. **(C)** Mental health disorder or Parkinson's self-report diagnosis by ELSQ score. N = 586 participants.

5.4.2 Demographic and self-report measures

The two study groups were well matched with respect to gender, age, education, ethnicity, relationship status, employment status and the presence of monetary worries (see table 5.3). However, high ELS participants had a self-reported lower social status coupled with higher depression scores in the BDI-II and elevated anhedonia scores in the SHAPS questionnaires. There was no difference between groups when participants were asked about stress they encountered in both the last year and their adult lives. When the BDI-II scores were classified into either minimal, mild, moderate or severe depression (Figure 5.5A, Beck et al., 1996) participants from the high ELS group were more likely to be in greater severity depression groupings (chi^2, $X^2(3) = 12.9$, $p = 0.005$). Similarly when SHAPS scores were classified into either normal (≤2) or abnormal (≥3) hedonic responses (Snaith et al., 1995) members of the high ELS group were more likely to have abnormal scores (Figure 5.5B, chi^2, $X^2(1) = 6.3$, $p = 0.012$).

Measure	No ELS (n = 65)	High ELS (n = 64)	Test statistic	p
Gender (% male)	44.6	37.5	$x^2(2) = 2.5$	0.28
Age (years)	37.3 ± 1.30	38.0 ± 1.24	U = 1936.0	0.50
Education (% graduates)	64.6	65.6	$x^2(5) = 4.9$	0.43
Ethnicity (% white)	95.4	82.8	$x^2(4) = 8.7$	0.070
Relationship status (% single)	18.5	28.1	$x^2(3) = 1.9$	0.60
Employment status (% full time)	64.6	60.9	$x^2(5) = 3.5$	0.61
Monetary concerns (% agree / strongly agree)	36.9	56.3	$x^2(3) = 4.4$	0.22
ELSQ	0 ± 0	4.36 ± 0.17	-	-
Social status	6.2 ± 0.17	5.2 ± 0.21	U = 1397.5	**0.001**
BDI-II	9.4 ± 1.0	15.2 ± 1.22	U = 1315.5	**0.0003**
SHAPS	1.4 ± 0.25	2.56 ± 0.32	U = 1496.5	**0.004**
SHAPS-C	24.3 ± 0.67	26.4 ± 0.86	$t_{119.4} = -1.92$	0.057
Lifetime stress	472.8 ± 22.4	529.2 ± 23.9	$t_{127} = -1.72$	0.088
Last year stress	111.4 ± 12.3	139.8 ± 17.0	U = 1939.5	0.51

Table 5.3. Demographic and self-report measures in the study population. Values are shown for each group as mean ± standard error with significant p values indicated in bold.

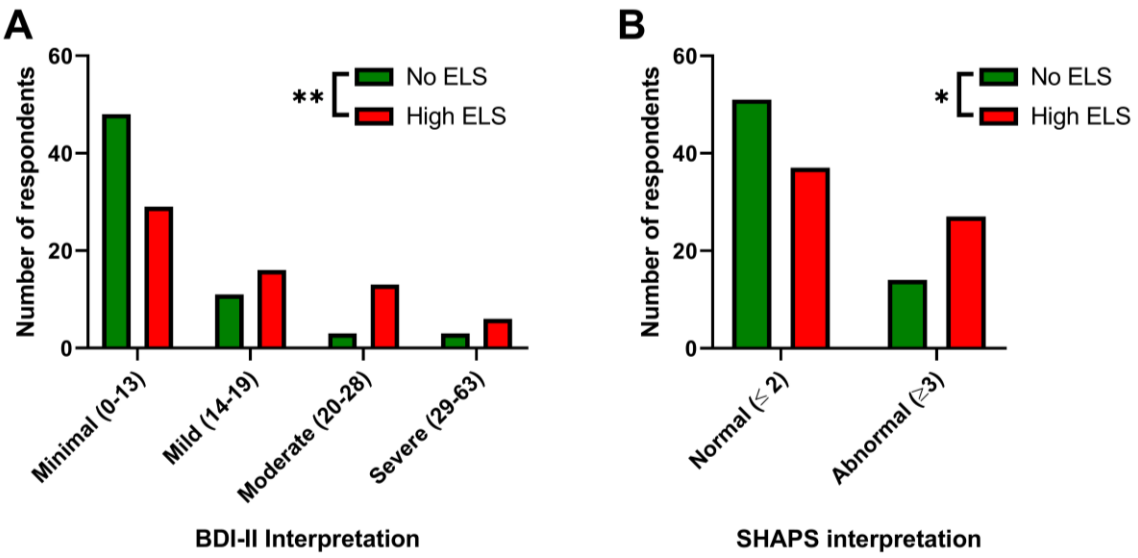


Figure 5.5 Interpretation of BDI-II and SHAPS scores in the no and high ELS populations. Scores were interpreted following Beck et al., 1996 and Snaith et al., 1995. **(A)** BDI-II split by severity of depression and **(B)** SHAPS split by normal or abnormal hedonic responses. N = 129 participants (65 no ELS, 64 high ELS)

5.4.3 Probabilistic reward task

Neither group of participants showed a response bias towards the more highly rewarded stimulus in any block (Figure 5.6A) neither was there evidence for a response bias developing between blocks (Figure 5.6B). However, participants with a history of high ELS did show an impaired ability to discriminate between stimuli (Figure 5.6C, ANOVA, $F_{1,127} = 4.8$, $p = 0.030$). Secondary analysis revealed that this difference between groups appeared to be driven by differences in depression symptomology with the effect of ELS disappearing when PCA component 1 was included in the analysis (ANCOVA, PCA1: $F_{1,126} = 6.08$, $p = 0.015$; ELS: $F_{1,126} = 1.7$, $p = 0.19$). Exploratory analysis further revealed main effects of lifetime stress with higher lifetime stress corresponding to increased discrimination ability (GLMM, $Z = 2.6$, $p = 0.007$). An effect of gender was also revealed (GLMM, $Z = 2.04$, $p = 0.04$) with males showing increased discrimination ability. There was no difference between groups in response latencies (Figure 5.6D) but when total points gained during the task were analysed a group difference emerged (Figure 5.6E, Mann-Whitney U, $U = 1692$, $p = 0.045$). However, again secondary analysis revealed that this was driven by PCA component 1 (ANCOVA, PCA1: $F_{1,126} = 5.6$, $p = 0.019$, ELS: $F_{1,126} = 0.9$, $p = 0.35$).

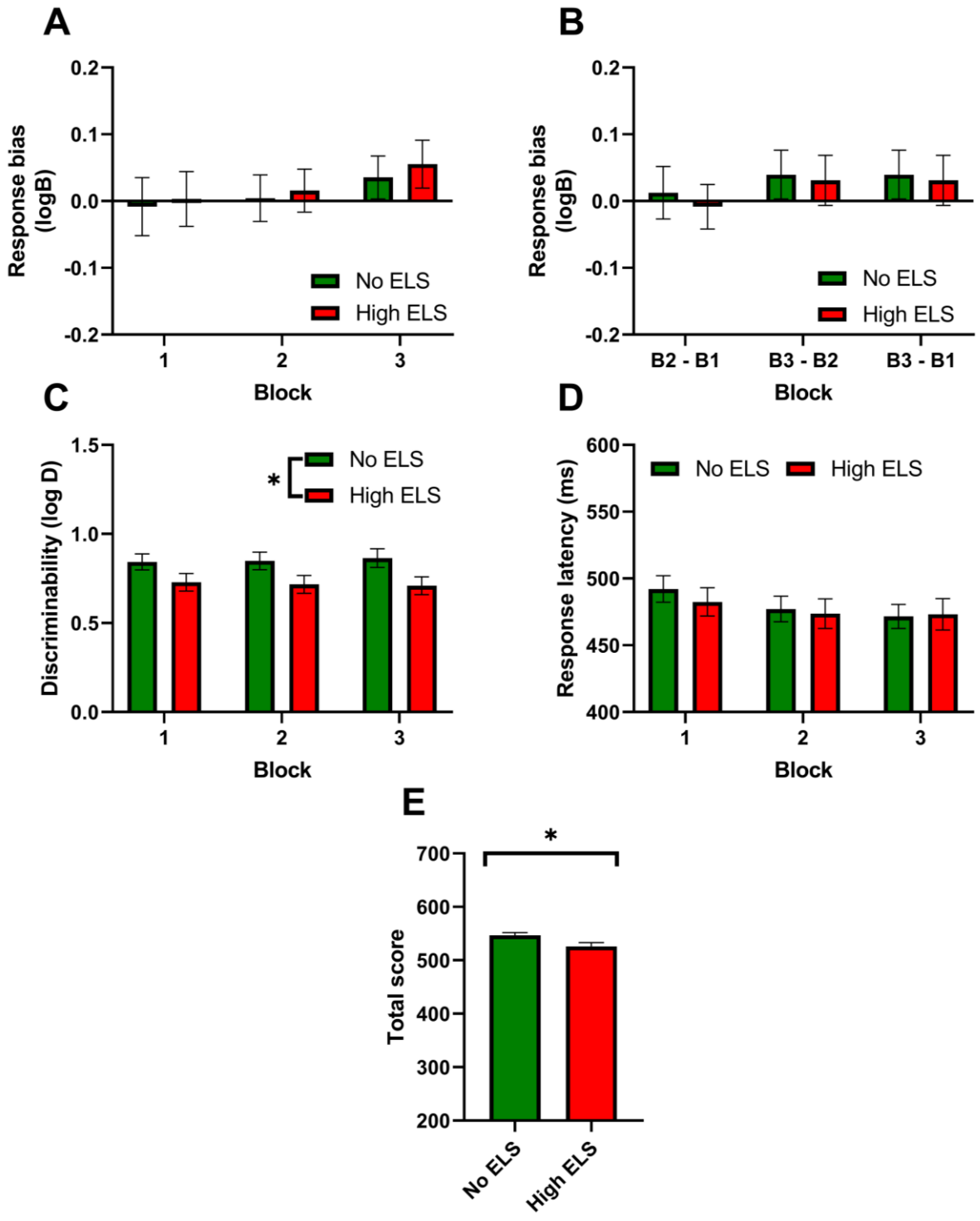


Figure 5.6 Participants with a history of ELS show decreased discriminability in the probabilistic reward task. **(A)** Response bias to the more highly rewarded stimulus, **(B)** response bias development between blocks, **(C)** discriminability between long and short face lengths, **(D)** average response latency and **(E)** total points gained by participants in the task. N = 129 participants (65 no ELS, 64 high ELS)

Current trial	Previous trial	No ELS	High ELS	Test statistic	p
Lean	Rich - rewarded	16.8 ± 2.1	18.4 ± 2.2	U = 1966	0.59
Lean	Rich - not rewarded	16.1 ± 1.8	18.6 ± 1.8	U = 1744	0.11
Lean	Lean - rewarded	19.3 ± 2.1	20.5 ± 2.0	U = 1928	0.47
Lean	Lean - not rewarded	16.8 ± 1.6	21.2 ± 1.8	U = 1928	0.097
Rich	Rich - rewarded	13.1 ± 1.5	18.3 ± 2.1	U = 1697.5	0.071
Rich	Rich - not rewarded	14.2 ± 1.6	20.0 ± 2.1	U = 1597.5	**0.023**
Rich	Lean - rewarded	13.1 ± 1.4	15.5 ± 1.7	U =1814	0.330
Rich	Lean - not rewarded	14.2 ± 1.4	19.6 ± 1.9	U = 1644.5	**0.040**

Table 5.4 Miss-rates, the chance of mis-categorising a stimulus, by previous trial. Data is shown as mean ± standard error and significant p-values are shown in bold.

In line with Pizzagalli et al., 2008 the probability of misclassifying a stimulus based upon the preceding trial outcome was analysed (Table 5.4). Participants with a history of high levels of ELS were more likely to misclassify rich stimuli if either the previous trial was a not rewarded rich trial or a lean not rewarded trial.

5.4.4 Probabilistic reversal learning task

There was no difference between groups in the number of rule changes participants were able to complete within the time allowed (Figure 5.7A). There was additionally no difference in accuracy between groups (Figure 5.7B), however participants with a history of high ELS did have a slower average response latency (Figure 5.7C, RM-ANOVA, $F_{1,126} = 5.03$, $p = 0.027$) with both groups getting equally faster over the course of the three blocks (RM-ANOVA, $F_{1.88,236.7} = 16.1$, $p < 0.0001$). Secondary analysis revealed little effect of depression symptomology (RM-ANCOVA, PCA1: $p > 0.05$) with the main effect of ELS persisting (RM-

ANCOVA, ELS: $F_{1,125} = 4.9$, $p = 0.028$). Exploratory analysis on overall reaction times did not replicate a main effect of group but did find an overall effect of age where older participants had slower reaction times (GLMM, $Z = 2.8$, $p = 0.005$). This analysis also indicated a trend towards an interaction between group and lifetime stress (GLMM, $Z = 1.55$, $p = 0.065$) but further investigation did not reveal an effect of lifetime stress in either group. Likewise with rule changes, there were no differences between groups in either reversal errors (perseverative errors following reversal) or the total score achieved by participants in the task (Figure 5.7D and E respectively).

Participants with a history of high ELS exhibited reduced positive feedback sensitivity (Figure 5.8A, RM-ANOVA, $F_{1,122} = 10.4$, $p = 0.002$) which persisted once PCA component 1 was included in the analysis (RM-ANOVA, $F_{1,121} = 6.6$, $p = 0.01$). Exploratory analysis revealed an interaction between ELS and both lifetime stress (GLMM, $Z = -2.15$, $p = 0.031$) and last year stress (GLMM, $Z = -1.99$, $p = 0.047$). Further investigation revealed effects of both stress types in the low ELS group only (GLMM, lifetime stress: $Z = -2.35$, $p = 0.019$, last year stress: $Z = -2.2$, $p = 0.026$) whereby higher lifetime stress led to greater positive feedback sensitivity but higher stress in the last year was associated with decreased win-stay probability. However it should be noted that although all suggested terms were removed from the model the overall model was a poorer fit than the null when measured by AIC (Δ AIC = 7.3, $X^2(13) = 18.7$, $p = 0.13$).

The effect of ELS upon positive feedback sensitivity was consistent across feedback that either matched (true feedback) or clashed (misleading feedback) with the underlying task rules (Figure 5.8B, Mann-Whitney U, true: $U = 1443$, $p = 0.03$; misleading: $U = 1337$, $p = 0.005$). This effect appeared to be constrained to positive feedback sensitivity with no corresponding changes in lose-shift probability between no and high ELS groups (Figures 5.8C and D).

When initial learning in the PRLT task was assessed it was apparent that although ELS and control participants performed similarly during the practice phase there was a learning deficit during acquisition of the first reversal criterion in block 1 as evidenced by increased errors to criterion (Figure 5.9A, Mann-Whitney U, $U = 1580$, $p = 0.045$) and decreased accuracy (Figure 5.9B, Mann-Whitney U, $U = 1584$, $p = 0.036$).

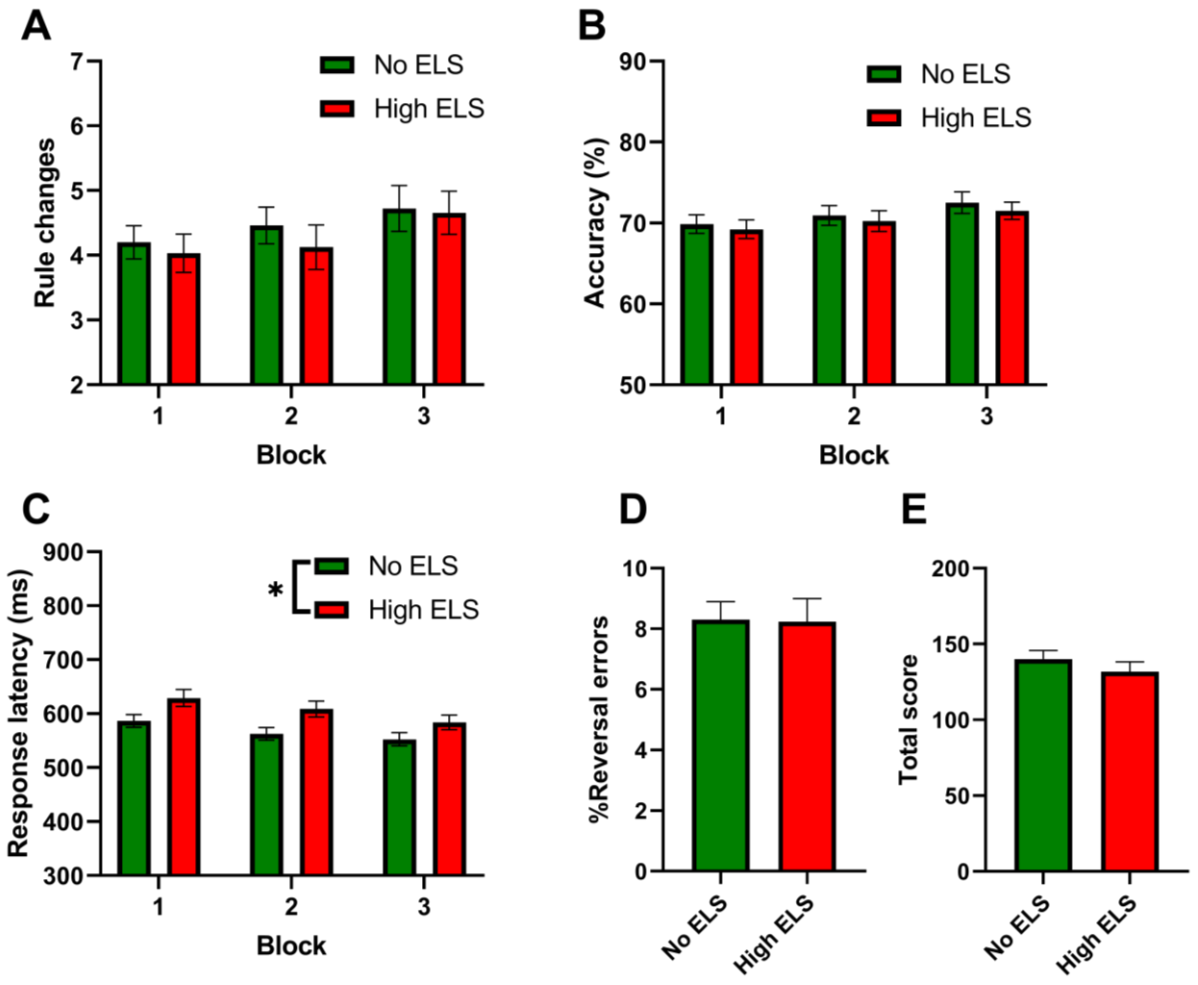


Figure 5.7 Overall reward learning measures in the PRLT are not different between groups. (A) Rule changes, **(B)** accuracy, **(C)** average response latency, **(D)** reversal errors as a percentage of total trials, these as perseverative errors following a rule change. **(E)** Total score. N = 129 participants (65 no ELS, 64 high ELS)

Both groups of participants however performed equally well at achieving criterion for a second and third reversal. Unlike the overall measures there was no difference in win-stay probability between groups (Figure 5.9C), however there was a trend for high ELS participants to show increased negative feedback sensitivity in the practice phase (Figure 5.9D, Mann-Whitney U, U = 1532, p = 0.052).

Data were additionally analysed utilising a Q-learning reinforcement learning model which suggested that high ELS participants trended towards having a lower learning rate compared

to the no ELS study population (Figure 5.10A, t-test, t_{127} = 1.78, p = 0.077). Secondary analysis revealed no effect of PCA component 1 upon learning rate but abolished any effect of ELS. In exploratory analysis a main effect of ELS was observed (GLMM, Z = 2.1, p = 0.037) with the addition of PC1 impairing model fit (ΔAIC = 1.69, $X^2(1)$ = 0.31, p = 0.57). Additionally, a relationship between stress in the last year and learning rate was observed whereby increased stress in the last year decreased learning rate (GLMM, Z = -2.3, p = 0.024). There was no difference in choice variability (Figure 5.10B) or accuracy compared to a model predicted perfect strategy (Figure 5.10C) between groups.

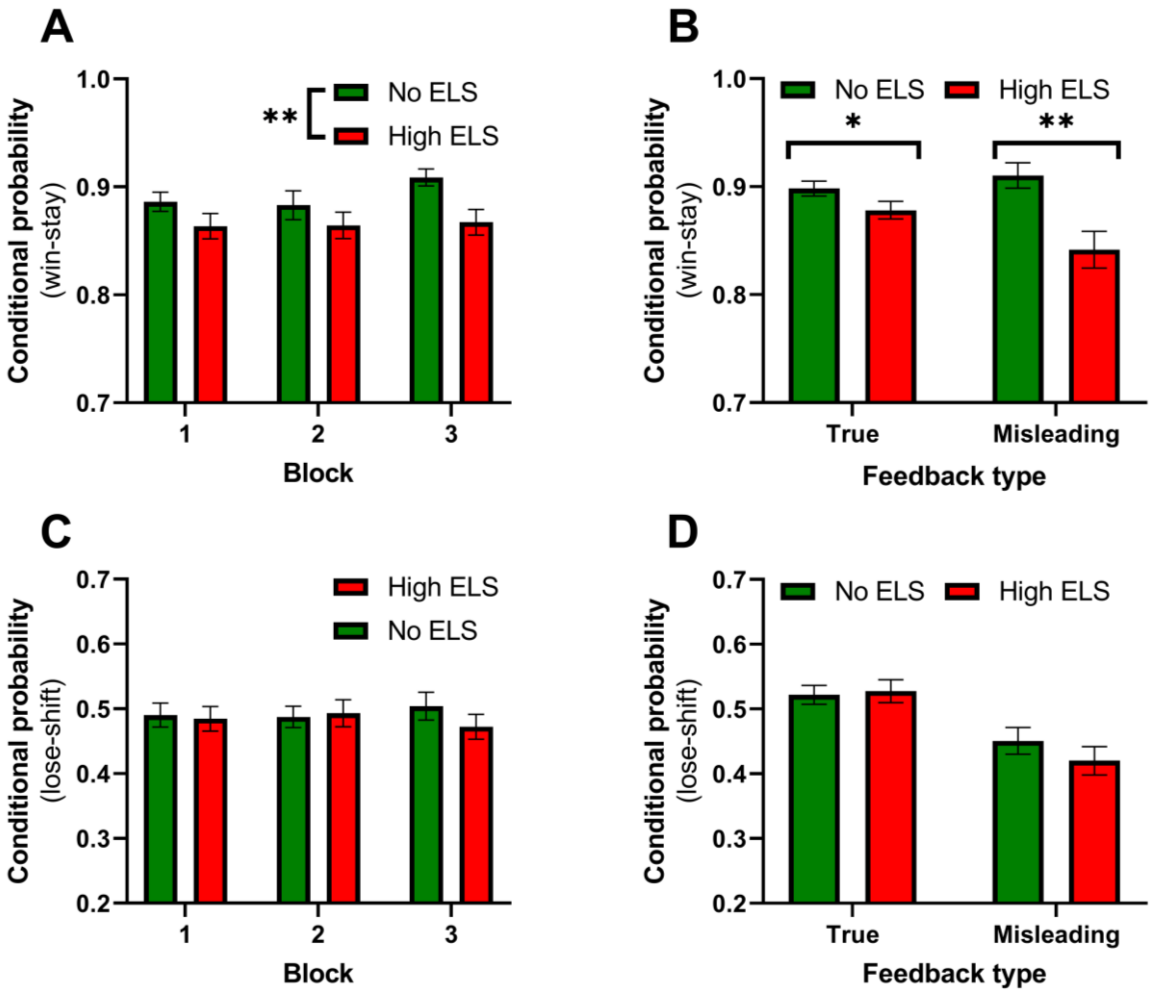


Figure 5.8 High ELS participants exhibited lower positive feedback sensitivity than those without a history of ELS. Win-stay probability by block **(A)** and **(B)** subdivided into true and misleading feedback. Lose-shift probability by block **(C)** and **(D)** subdivided into true and misleading feedback. N = 129 participants (65 no ELS, 64 high ELS)

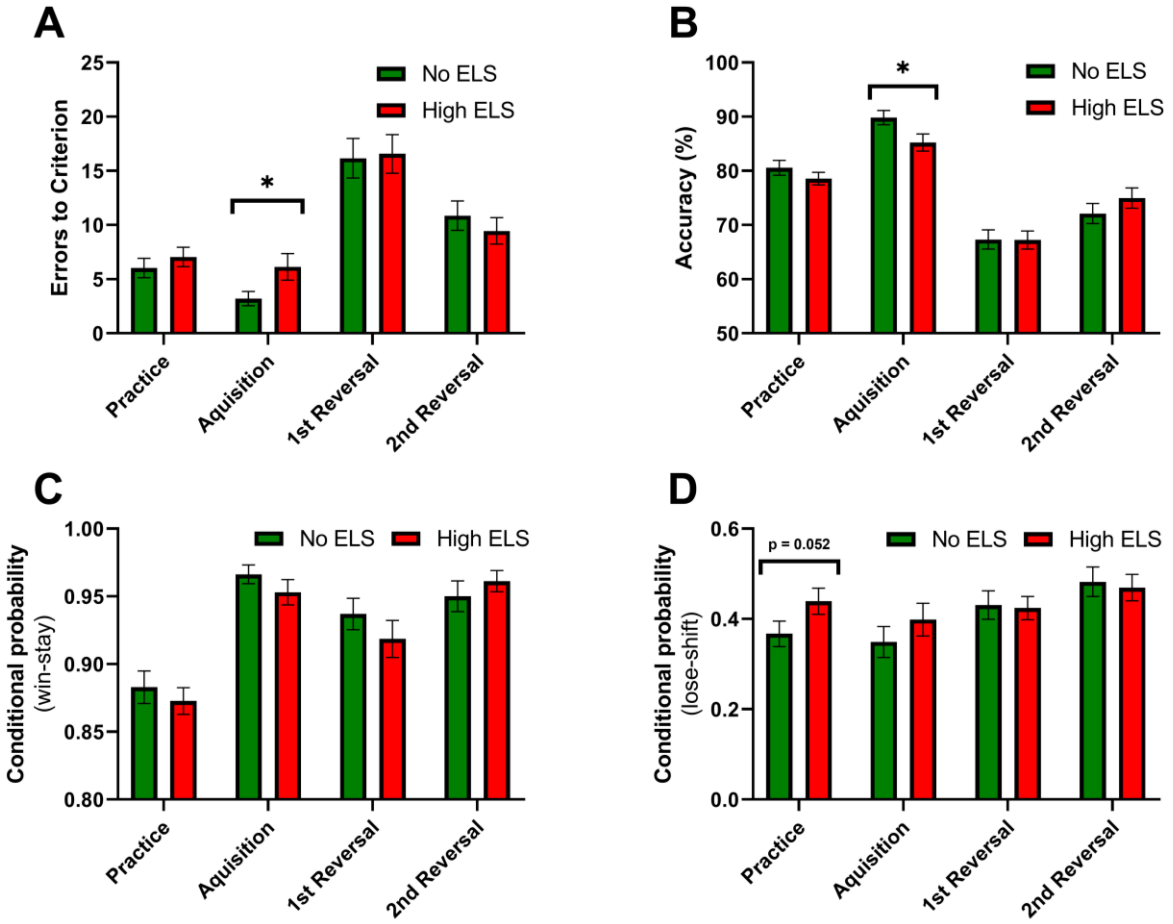


Figure 5.9 High ELS participants show impaired learning in the acquisition phase of block 1. (A) Errors made while reaching criterion for each phase, **(B)** accuracy within each phase, **(C** and **D)** win-stay and lose-shift probabilities for each phase of block 1 and practice respectively. N = 129 participants (65 no ELS, 64 high ELS)

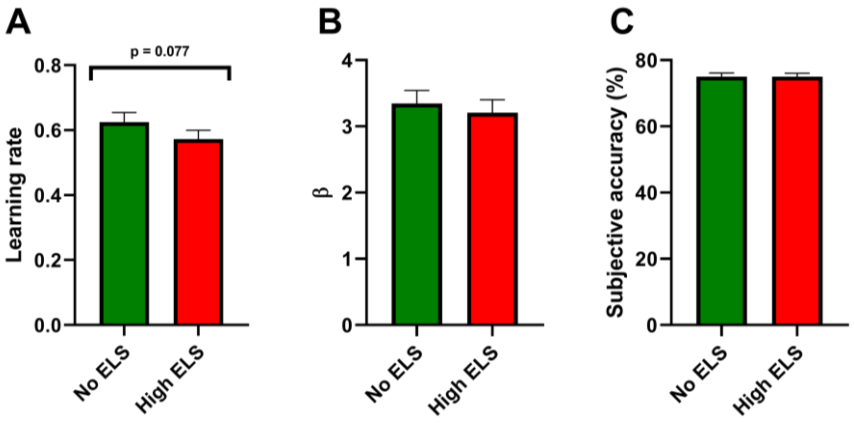


Figure 5.10 Q-learning analysis revealed that high ELS participants trended towards having a lower learning rate during the PRLT. (A) Learning rate, **(B)** choice variability as measured by the ß parameter and **(C)** accuracy compared to a model predicted perfect strategy. N = 129 participants (65 no ELS, 64 high ELS)

5.5 Discussion

This study was designed to test the hypothesis that healthy adult participants with a history of high early life stress have reward learning deficits with these deficits hypothesised to pre-dispose to the development of depression. Nearly 600 participants were screened for ELS and asked to self-report if they had a diagnosis of a mental health condition or Parkinson's disease. In agreement with Cohen et al., 2006 ELS was highly prevalent in the population with 79.0% of participants experiencing one or more ACE and 44.4% experiencing three or more. Congruent with multiple previous studies reporting associations between ELS and MDD (Agid et al., 1999; Green et al., 2010; Lahti et al., 2016), people with a higher ELSQ score were more likely to self-report a diagnosis of a mental health disorder or Parkinson's. Because the incidence of Parkinson's disease is extremely low in participants between 25-65 years of age (incidence rate = 0.004-0.03%, Parkinson's UK, 2017) it is likely that these self-report diagnoses were almost entirely mental health disorders.

Participants who did not self-report a mental health or Parkinson's diagnosis and who experienced either zero or 3 or more ACEs were invited to take part in the full study. However, participants with a history of high ELS had higher self-report depression and anhedonia symptoms as measured by the BDI-II and SHAPS questionnaires in addition to having lower reported social status. Indeed 54.7% of high ELS and 26.2% of no ELS participants had either mild, moderate or severe depression symptoms while 42.2% of high ELS and 21% of no ELS participants had abnormally high levels of anhedonia. Although it is not possible to diagnose depression without a clinical assessment, these data are in agreement with other studies suggesting a large societal burden of un-diagnosed depression (Lewis et al., 2019; Li et al., 2009; Lotfaliany et al., 2018) with one study in the general population finding up to 49% of depression was undiagnosed (Asami et al., 2015). It should be noted that the present study was undertaken during the Covid-19 global pandemic with it being estimated that levels of depression had doubled during this period (Office for National Statistics, 2020b). This is a major limitation of the present study as depression and anhedonia are well known to reduce reward learning in both the PRLT (Murphy et al., 2003) and PRT (Pizzagalli et al., 2005, 2008; Vrieze et al., 2013).

5.5.1 Probabilistic reward task

In contrast to previous studies employing the PRT neither groups showed a response bias toward the more highly rewarded stimulus (Pizzagalli et al., 2005, 2008) suggesting a general failure of all participants to modulate their responses as a function of reward. There are no previous studies carrying out the PRT online making a direct comparison challenging. However one potential reason for this failure may be due to participants being informed high performance would lead to a bonus payment with the actual reward in the task being points. This compares to previous studies where direct monetary compensation was displayed in the task (Pizzagalli et al., 2005). This lack of response bias indicates that participants solved the task in a different manner using potentially different cognitive processes. This makes comparison of other task measures to previous literature challenging. Participants with high levels of ELS did show impairments in discrimination, a measure of task difficulty, which was driven by changes in depression symptomology as opposed to ELS directly. Discriminability in the no ELS cohort of the present study (overall mean = 0.85 ± 0.05) is higher than that reported by Pizzagalli et al., 2005 (≈ 0.7 – 0.75), a level more similar to the high ELS cohort seen here (overall mean = 0.70 ± 0.04). This would indicate that no ELS participants found the task relatively easier than previous data while high ELS participants performed at a similar level.

5.5.2 Probabilistic reversal learning task

Participants also completed the probabilistic reversal learning task where participants with a history of ELS displayed decreased positive feedback sensitivity independent of depression symptomology as measured by win-stay probability compared to controls. This effect was also specific to positive feedback sensitivity with no changes observed in lose-shift probability. Blunted striatal responses to reward in participants with a history of ELS has been previously reported (Hanson et al., 2015, 2016) which might underly the decreased positive feedback sensitivity observed in the present study. Consistent with the present study, women with MDD and a history of childhood sexual abuse have also been found to have impaired performance upon trials requiring use of previously rewarded information but not those requiring previously punished information in the probabilistic stimulus selection task (Pechtel and Pizzagalli, 2013). These findings are also interesting when contrasted to depressed patients who have been reported to show increased negative feedback sensitivity

alongside attenuated positive feedback sensitivity (Elliott et al., 1997; Foti and Hajcak, 2009; Herzallah et al., 2013; Mueller et al., 2015; Webb et al., 2017).

Although no previous comparable studies have been carried out in humans there are interesting comparisons that can be made with literature concerning reversal learning in animal models of ELS. Marmoset offspring who were maternally separated on postnatal days 2-28 showed no change compared to matched controls when learning simple visual discrimination but were impaired when the contingencies reversed (Pryce et al., 2004). Although this was not a probabilistic task this is similar to that seen in both depressed and bipolar patients in the human PRLT (Gorrindo et al., 2005; Murphy et al., 2003) who acquire the initial rule successfully but then are impaired following reversal. This compares to ELS participants in the present study who performed equally well in the practice and reversal phases but showed a deficit in acquisition of the first rule in block 1. This suggests a potential impairment in the ability to generalise the task rules between the practice and acquisition phase. However this contrasts to results from Pechtel and Pizzagalli, 2013 using the probabilistic stimulus selection task who reported that women with remitted MDD and CSA and controls learnt the acquisition at the same rate.

Another measure that is commonly found to differ between depressed patients and controls is the probabilistic switch rate, otherwise known as sensitivity to misleading negative feedback (Murphy et al., 2003; Taylor Tavares et al., 2008). Unlike depressed patients, participants with high ELS did not show increased switching in response to misleading negative feedback. There were no effects of ELS upon the overall reward learning measures of rule changes and accuracy in the present study, however when data were analysed with a Q-learn reinforcement learning model a trend towards decreased learning rate was observed which was strengthened in exploratory analysis. Decreased associative learning has been previously observed in juveniles previously exposed to physical abuse (Hanson et al., 2017). There is mixed evidence in depression studies as to whether learning rate differs between patients and controls with many studies finding a decrease alongside others finding no change (Chen et al., 2015). However compared to previous studies utilising a similar model (Grogan et al., 2017) participants in the present study learnt at an equal rate from positive and negative feedback making a single learning rate model fit better.

5.5.3 Little evidence for an interaction between stress and reward processing deficits in ELS

One of the hypotheses of this study was that stress in adult life would modulate the relationship between reward processing deficits and ELS. There was little evidence that this was the case with lifetime stress being found to increase discrimination ability in the PLT but reduce learning rate in the PRLT. Crucially these differences were constant across all participants. Acute stress has been previously reported to decrease model free learning rate (Park et al., 2017) in other learning tasks but not discriminability in the PRT (Bogdan et al., 2010; Bogdan and Pizzagalli, 2006). Interestingly a previous study investigated the interaction between acute and chronic stressors and found that participants with higher stressful life events in the last 2 years shift from model-based to model-free learning after acute stress induction (Radenbach et al., 2015). However, no studies have previously looked at the longer-term effects of chronic stress upon these measures in the same tasks.

There was an interaction between positive feedback sensitivity and ELS whereby stress only influenced positive feedback sensitivity in participants without a history of ELS. Higher lifetime stress led to greater positive feedback sensitivity but higher stress in the last year was associated with decreased win-stay probability. There are few previous studies investigating similar constructs but Berghorst et al., 2013 reported that after stress induction those who had higher cortisol reactivity and self-reported negative affect had lower reward but not punishment sensitivity. Following acute stress it has also been reported that participants show decreased negative feedback sensitivity (Petzold et al., 2010). However, the findings from the present study clash with the hypothesis that those with ELS not controls would be sensitised to the effects of stress upon reward learning. This is consistent with the match/mismatch theory which suggests that ELS primes for a stressful adulthood (Hartmann and Schmidt, 2019). An example of this is a finding that children with moderate levels of ELS show reduced HPA activation to an induced stressor compared to those with no or severe ELS (Gunnar et al., 2009; Hartmann and Schmidt, 2019). It is worth still noting other studies report that those with ELS are more susceptible to stress induced depressive episodes than controls (Hammen et al., 2000; Mclaughlin et al., 2010) indicating some level of enhanced stress vulnerability. Additionally, it is worth noting that due to the relatively poor model fit for this exploratory analysis that these findings should be taken as preliminary due to the risk of data overfitting.

5.5.4 Limitations

As previously discussed, the major limitation of this study is the high proportion of undiagnosed depression in the high ELS cohort, potentially due the study being carried out during the Covid-19 global pandemic. This is also likely to mean that participants had experienced higher levels of stress than if the population had been studied in a normal period. Utilisation of the online testing platform also creates another limitation as it is not possible to ensure that participants are completing the tasks in as controlled an environment as would be possible by laboratory testing. Another potential issue with this study, although deliberate in order to ensure that all participants were more neurodevelopmentally adult (Somerville, 2016), is the fact that all participants were over the age of 25. However, it is estimated that around 75% of adults suffering from mental health disorders experienced the onset of symptoms before the age of 24 (Kessler et al., 2005). This means that the study population is potentially biased towards those protected from mental health disorders as those who have reached age 25 without diagnosis may be a more protected population than the population of those who have experienced ELS. Finally, this study was only powered to detect group differences with two groups therefore this means that ANCOVA analysis and exploratory analysis is likely to be underpowered meaning that the learning rate findings should be taken as preliminary.

5.5.5 Conclusions

There is limited evidence for reward processing deficits in participants with a high level of early life stress in the probabilistic reward task with any changes being more dependent upon depression symptomology than ELS. However, there does appear to be evidence for a specific impairment in positive feedback sensitivity in ELS which did not appear to be moderated by depression scores within the PRLT. There was additionally evidence of impaired reward learning in the PRLT, both overall as evidenced by learning rate and selectively during the acquisition phase of block 1. There was little support for a moderating effect of stress upon the effects of ELS upon reward learning. Further studies are needed that better control for depression symptomology in the recruited population to more completely understand if reward processing deficits are present in otherwise healthy persons with a history of ELS and mediate the link between ELS and depression.

Chapter 6

General Discussion

6.1 A framework for assessing the overall hypothesis

Depression is a debilitating and highly prevalent disease with a complex aetiology. Early life stress has been identified as one of the most important risk factors for the development of depression therefore has been extensively investigated in this dissertation. Work in this thesis was designed to contribute evidence towards the hypothesis that early life stress causes individuals to have a high risk of developing depression through divergent brain development due to glucocorticoid exposure leading to an altered function in core reward circuitry which culminates in impaired reward learning as an intermediate phenotype of depression (Vrieze et al., 2013).

Key findings in this thesis advance elements of the proposed theory. Human participants with a history of ELS showed specific deficits in elements of reward learning and positive feedback sensitivity when measured in the probabilistic reversal learning task, a task that was also extensively characterised in rats. Within rats this task was found to be sensitive to manipulations of 5-HT but all other manipulations led to generalised impairments in reward learning. There was also evidence for altered circuit dynamics in the rat hippocampus following maternal separation, a region known to be important in reward learning, with increased NMDAR function observed. However, these are only small pieces of a much larger puzzle in understanding how ELS leads to an increased risk of developing depression and gaining wider acceptance into the validity of the proposed hypothesis. For general support of this hypothesis the data in this thesis combined with that in the literature would have to:

- Provide evidence that ELS causes reward learning deficits similar to those seen in people at risk of depression.
- Give confidence that animal models of ELS are robust and generate a phenotype matching that seen in humans
- Reassure that translational reward learning assays in animals and man are able to measure similar underlying constructs
- Demonstrate changes in brain reward circuitry caused by ELS that were sufficient to impair reward learning in animal models of ELS
- Show reversal of reward learning impairments through modulation of brain reward circuitry alterations

If these five tests were met this would have important implications for the aetiology of depression and provide vital directions for future research into new therapeutic avenues to treat depression. It is also worth stating that this framework is based upon the assumption that the reward learning theory of depression is valid and important in the aetiology of depression (Admon and Pizzagalli, 2015). While there has been plenty of evidence in support of this (discussed in section 1.2.3) the theory has still not reached maturity. For the full validation of this theory it would be beneficial for studies to have been conducted showing that improving reward learning is sufficient to protect from depressive risk. The following sections will now discuss what progress has been made to address each question and if the data available supports or detracts from the test.

6.1.1 Test 1: Robust evidence for reward learning impairments in ELS

The first and most important facet of the theory to examine is whether reward learning impairments are present in people with a history of ELS but prior to the emergence of depressive symptoms. These reward learning impairments should also be of a similar kind to those seen in both at risk and depressed patients (Vrieze et al., 2013). If there was no evidence for this being the case, then this would be an immediate disqualification of the proposed theory.

Work within this thesis demonstrated that healthy participants with a history of ELS do show alterations in reward processing as evidenced by decreased positive feedback sensitivity in the probabilistic reversal learning task. This was combined with a reduced learning ability during the acquisition phase of block 1. However these were different alterations compared to those observed in depressed patients where changes specific to misleading negative feedback sensitivity and reversal errors have been observed (Murphy et al., 2003; Taylor Tavares et al., 2008). Additionally, other reinforcement learning tasks have observed increased overall negative feedback sensitivity in MDD patients (Halahakoon et al., 2020). This means that for the data in this thesis to support the overall hypothesis future experiments are needed exploring long term consequences of these specific reward learning impairments and how they relate to depression vulnerability. If future studies do not link these specific changes to depression vulnerability, then this would be sufficient to disprove the suggested theory.

Although a trend towards reduced reward learning was consistent with previous studies (Halahakoon et al., 2020) the task with the most evidence linking reward learning with depression as an intermediate phenotype is the probabilistic reward task (Admon and Pizzagalli, 2015). Due to both the fact that this task was not successfully used within this thesis and a lack of published literature then this must be a priority for future investigation. Longitudinal studies using the PRT to assess persons with ELS for reward learning prior to the development of depression will be crucial to answer this first test in the framework.

Overall the assessment of this facet of the theory is hampered by a lack of quality data. The current data does not allow either the confirmation or rejection of reward processing deficits as an intermediate phenotype following ELS. Although the results from the present studies show a distinct pattern in ELS compared to depressed patients this needs both replicating and follow-up to understand its significance.

6.1.2 Test 2: The availability of reliable animal models of ELS

To progress the links between proposed reward learning impairments in ELS and specific neural circuit changes this will require the use of reliable animal models of ELS. As seen in Chapter 2 this is not straightforward where the LNBM, a model requiring the least intervention compared to others, did not show findings congruent with previous literature (Rice et al., 2008; Wang et al., 2012). Although a ELS phenotype was generated using the MS180 model in Chapter 4, with animals showing increased anxiety in the NSFT, other measures such as BrdU neurogenesis or restraint stress corticosterone were not consistent with previous literature (Stuart et al., 2019). From published protocols animal models of ELS look deceptively easy to implement. However, developing rodents are exceptionally sensitive to a myriad of stressors from a range of sources including the experimenter, animal facility staff, building maintenance and surrounding animals. Other issues can also arise from the animal strain and species used with it being apparent there are clear differences between both mice and rats and different strains (Bonapersona et al., 2019; Millstein and Holmes, 2007; Mirescu et al., 2004). This is combined with the vast variety of small changes made between different laboratories such as changes in separation schedule in maternal separation, control conditions (animal facility rearing vs short handling) and husbandry conditions. Only when all these factors are successfully managed is it possible to generate believable animal models of ELS with a sufficient effect size for further investigation. These factors likely underly the huge heterogeneity within the published literature in addition to

a demonstrable publication bias (Bonapersona et al., 2018, 2019). For the field to move forwards the introduction of a standardised protocol with extensive buy in from researchers will be needed to produce reliable and comparable results between laboratories. Although efforts have been made to standardise the LNBM model (Walker et al., 2017) it is too early to see if this has had any impact and represents relatively few studies compared to the other ELS models (see figure 1.5). By reducing variability and heterogeneity this would also have important implications for the 3Rs (Russel and Burch, 1959) by reducing the number of failed studies.

Additionally current ELS models in rodents do not compare well to traditional validity criteria (see table 6.1 for overview). Current models show limited face validity by the extremeness of the measures taken (extreme scarcity in the LNBM model and extensive separations in the MD and MS models) while being carried out at different neurodevelopmental timepoints and having different phenotypic outcomes (e.g. differences in CORT reactivity and important sex differences). Construct validity is relatively good in ELS models with the core mechanism of elevated CORT concentrations binding to glucocorticoid receptors to remodel brain development being consistent. However there has been a general lack of predictive validity with a myriad of papers in animals reporting reversal of a ELS phenotype following manipulations (non-exhaustive examples: Couto et al., 2012; Danielewicz et al., 2017; Gosselin et al., 2010; Leventopoulos et al., 2009; Maciag et al., 2002; Wilber et al., 2010) which have never been reported in humans implying a lack of confidence in the translatability of findings between animals and man. Generalisability of animal ELS models is relatively poor with the same model in mice and rats and different strains leading to divergent results (Bonapersona et al., 2019; Millstein and Holmes, 2007; Tan et al., 2017). The previously discussed high heterogeneity is also to the detriment of the generalisability of animal ELS models.

However, trying to model as complex a manipulation such as early life stress which in humans is often a result of socioeconomic factors and dysfunctional child-caregiver interactions is always going to be a challenge in rodents. Although animal ELS models compare relatively poorly to traditional validity measures it is also important to be pragmatic that this is currently the best approach in modelling an extremely intricate condition. Within the current framework assessing the validity of the reward learning ELS theory the models still have enough utility to provide useful insights. Through the development of better models and standardised methodologies this would enhance the

ability of animal ELS models to provide useful predictions to treat humans with depression and a history of ELS.

Type of validity	Description
Face	How similar the animal model and human condition subjectively look
Construct	How theoretically similar are the mechanisms underlying the animal model and the human condition
Predictive	To what degree can the animal model predict outcomes in the human condition and vice-versa
Generalisability	How widely valid are findings in the animal model across a wide range of scenarios and experimental conditions

Table 6.1 Type of validity relevant to animal models of ELS. Descriptions written using insights from Belzung and Lemoine, 2011 and Staay et al., 2009.

6.1.3 Test 3: Translational reward learning assays measuring the same constructs in animals and man

Once reward learning deficits in humans with ELS have been confirmed and a refined way to model ELS in animals is achieved then the next critical step would be to assess reward learning in animals with a history of ELS using translational reward learning tasks. Translational tasks that measure the same construct in man and animal are key for the predictive validity of findings and to enhance the probability that pre-clinical research can translate into clinical implications (Jensen and Amara, 2014). In Chapter 3 a translational reward learning assay, the probabilistic reversal learning task, that has previously been used in depressed patients (and participants with a history of ELS in Chapter 5) was assessed in rats using pharmacological treatment. While initially animals' performance was modulated by citalopram treatment, over time it appeared that responses shifted to drugs causing non-specific behavioural impairments that manifested as reward learning impairments through decreased motivation. This is similar to that seen in many operant behavioural tasks where animals switch from an instrumental learning strategy to a procedural strategy with repeated testing (Robinson, 2018). Although repeated testing is critical in the pursuit of the

3Rs this is different to experiments traditionally carried out in humans where participants only complete a single session where they are expected to learn the task within this period. However, although this means that the PRLT is unsuitable for long term repeated testing this means that well designed experiments with a single manipulation based upon extensive evidence could still be successfully carried out. While multiple studies testing pharmacological interventions have now been carried out in rodents (Bari et al., 2010; Drozd et al., 2018; Noworyta-Sokolowska et al., 2019; Wilkinson et al., 2020) there is a lack of corresponding studies in humans. This means that it is difficult to ensure that both human and rodent tasks are measuring similar constructs. Indeed while in chapter 3 it was observed that increasing synaptic 5-HT through citalopram administration increases positive feedback sensitivity in rats, human data has shown that tryptophan supplementation increases negative feedback sensitivity with no effect on positive feedback (Thirkettle et al., 2019).

Additionally, other translational tasks have been carried out in animals in particular the probabilistic reward task. While the original translation of the task from man to animal suffered from extremely slow training (Der-Avakian et al., 2013) a recent version using touchscreen visual discrimination has improved upon the original version with success (Kangas et al., 2020). Within the PRT the fact that administration of pramipexole impairs response bias in both humans and rodents suggests predictive validity between the two versions (Der-Avakian et al., 2013). However wider validation under a range of conditions has not yet been carried out.

These factors suggest that further work is needed to ensure that the translational tasks measure similar constructs in man and animal. This is critical to avoid the translational failures that have beset other pre-clinical models in the depression field (Robinson, 2018). However current tasks do show great promise and well-designed experiments coupled with cautious interpretation could provide valuable insights when trying to reverse reward learning deficits using well defined hypotheses from investigations of brain circuit changes.

6.1.4 Test 4: Demonstrable changes in brain reward circuitry

Early life stress reprograms the developing brain through its action upon glucocorticoid receptors in key brain regions; loci include the hippocampus, prefrontal cortex and amygdala. If people with ELS have reward learning deficits that pre-dispose to depression,

then this must be underlain by neural circuit changes in brain reward circuitry. In Chapter 4 electrophysiological parameters in the hippocampus, a key component of the limbic system, were investigated. Although work was heavily underpowered due to Covid-19 disruption, ELS animals appeared to show changes in NMDA receptor function which would be hypothesised to manifest as changes in hippocampal LTP. However changes observed didn't appear to localise to the ventral end of the hippocampus which might be expected for a purely affective phenotype (Fanselow and Dong, 2010). This either means that changes were non-selective and may not relate to reward learning or another possibility is that broad hippocampal dysfunction is a consequence of ELS with ventral impairment affecting reward learning and dorsal impairment being more specific to spatial memory impairments (Cao et al., 2014; Fanselow and Dong, 2010; Wang et al., 2011). There are also numerous other papers suggesting alterations in reward circuitry following ELS with changes in the amygdala (Arnett et al., 2015; Birnie et al., 2020; Bolton et al., 2018; Ono et al., 2008), lateral septum (Shin et al., 2018), hippocampus (Hulshof et al., 2011; Köhler et al., 2019; Marais et al., 2008) and orbitofrontal cortex (Goodwill et al., 2018) amongst other frontolimbic regions reported (Cohodes et al., 2020; Wang et al., 2012).

This broad body of evidence provides great support for the theory that ELS reprograms brain reward circuitry. However the functional roles of many of these changes upon reward learning have not been determined nor have any of these findings been investigated in man.

6.1.5 Test 5: Amelioration of reward learning deficits through neural circuit interventions

While great in support of the proposed theory, the fact that so many reward learning regions show changes compared to controls creates its own problem. With evidence that dysfunction is wide ranging across multiple different neurotransmitter systems, brain regions and circuit types this suggests that targeting one specific intervention is unlikely to be sufficient to compensate for the weight of all the other abnormalities. However, it may be the case that dysfunction can be grouped into broader circuits where for example manipulating amygdala circuits may reduce anxiety but have no effect on other elements of the ELS phenotype. One example of this is that Drd3 mediated signalling in the lateral septum has been found to be downregulated following early social deprivation and mediate social dysfunctions (Shin et al., 2018). Restoration of this Drd3 function with optogenetic activation was sufficient to restore social behaviour. ELS should therefore be viewed through the prism similar to how

polygenic or other neurodevelopmental disorders are where treatment is much more based upon specific systems, pathways and symptom clusters.

Complex reward learning requires functional circuitry in the mesolimbic system, hippocampus and prefrontal cortex amongst other regions (Russo and Nestler, 2013) with all of these regions impacted by ELS (Loi et al., 2015; Murthy and Gould, 2020; Tottenham and Sheridan, 2009). This means that it is unlikely that specific manipulations will be identified that are able to reverse these changes to increase reward learning in a manner able to protect from psychiatric disorders. It is however possible that a single change, for example changes in LTP in a pathway of the ventral hippocampus, could be the integrative locus of reward circuit changes and sufficient to impair reward learning. It should however be noted that there are critical limitations in current technology necessary to leverage any potential findings into patient benefit. Current experimental approaches to alter specific neural circuit activity rely extensively upon optogenetics, viral mediated gene expression and targeted drug infusions. To justify the use of these techniques in humans there has to be a strong risk-benefit argument which would be difficult to argue for with ELS. Patients would not be willing and healthcare providers would not pay for invasive surgery for the implantation of optogenetic probes to reduce the risk of developing psychiatric disorders. However if predictive models of psychiatric risk improve to the point where accuracy is extremely high these approaches may have more merit as is the case with prophylactic mastectomies in the case of BRCA 1/2 mutations to protect from breast cancer (t'Kint de Roodenbeke et al., 2020).

However in the context of the given framework for assessing how feasible the theory of ELS reprogramming reward learning circuits to predispose to depression the previously discussed findings do not invalidate the theory. It is more than possible that findings will be made in animal models upon specific elements of reward learning behaviour. However, the issue will be translating those findings to humans in a proportionate and tolerable intervention that shows efficacy.

6.2 Broader perspectives on ELS and depression

It is also important to remember that depression is a heterogenous disease with a complex aetiology and pathogenesis of which reward learning likely only plays one part (see Figure

1.2). There is an abundance of evidence that ELS impacts monoaminergic neurotransmission (Diamantopoulou et al., 2018; Kloke et al., 2013; Leventopoulos et al., 2009; Ohta et al., 2014), epigenetic regulation (Kundakovic et al., 2015; Turecki et al., 2014; Turecki and Meaney, 2016), neurotrophic signalling (Kanatsou et al., 2017; Korosi et al., 2012; Maccari et al., 2014; Mirescu et al., 2004; Oomen et al., 2010), immune regulation (Abbink et al., 2019; Brenhouse et al., 2019; Fagundes et al., 2013) and HPA axis function (Dashkalakis and Yehuda, 2015; Maccari et al., 2014). These factors may all interact upon a locus of reward learning but it is more likely that they to some degree interact and another degree work independently to cause the myriad of psychiatric risk that people with a history of ELS suffer. While reward learning may be important it is important to be cognizant that it also may be less significant in the development of mental health conditions compared to the many other changes ELS causes in both brain function and behaviour. Another factor that is important to consider is that depressed patients also show deficits in other aspects of reward processing such as option valuation (Halahakoon et al., 2020). These may also be important in the aetiology of the disease and be relevant to ELS.

With the previously discussed issues in implementing precision-based circuit interventions to reduce psychiatric risk in suffers of ELS then at the current time it would be most prudent to focus upon the most well established and successful interventions: socioeconomic. The most successful intervention to reduce risk has been environmental enrichment in animals (Francis et al., 2002) and education in humans (Friedman et al., 2015). Additionally with stress being a major trigger of depressive episodes and those with ELS being more sensitive to stress (Hammen et al., 2000; Sullivan et al., 2000) this would seem an obvious locus for intervention. Animal studies have also shown that dietary interventions can help protect following ELS (Rincel et al., 2016, 2018) with malnutrition being a major source of stress in children (Hoeijmakers et al., 2015). With societal measures that reduce stress in the population through enhanced social security, support for families of low socioeconomic status and high-quality healthcare this could help prevent countless cases of psychiatric disorders. Finally, through implementation of these measures and combined with enhanced welfare monitoring of children this may help to prevent the incidence of ELS in the first place; a far better proposition than post hoc preventative treatment.

6.3 Relevance to other psychiatric disorders

Early life stress not only predisposes to depression but a wide range of other psychiatric disorders including schizophrenia, addiction and anxiety (Agid et al., 1999; Andersen et al., 2008; McCauley et al., 1997; Phillips et al., 2005). Reward learning deficits have also been suggested to be an important biomarker in schizophrenia (Deserno et al., 2013; Gold et al., 2008) and pathological responses to reward are at the core of addiction disorders (Keiflin and Janak, 2015). Interestingly schizophrenia is associated with a failure to accurately represent reward value while dysfunctional reward prediction errors underly much of addiction behaviour (Keiflin and Janak, 2015; Redish, 2004). The fact that ELS can predispose to such opposite outcomes suggests that other factors are important in determining which disorder presents in patients. It is likely that interactions between genetics, epigenetics and the sequelae of childhood adversity underly the manifestation of psychiatric disorders in later life. Efforts have been made to create polygenic risk scores for predicting the risk of psychiatric illness following ELS but currently it is not possible to distinguish between different disorders using this approach (Belsky et al., 2019; Bischoff et al., 2017; Graffi et al., 2018). Efforts have also been made to investigate interactions between ELS and single gene mutations however often these studies have been hampered by extremely small effect sizes or an extremely low occurrence of the risk allele (Fogelman and Canli, 2019; Wang et al., 2017). It may though be possible to assess trajectory based upon specific patterns of reward learning alterations. However, this would require gene by environment interactions to create specific reward learning deficits following ELS which themselves relate to the specific disorders which is unlikely. Interactions between genotype and prenatal environment have been reported upon hippocampal and amygdala development (Ong et al., 2019). These hippocampal alterations are likely to also be important in the aetiology of the other conditions that ELS predisposes to other than depression. For example glutamatergic dysfunction has been found to be important in schizophrenia (Balu, 2016; Rubio et al., 2012) with the observed AMPA/NMDA changes following ELS in Chapter 4 being potentially relevant to increased schizophrenia risk. The findings of this thesis may be therefore relevant to multiple disorders, especially if future work observes hippocampal dysfunction at the core of ELS mediated psychiatric risk.

6.4 Conclusions

It is too early to conclude that early life stress mediates increased psychiatric risk through reprogramming of brain reward circuitry to create reward learning deficits. However work contained in this thesis has supported this theory and provided valuable information which will be of use to future investigators. There do appear to be reward learning deficits in ELS with it being possible, although not yet investigated, to use translational tasks in animals to assess these. With the wide-ranging neurodevelopmental effects of ELS there are an abundance of alterations in reward processing circuitry that may underly these reward processing changes with the challenge being more finding specific loci for intervention that are sufficient to reverse reward learning impairments. Previous work has shown the reversal of specific ELS phenotypic components although the greatest challenge going forward is the lack of availability of non-invasive technology to alter specific neural circuits. Until this technology is ready the best available interventions appear to be socioeconomic in nature. In the future by combining these interventions with insights from neural circuit changes it may enable those with stressful childhoods to live a full and happy adult life free from the debilitating consequences of mental health conditions.

Appendix I: Wilkinson et al., 2020 (BioRxiv)

Investigation of reward learning and feedback sensitivity in non-clinical participants with a history of early life stress.

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Abstract

Background: Early life stress (ELS) is an important risk factor for the development of depression. Impairments in reward learning and feedback sensitivity have been suggested. to be an intermediate phenotype in depression aetiology. We therefore hypothesised that healthy adults with a history of ELS would have impairments in reward learning and feedback sensitivity.

Methods: We recruited 64 adult participants with high levels of ELS and no diagnosis of a current mental health disorder in addition to 65 controls. Participants completed two online reward learning tasks: the probabilistic reversal learning task (PRLT) and probabilistic reward task (PRT). Participants also completed depression, anhedonia, social status and stress scales with PRLT data being additionally analysed utilising a reinforcement learning model.

Results: Participants with high levels of ELS showed decreased positive feedback sensitivity (PFS) in the PRLT compared to controls. High ELS participants also tended towards possessing a decreased model-free learning rate which strengthened in subsequent analysis. This was coupled with a decreased learning ability in the acquisition phase of block 1 following the practice session. Neither groups of participants showed a reward induced response bias in the PLT however high ELS participants exhibited decreased discrimination ability between stimuli; this was however accounted for by depression symptomology in further analysis.

Conclusions: These data suggest that healthy participants without a mental health diagnosis and high levels of ELS show deficits in PFS and reward learning in the PRLT that are distinct from depressed patients. These deficits may be relevant to an increased vulnerability to depression.

1. Introduction

Early life stress (ELS) is a major known risk factor for the development of depression (Agid et al., 1999; Green et al., 2010; Lemoult et al., 2019; McCauley et al., 1997). Elevated levels of childhood stress lead to widespread functional and morphological alterations in the adult brain with the hippocampus, amygdala and prefrontal cortex being most impacted (Cohodes et al., 2020; Tottenham, 2009). This not only renders those with a history of ELS vulnerable to depression but may also lower the threshold of stress required to precipitate depression (Hammen et al., 2000). However, how ELS influences the developing brain to predispose individuals to psychiatric illness is not yet understood.

Reward learning deficits have been proposed to be an intermediate phenotype in the aetiology and maintenance of depression (Halahakoon et al., 2020; Pizzagalli et al., 2008; Vrieze et al., 2013; Whitton et al., 2015). Depressed patients show decreased reward sensitivity in the probabilistic reward task (PRT, Pizzagalli et al., 2008), a test of reward learning. These deficits have been observed to both predict the risk of disease development (Bress et al., 2013) and persistence (Pechtel et al., 2013; Vrieze et al., 2013). Utilising a different reward learning assay, the probabilistic reversal learning task (PRLT), depressed patients show impaired accuracy following probabilistic rule reversal and increased sensitivity to probabilistic negative feedback (Murphy et al., 2003; Taylor Tavares et al., 2008). Acute stress has also been observed to impair reward learning (Berghorst et al., 2013; Bogdan and Pizzagalli, 2006) suggesting a potential link between stress, reward processing deficits and depression aetiology.

Previous studies have investigated reward processing deficits in people who have experienced ELS. Hanson et al., 2017 recruited adolescents with a history of physical abuse who then completed a probabilistic learning task where they showed lower associative learning compared to controls. Changes in reward learning have also been reported within another probabilistic reward task, the probabilistic stimulus selection task (PSST), by Pechtel and Pizzagalli, 2013. Women with a history of childhood sexual abuse and a diagnosis of MDD showed decreased performance on trials requiring learning of previously rewarded information compared to MDD only and control groups. Although these studies provide valuable insights, they use different tasks to those previously used to study depressed populations making direct comparisons difficult. Additionally, studies are needed in adults without a current mental health diagnosis to understand if any reward processing changes are present prior to the development of mental health disorders.

In this study it was hypothesised that ELS leads to alterations in reward processing and feedback sensitivity in an otherwise healthy adult population. Two groups of adult participants that reported no diagnosis of a mental health condition or Parkinson's disease were recruited online and completed a survey of adverse childhood experiences (Cohen et al., 2006) before being split into high and no ELS groups. Participants completed the PRT and PRLT with PRLT data additionally being analysed using a Q-learning model to probe reward learning parameter changes. Participants were asked about stress exposure to enable exploratory analysis investigating if life stress interacts with ELS to cause reward processing deficits. By understanding the links between ELS and reward processing deficits as a

hypothesised intermediate phenotype in depression this aims to provide insights into how a person with a history of ELS is rendered at higher risk for depression.

2. Methods

All procedures were approved by the Faculty of Life Sciences and Faculty of Science Research Ethics Committee at the University of Bristol and the study protocol was pre-registered (www.osf.io/gvy65). All participants provided full written consent for the collection, analysis and publication of their data which is available open access and were reimbursed at a rate of £6.00 per hour.

2.1 Participants

586 participants were recruited using the Prolific (www.prolific.co) online platform to complete an online screening questionnaire (see supplementary figure 1 for study overview). These participants were 25 - 65 years of age, fluent in English, resident in the UK and had no mild cognitive impairments or dementia. Participants completed the early life stress questionnaire (ELSQ, Cohen et al., 2006) while also being asked to self-report if they had a diagnosis of a mental health condition or Parkinson's disease.

Participants who met the inclusion criteria for high ELS or no ELS and did not report a diagnosis of a mental health disorder or Parkinson's were then invited to take part in a second phase of the experiment online within a week of screening and were allocated into two groups. A no ELS group (n = 65) contained people scoring 0 on the ELSQ while a high ELS group (n = 64) consisted of those who scored ≥3 (estimated to be the top tercile of the population from Cohen et al., 2006). In this second phase of the experiment participants entered demographic information before completing the MacArthur Scale of Subjective Social Status (Adler et al., 2000), Beck's depression inventory II (BDI-II, Beck et al., 1996), the Snaith Hamilton pleasure scale (SHAPS, Snaith et al., 1995) and the Holmes and Rahe stress scale (Holmes and Rahe, 1967). The SHAPS was additionally scored using the SHAPS-C criteria (Ameli et al., 2014) while for the stress scale participants were asked if each event occurred in either their adult life or the last year. For all stages of the experiment participants were instructed to use a desktop or laptop only and that they should be in a quiet place with minimal distractions. Sample size was estimated for a medium effect size (Cohen's d = 0.5) and 80% power for a t-test at 64 participants per group.

2.2 Behavioural testing

Following completion of self-report measures, participants completed the Probabilistic reversal learning task (Cools, Clark, Owen, & Robbins, 2002; Waegeman, Declerck, Boone, Seurinck, & Parizel, 2014) followed by the Probabilistic reward task (Pizzagalli et al., 2005). To complete the tasks participants were required to download and install the Millisecond Inquisit web player (Millisecond, US) which ran both tasks using Millisecond Inquisit v6.2.1.

Participants were instructed they were able to earn an additional £2.00 for high performance on the behavioural tasks.

2.2.1 Probabilistic Reversal Learning task

The PRLT was conducted as previously described (Cools et al., 2002; Waegeman et al., 2014) using the task from the Millisecond test library (Millisecond, 2020a). Participants were instructed to choose between a "lucky" (rich) and "unlucky" (lean) pattern to maximise points. Selection of the rich stimulus enabled participants to gain a point 80% of the time and lose a point 20% of the time with the lean stimulus having opposite contingencies. If no stimulus was chosen within 2s then this was classed as incorrect and participants lost a point. After meeting the reversal criterion, the contingencies reverse such that the rich stimuli becomes lean and vice versa. This criterion was set randomly between 10 to 15 consecutive correct rich choices to stop participants counting to the criterion. Participants first completed a practise phase where they had to achieve the criterion for a single reversal before proceeding to the main task which was completed in three blocks each limited to 9 minutes. Participants who did not pass the practice phase were excluded from analysis. Data was analysed as previously described (Wilkinson et al., 2020) with win-stay and lose-shift probabilities being calculated as measures of positive and negative feedback sensitivity respectively. These were subdivided into either true, feedback that matches with the underlying task rules, or misleading feedback, that which is opposite to the underlying task rule. The number of rule changes, accuracy and response latency per block were additionally analysed. A Qlearn reinforcement learning model was applied to data as previously described

(Grogan et al., 2017; Wilkinson et al., 2020) to give estimates of learning rate, accuracy compared to a model predicted perfect strategy (subjective accuracy) and beta, a measure of choice variability. Additionally, data per phase (practice, acquisition of the first rule in block 1 and the following two reversals) was analysed consisting of participant accuracy, errors to criterion and win-stay / lose-shift probability.

2.2.1 Probabilistic Reward Task

The PRT was conducted as previously described (Pizzagalli et al., 2005) using the task from the Millisecond test library (Millisecond, 2020b). Participants were instructed to identify whether the mouth of a presented cartoon face was long or short to win points over 3 blocks of 100 trials. Participants were shown a face before a mouth was rapidly presented for 100ms with participants given up to 1750ms to respond. Feedback was not provided on every trial but unknown to participants one mouth was rewarded with points three times more often than the other (rich = 60%, lean = 20%). Response key and rich/lean stimuli assignments were counterbalanced across participants and responses that were quicker than 150ms or slower than 1750ms were excluded from analysis. Additional responses that differed by more than 3 standard deviations from the mean following natural log transformation of latencies for each participant were excluded from analysis. Response bias (logB), a measure of reward learning, and discriminability (logD), a measure of task difficulty, were calculated as described previously (Pizzagalli et al., 2005).

2.3 Data Analysis

Demographic and self-report measures were compared between groups using either X^2, t-tests or Mann-Whitney U tests where appropriate. The primary analysis for each measure was a direct comparison between no ELS and high ELS groups. Where data was not normally distributed then efforts were first made to transform data to normality and where this was not possible Mann-Whitney U tests were completed. Win-stay by block data was transformed using the bestNormalize package in R (Peterson and Cavanaugh, 2019). Where measures were split by a within subject factor such as block or feedback type these were analysed with repeated measures ANOVAs. Where Mauchly's test identified a violation of the Sphericity assumption then this was corrected using the Huynh-Feldt correction. T-tests were used for direct group comparisons.

Due to differences in social status, BDI-II score and SHAPS score between the no ELS and high ELS groups, principal component analysis (PCA) was conducted to reduce the dimensionality of these variables to account for depression symptomology as an analysis stage (see supplementary tables 1 and 2). Because only principal component 1 (PC1) differed between groups and explained 94.6% of variance this was used in ANCOVAs (analysis of covariance) to analyse whether parameter changes were due to ELS or due to changes in depression symptomology accounted for by the PC1 component. To understand if stress and gender interacted with ELS to modify reward learning, exploratory analysis was also undertaken using generalised linear mixed models (GLMMs) containing the factors: gender, ELS, lifetime stress, last year stress and age. GLMMs were fit using the glmmTMB package in R 4.0 (Brooks et al., 2017; R Core Team, 2020) with model refinement conducted utilising stepwise deletion based

upon Akaike information criterion before being compared with a null model to protect against overfitting. PC1 was also added to each model following final model selection to assess the effects of depression symptomology.

Statistical analysis was conducted in SPSS v26 (IBM, US), MATLAB 2018a (Mathworks, USA) and R 4.0 (R Core Team, 2020) with output graphics constructed in GraphPad Prism 8 (GraphPad, US). All data is shown as mean ± SE with a bar and stars showing a main effect of ELS in the primary analysis. * ≤ 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

3. Results

Early life stress was highly prevalent in the study population with only 21.0% of participants having no adverse childhood experiences (ACEs) and 44.4% of the population suffering three or more ACEs in their childhood (see supplementary figure 2). 16.0% of respondents self-reported a diagnosis of a mental health disorder or Parkinson's with this being associated with a higher ELSQ score (Mann-Whitney, $U = 15725$, $p < 0.0001$).

The two study groups were well matched with respect to gender, age, education, ethnicity, relationship status, employment status and the presence of monetary worries (see table 1). However, high ELS participants had a self-reported lower social status coupled with higher depression scores in the BDI-II and elevated anhedonia scores in the SHAPS questionnaires.

There was no difference between groups when participants were asked about stress they encountered in both the last year and their adult lives. When the BDI-II scores were classified into either minimal, mild, moderate or severe depression (see supplementary figure 3, Beck et al., 1996) participants from the high ELS group were more likely to be in greater severity depression groupings (chi^2, $X^2(3) = 12.9$, $p = 0.005$). Similarly when SHAPS scores were classified into either normal (≤2) or abnormal (≥3) hedonic responses (Snaith et al., 1995) members of the high ELS group were more likely to have abnormal scores (see supplementary figure 3, chi^2, $X^2(1) = 6.3$, $p = 0.012$).

Measure	No ELS (n = 65)	High ELS (n = 64)	Test statistic	p
Gender (% male)	44.6	37.5	$x^2(2) = 2.5$	0.28
Age (years)	37.3 ± 1.30	38.0 ± 1.24	U = 1936.0	0.50
Education (% graduates)	64.6	65.6	$x^2(5) = 4.9$	0.43
Ethnicity (% white)	95.4	82.8	$x^2(4) = 8.7$	0.070
Relationship status (% single)	18.5	28.1	$x^2(3) = 1.9$	0.60
Employment status (% full time)	64.6	60.9	$x^2(5) = 3.5$	0.61
Monetary concerns (% agree / strongly agree)	36.9	56.3	$x^2(3) = 4.4$	0.22
ELSQ	0 ± 0	4.36 ± 0.17	-	-
Social status	6.2 ± 0.17	5.2 ± 0.21	U = 1397.5	**0.001**
BDI-II	9.4 ± 1.0	15.2 ± 1.22	U = 1315.5	**0.0003**
SHAPS	1.4 ± 0.25	2.56 ± 0.32	U = 1496.5	**0.004**
SHAPS-C	24.3 ± 0.67	26.4 ± 0.86	$t_{119.4} = -1.92$	0.057
Lifetime stress	472.8 ± 22.4	529.2 ± 23.9	$t_{127} = -1.72$	0.088
Last year stress	111.4 ± 12.3	139.8 ± 17.0	U = 1939.5	0.51

Table 1. Demographic and self-report measures in the study population. Values are shown for each group as mean ± standard error with significant p values indicated in bold.

3.1 Probabilistic reversal learning task

There was no difference between groups in either the number of rule changes participants were able to complete (Figure 1A) or accuracy (Figure 1B). However participants with a history of high ELS did have a slower average response latency (Figure 1C, RM-ANOVA, $F_{1,126} = 5.03$, $p = 0.027$) with both groups getting equally faster over the course of the three blocks (RM-ANOVA, $F_{1.88,236.7} = 16.1$, $p < 0.0001$). Secondary analysis revealed little effect of depression symptomology (RM-ANCOVA, PCA1: $p > 0.05$) with the main effect of ELS persisting (RM-ANCOVA, ELS: $F_{1,125} = 4.9$, $p = 0.028$). Exploratory analysis on overall reaction times did not replicate a main effect of group but did observe older participants having slower reaction times (GLMM, $Z = 2.8$, $p = 0.005$). This analysis also indicated a trend towards an interaction between group and lifetime stress (GLMM, $Z = 1.55$, $p = 0.065$) but further investigation did not reveal an effect of lifetime stress in either group.

When data was analysed using the Q-learning reinforcement learning model a trend emerged towards high ELS participants having a lower learning rate compared to the no ELS study population (Figure 1D, t-test, $t_{127} = 1.78$, $p = 0.077$). Secondary analysis revealed no effect of PCA component 1 upon learning rate but abolished any effect of ELS. In exploratory analysis a main effect of ELS was observed (GLMM, $Z = 2.1$, $p = 0.037$) with the addition of PC1 impairing model fit ($\Delta AIC = 1.69$, $X^2(1) = 0.31$, $p = 0.57$). Additionally, a relationship between stress in the last year and learning rate was observed whereby increased stress in the last year decreased learning rate (GLMM, $Z = -2.3$, $p = 0.024$). There was no difference in choice

variability (Figure 1E) or accuracy compared to a model predicted perfect strategy (Figure 1F) between groups.

Participants with a history of high ELS exhibited reduced positive feedback sensitivity (PFS, Figure 2A, RM-ANOVA, $F_{1,122}$ = 10.4, p = 0.002) which persisted once depression symptomology was accounted for using PCA component 1 (RM-ANOVA, $F_{1,121}$ = 6.6, p = 0.01). Exploratory analysis revealed an interaction between ELS and both lifetime stress (GLMM, Z = -2.15, p = 0.031) and last year stress (GLMM, Z = -1.99, p = 0.047). Further investigation revealed effects of both stress types upon PFS in the low ELS group only (GLMM, lifetime stress: Z = -2.35, p = 0.019, last year stress: Z = -2.2, p = 0.026) whereby higher lifetime stress led to greater PFS but higher stress in the last year was associated with decreased PFS. However it should be noted that although all suggested terms were removed from the model the overall model was a poorer fit than the null when measured by AIC (Δ AIC = 7.3, $X^2(13)$ = 18.7, p = 0.13).

The effect of ELS upon PFS was consistent across feedback that matched (true feedback) or clashed (misleading feedback) with the underlying task rules (Figure 2B, Mann-Whitney U, true: U = 1443, p = 0.03; misleading: U = 1337, p = 0.005). This effect appeared to be constrained to PFS with no corresponding changes in lose-shift probability between no ELS and high ELS groups (Figures 2C and D).

When initial learning in the PRLT task was assessed it was apparent that although ELS and control participants performed similarly during the practice phase there was a learning deficit during acquisition of the first reversal criterion in block 1 as evidenced by increased errors to criterion (Figure 3A, Mann-Whitney U, $U = 1580$, $p = 0.045$) and decreased accuracy (Figure 3B, Mann-Whitney U, $U = 1584$, $p = 0.036$). Both groups of participants however performed equally well at achieving criterion for a second and third reversal. Unlike the overall measures there was no difference in win-stay probability between groups (Figure 3C), however there was a trend for high ELS participants to show increased negative feedback sensitivity (NFS) in the practice phase (Figure 3D, Mann-Whitney U, $U = 1532$, $p = 0.052$).

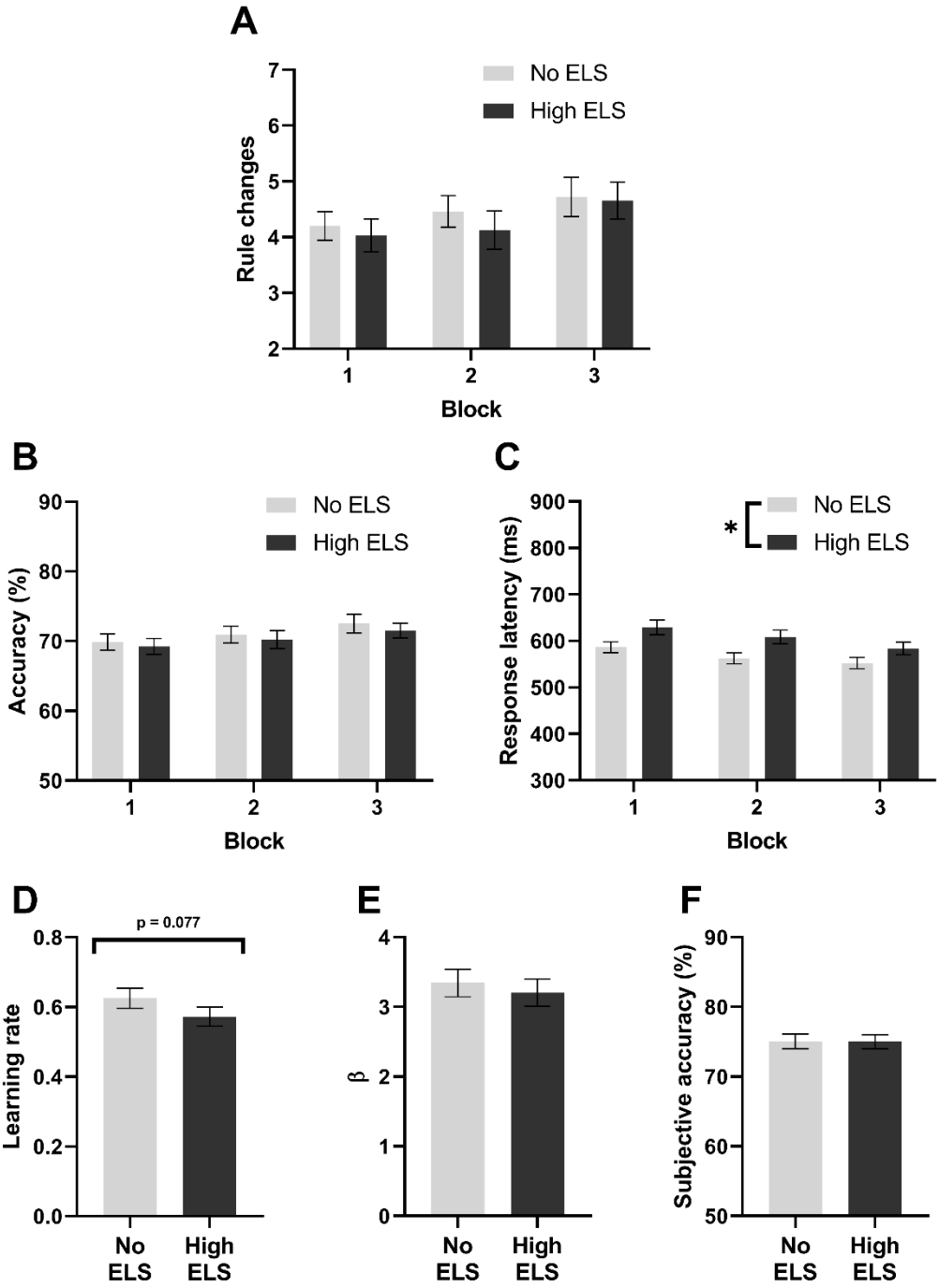

Figure 1. Overall reward learning and reinforcement learning in the PRLT. (A) Rule changes within each block, **(B)** accuracy by block and **(C)** average response latency per block. From the Q-learn reinforcement learning model: **(D)** learning rate, **(E)** β, the inverse of the softmax temperature and a measure of choice variability and **(F)** subjective accuracy, participant accuracy compared to a model predicted perfect strategy.

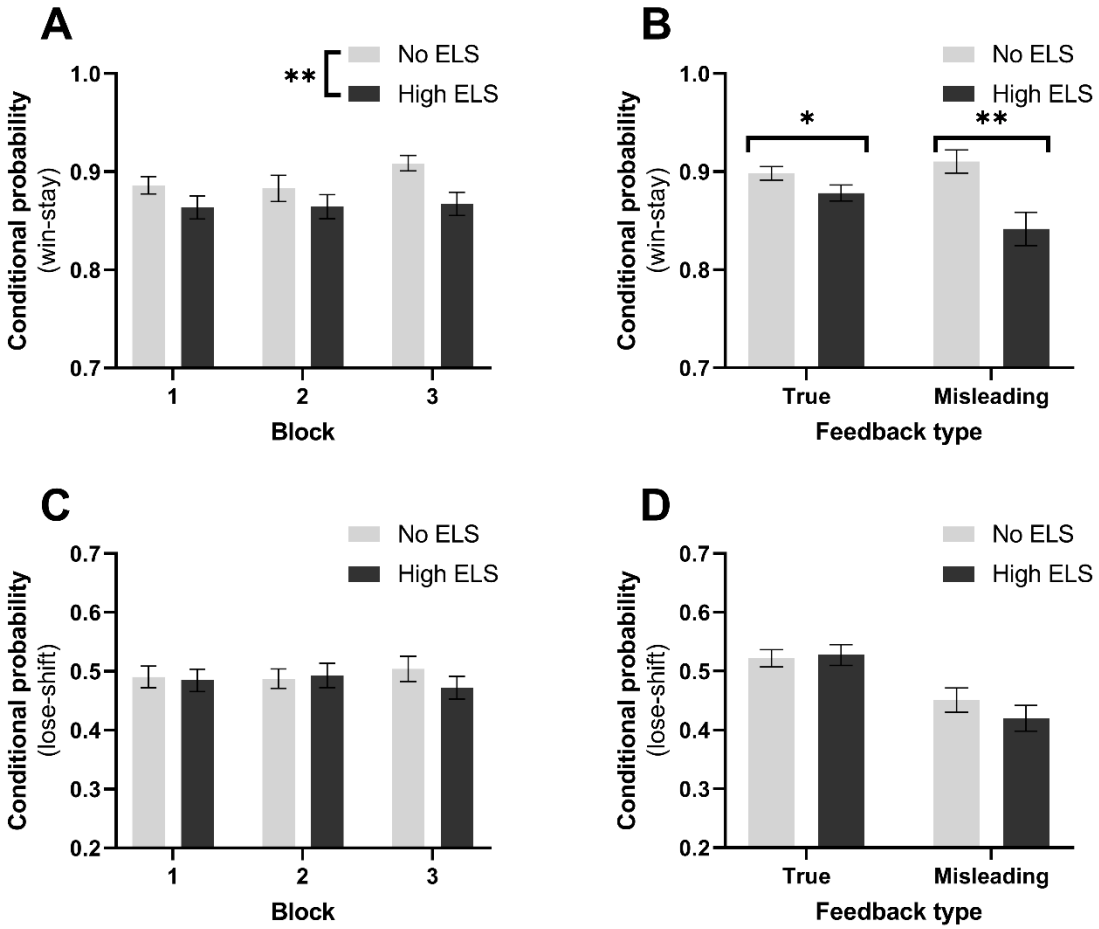

Figure 2. High ELS participants exhibited lower positive feedback sensitivity than those without a history of ELS. (A) Win-stay probability, **(B)** Lose-shift probability, **(C** and **D)** win-stay and lose-shift probability respectively subdivided into true and misleading feedback.

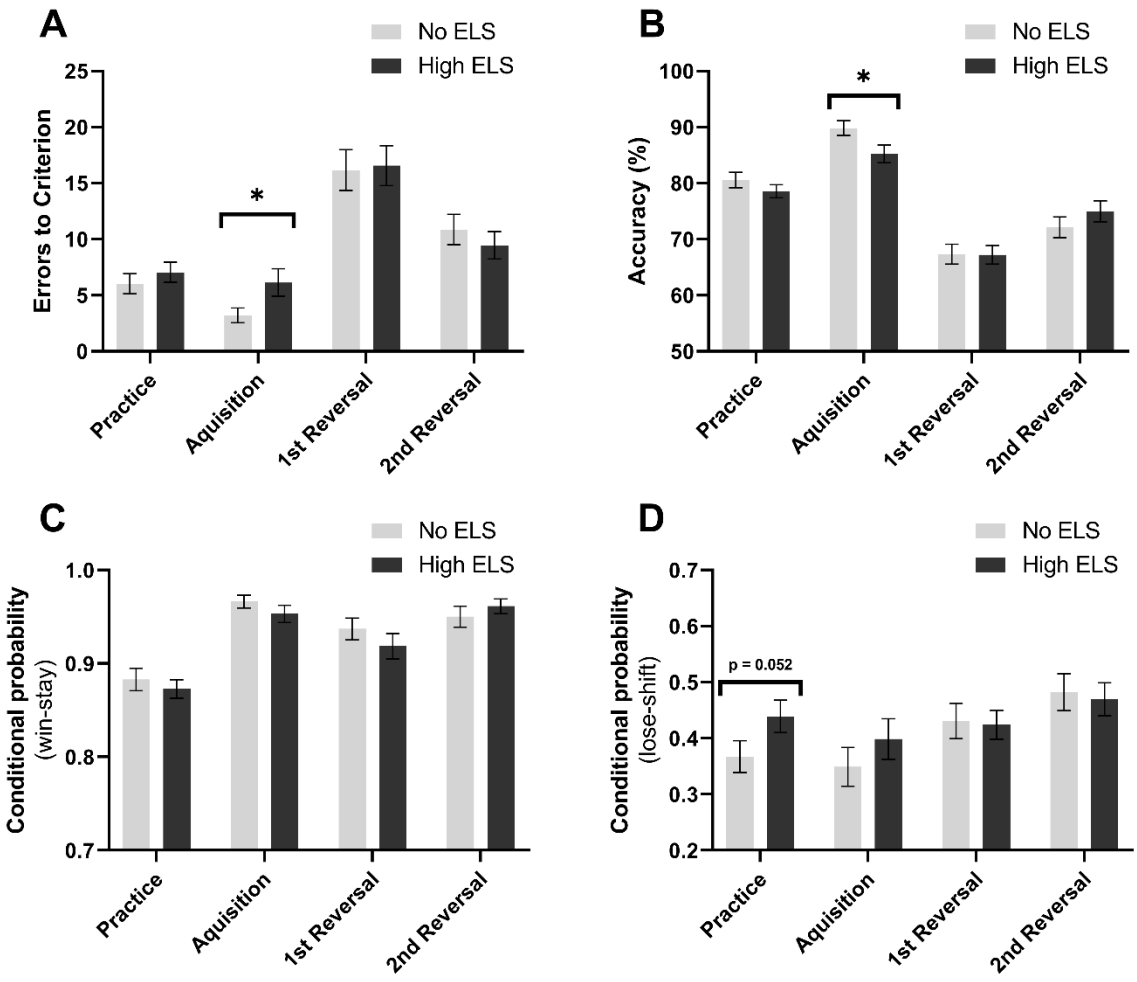

Figure 3. High ELS participants show impaired learning in the acquisition phase of block 1. **(A)** Errors made while reaching criterion for each phase, **(B)** accuracy within each phase, **(C** and **D)** win-stay and lose-shift probabilities for each phase of block 1 and practice respectively.

3.2 Probabilistic reward task

Neither group of participants developed a response bias towards the more highly rewarded stimulus in any block (Figure 4A) nor was there evidence for a response bias developing between blocks (Figure 4B). However, participants with a history of high ELS did show an impaired ability to discriminate between stimuli (Figure 4C, ANOVA, $F_{1,127} = 4.8$, $p = 0.030$). Secondary analysis revealed that this difference between groups appeared to be driven by differences in depression symptomology with the effect of ELS disappearing when PCA component 1 was included in the analysis (ANCOVA, PCA1: $F_{1,126} = 6.08$, $p = 0.015$; ELS: $F_{1,126} = 1.7$, $p = 0.19$). Exploratory analysis further revealed a main effect of lifetime stress with higher lifetime stress corresponding to increased discrimination ability (GLMM, $Z = 2.6$, $p = 0.007$). An effect of gender was also revealed (GLMM, $Z = 2.04$, $p = 0.04$) with males showing increased discrimination ability. Finally, there was no difference between groups in response latencies (Figure 4D).

Consistent with Pizzagalli et al., 2008 the probability of misclassifying a stimulus based upon the preceding trial outcome was also analysed (supplementary table 3). Participants with a history of high levels of ELS were more likely to misclassify rich stimuli if either the previous trial was a not rewarded rich trial or a lean not rewarded trial with these measures roughly corresponding with rich lose-shift and lean lose-stay probability in the PRLT respectively.

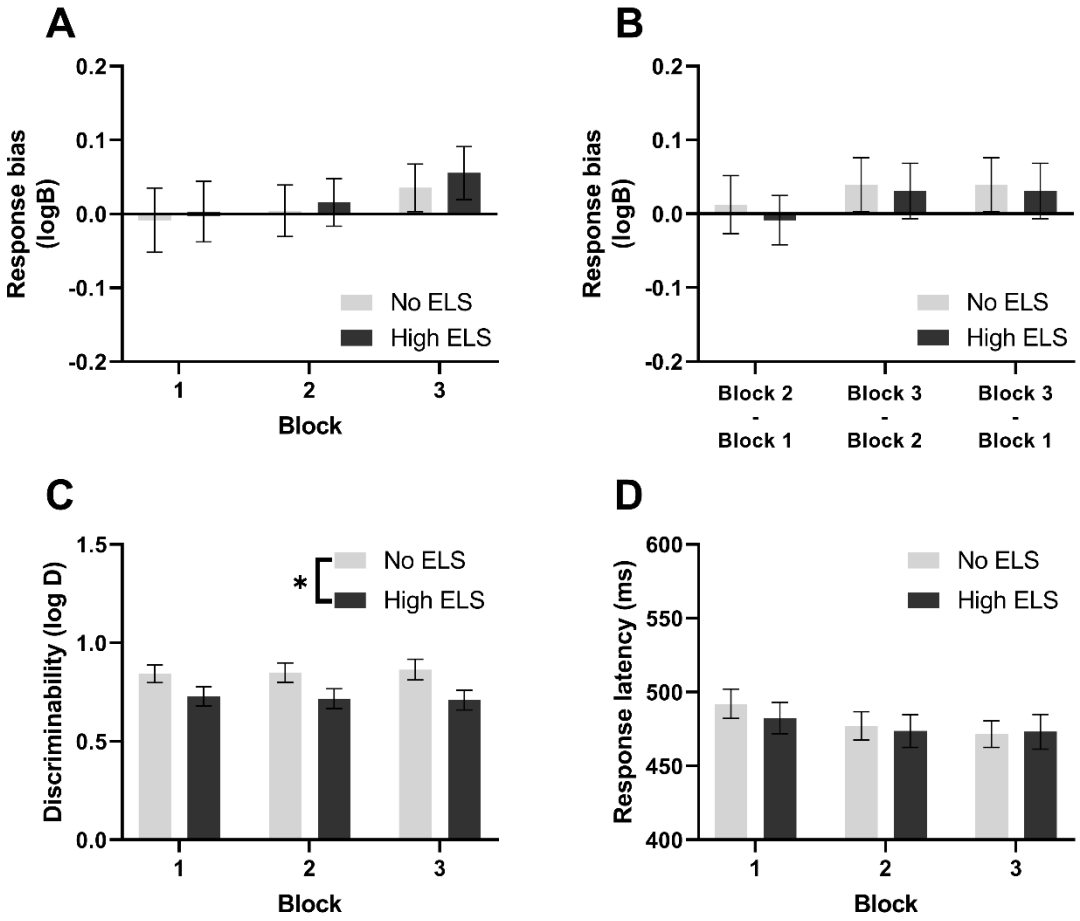

Figure 4 Participants with a history of ELS show decreased discriminability in the PRT. (A) Response bias to the more highly rewarded stimulus, **(B)** response bias development between blocks, **(C)** discriminability between long and short face lengths and **(D)** average response latency.

4. Discussion

This study was designed to investigate whether healthy adults with a history of ELS show alterations in reward processing and feedback sensitivity. Nearly 600 participants were screened; ELS was highly prevalent in the population with 79.0% of participants experiencing one or more ACE and 44.4% experiencing three or more.

Participants with a history of high ELS had higher self-report depression and anhedonia symptoms. Although participants stated they did not have a diagnosis of depression, 54.7% of high ELS and 26.2% of no ELS participants showed at least mild symptoms based upon the BDI-II questionnaire. BDI-II scores in no ELS (mean: 9.4 ± 1.0) and high ELS (mean: 15.2 ± 1.2) participants were higher than controls for similar studies (range 1.3-3.62, Pechtel & Pizzagalli, 2013; Pizzagalli, Bogdan, Ratner, & Jahn, 2007; Pizzagalli et al., 2008) but lower than depressed patients (mean: 32.1 ± 8.6, Pizzagalli et al., 2008) or participants described as having a high BDI (>16, Pizzagalli et al., 2007). These data consistent with a large societal burden of un-diagnosed depression (K Lewis et al., 2019; Li et al., 2009; Lotfaliany et al., 2018). It should be noted that the present study was undertaken during the Covid-19 global pandemic with it being estimated that levels of depression had doubled during this period (Office for National Statistics, 2020b). The high level of undiagnosed depression is a major limitation of this study as depression and anhedonia are well known to reduce reward learning in both the PRLT (Murphy et al., 2003) and PRT (Pizzagalli et al., 2005, 2008; Vrieze et al., 2013). It is also worth considering that 75% of adults with mental health conditions experience the onset of symptoms before aged 24 (Kessler et al., 2005). This means that the

study population, all 25 years of age or greater, is potentially biased towards those more protected from mental health disorders.

4.1 Probabilistic reversal learning task

In the PRLT participants with high ELS displayed decreased positive feedback sensitivity compared to controls as measured by win-stay probability. This finding was independent of depression symptomology and specific to PFS with no changes observed in lose-shift probability. Blunted striatal responses to reward in participants with a history of ELS have been previously reported (Hanson et al., 2015, 2016) which may underly the decreased PFS observed in the present study. Consistent with the present study, women with MDD and a history of childhood sexual abuse have also been found to have impaired performance in the PSST but only for trials requiring use of previously rewarded information and not those requiring use of previously punished information (Pechtel and Pizzagalli, 2013). Within the PRLT depressed patients have been observed to show increased sensitivity to misleading negative feedback (Murphy et al., 2003; Taylor Tavares et al., 2008). This was not observed in the high ELS cohort in the present study. In other tasks depressed patients have also been reported to show increased NFS alongside attenuated PFS (Elliott et al., 1997; Foti and Hajcak, 2009; Herzallah et al., 2013; Mueller et al., 2015; Webb et al., 2017). These findings suggest that ELS influences feedback sensitivity in the PRLT differently to depression with ELS decreasing PFS but not effecting NFS while depression has an opposite effect.

The PRLT also allows for assessment of reinforcement learning through the analysis of rule changes and accuracy in addition to parameters calculated through use of the reinforcement learning model. In contrast with our hypothesis, ELS did not affect rule changes which is surprising considering evidence that both depression and ELS can impair cognitive flexibility (Murphy et al., 2012; Zhou et al., 2020). Although rule changes was used as the main behavioural reward learning output, when data was analysed with the Q-learn model a trend towards decreased learning rate was observed in high ELS participants. This was became significant in exploratory analysis and decreased associative learning has been previously observed in juveniles previously exposed to physical abuse (Hanson et al., 2017). There is a lack of consistent evidence in depression studies as to whether model free learning rate differs between patients and controls (Chen et al., 2015; Robinson and Chase, 2017). These findings warrant future investigation due to this study being only powered to detect group differences between two groups meaning that ANCOVA and exploratory analysis is likely to be underpowered.

A slower response latency was also observed in high ELS participants which was specific to the PRLT with no congruent changes seen in the PRT. This discrepancy may be related to differing cognitive demands with the PRLT potentially requiring greater working memory.

No directly comparable studies have been carried out in humans. However, maternally separated marmosets, an animal model of ELS, showed no change in simple visual discrimination compared to controls but showed impairments when the contingencies reversed (Pryce et al., 2004). This is similar to that seen in both depressed and bipolar patients

in the human PRLT (Gorrindo et al., 2005; Murphy et al., 2003) who acquire the initial rule successfully but then are impaired following reversal. This compares to ELS participants in the present study who performed equally well in the practice phase and reversal phases but showed a deficit in acquisition of the first rule in block 1. This suggests a potential impairment in the ability to generalise the task rules between the practice and acquisition phase. However previous probabilistic learning studies did not include a practice phase meaning that this likely changed the way participants processed the start of block 1. This might explain the contrast with Pechtel and Pizzagalli, 2013 who reported that women with remitted MDD and ELS learnt acquisition in the PSST at the same rate as controls.

One of the hypotheses of this study was that stress in adult life would modulate the relationship between reward processing deficits and ELS. There was little evidence that this was the case except for an observed interaction between PFS and ELS whereby stress only influenced PFS in participants without a history of ELS. Higher lifetime stress led to greater PFS but higher stress in the last year was associated with decreased win-stay probability. There are few previous studies investigating similar constructs but Berghorst et al., 2013 reported that after stress induction those who had higher cortisol reactivity and self-reported negative affect had lower reward but not punishment sensitivity. Additionally, it is worth noting that due to the relatively poor model fit for this exploratory analysis that these findings should be taken as preliminary due to the risk of data overfitting.

4.2 Probabilistic reward task

In contrast to previous studies employing the PRT neither groups showed a response bias toward the more highly rewarded stimulus (Pizzagalli et al., 2005, 2008) suggesting a general failure of all participants to modulate their responses as a function of reward. There are no previous studies carrying out the PRT online but in this study we failed to replicate the main outcome measure. All aspects of the task were similar between the lab and online version except for participants being informed high performance would lead to a bonus payment with the actual reward in the task being points. Previous studies instead used direct monetary compensation in the task (Pizzagalli et al., 2005). It should also be noted that the online testing platform limits the ability to ensure that participants are completing the tasks in as controlled an environment as would be possible by laboratory testing providing another explanation for the high data variability. The lack of response bias indicates that participants solved the task in a different manner using potentially different cognitive processes making comparison to previous literature challenging. Nevertheless, participants with high levels of ELS did show impairments in discrimination, a measure of task difficulty which appeared to be driven by changes in depression symptomology as opposed to ELS specifically.

4.3 Conclusions

These data suggest that participants without a formal diagnosis of a mental health condition but a history of ELS show impairments in positive feedback sensitivity and reward learning in the PRLT compared to controls. These impairments may be important in understanding how ELS predisposes to depression with reduced reward learning being a key feature in MDD patients (Halahakoon et al., 2020). However, high levels of undiagnosed depression are a

potential confound and highlight a potential wider issue in terms of the number of people who meet criteria for MDD but are not formally diagnosed or receiving care. Future studies are needed to replicate these findings, investigate the neural circuit changes underlying these reward learning impairments and investigate whether these findings are directly related to psychiatric risk.

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6. Declarations

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- The authors declare no conflict of interest.
- All procedures were approved by the Faculty of Life Sciences and Faculty of Science Research Ethics Committee at the University of Bristol
- All participants consented to participation both prior to and following completion of the study

- All participants consented to publication of anonymised data
- Data will be made available open access at www.osf.io/gvy65 and by contacting the corresponding author
- Code will be made available open access at www.osf.io/gvy65 and by contacting the corresponding author

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Supplementary Material

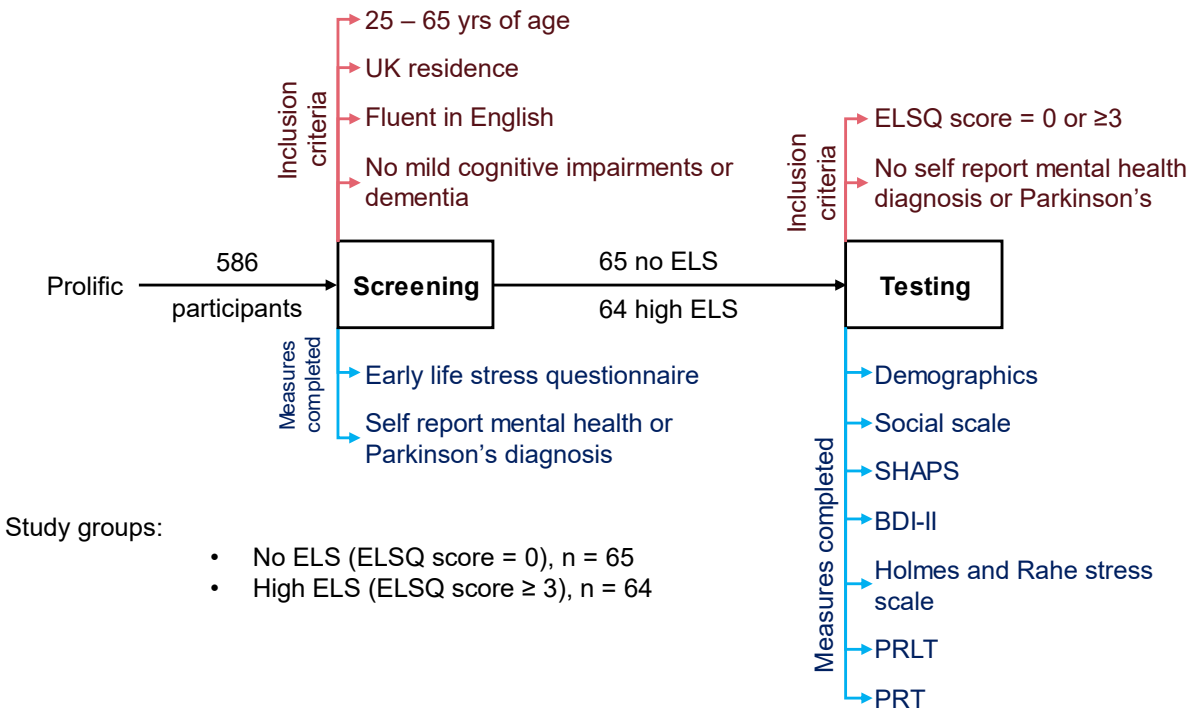

Figure S1 Study overview. Participants were screened by ELSQ score and then formed into two study groups: no ELS and high ELS.

Component	Explained variance (%)	No ELS	High ELS	Test statistic	P value
1	94.6	4.32 ± 0.24	5.65 ± 0.25	t_{127} = -3.86	**0.0002**
2	3.4	-0.19 ± 0.21	0.20 ± 0.24	t_{127} = -1.22	0.226
3	2.0	0.21 ± 0.15	-0.22 ± 0.18	t_{127} = 1.79	0.076

Table S1 Principal component analysis of social scale, SHAPS and BDI-II scores. The mean ± standard error are shown for each group with the relevant statistical comparison.

	Principal component		
	1	**2**	**3**
Social scale	-0.07	-0.40	0.91
BDI-II	0.98	-0.18	-0.003
SHAPS	0.17	0.90	0.40

Table S2 Principal component analysis component loadings.

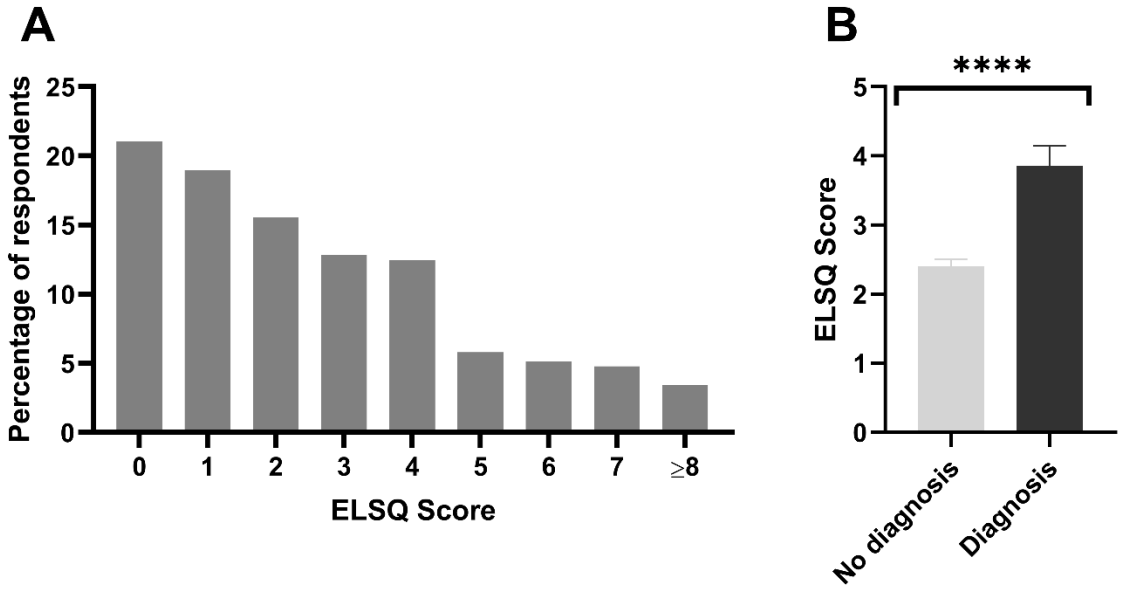

Figure S2 Early life stress in an online study population. (A) ELSQ scores in the study population. (B) Mental health disorder / Parkinson's self-report diagnosis by ELSQ score. N = 586 participants.

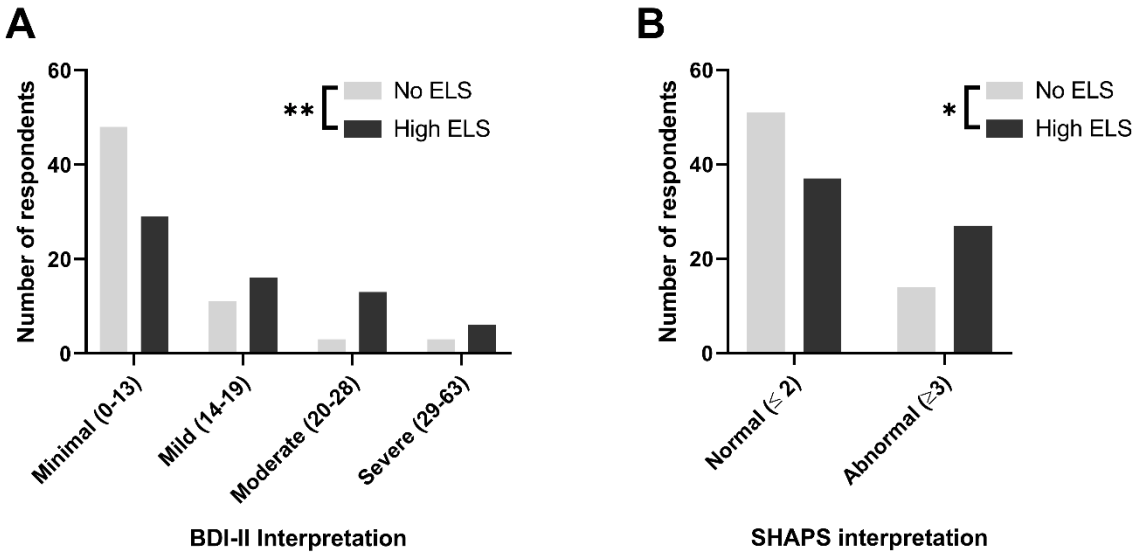

Figure S3 Interpretation of BDI-II and SHAPS scores in the no and high ELS populations. Scores were interpreted following Beck et al., 1996 and Snaith et al., 1995. (A) BDI-II split by severity of depression and (B) SHAPS split by normal or abnormal hedonic responses. N = 129 participants (65 no ELS, 64 high ELS).

Current trial	Previous trial	No ELS	High ELS	Test statistic	p
Lean	Rich - rewarded	16.8 ± 2.1	18.4 ± 2.2	U = 1966	0.59
Lean	Rich - not rewarded	16.1 ± 1.8	18.6 ± 1.8	U = 1744	0.11
Lean	Lean - rewarded	19.3 ± 2.1	20.5 ± 2.0	U = 1928	0.47
Lean	Lean - not rewarded	16.8 ± 1.6	21.2 ± 1.8	U = 1928	0.097
Rich	Rich - rewarded	13.1 ± 1.5	18.3 ± 2.1	U = 1697.5	0.071
Rich	Rich - not rewarded	14.2 ± 1.6	20.0 ± 2.1	U = 1597.5	**0.023**
Rich	Lean - rewarded	13.1 ± 1.4	15.5 ± 1.7	U =1814	0.330
Rich	Lean - not rewarded	14.2 ± 1.4	19.6 ± 1.9	U = 1644.5	**0.040**

Table S3 Miss-rates, the chance of mis-categorising a stimulus, by previous trial. Data is shown as mean ± standard error and significant p-values are shown in bold.

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