

Antidepressant-Like Effects of Ketamine on Fear Conditioning and Extinction

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Master of Arts
in the Department of Psychology
University of Saskatchewan
Saskatoon

By
Brian D. Kulyk

© Copyright Brian D. Kulyk, December 2016. All rights reserved.

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Psychology
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5A5
Canada

ABSTRACT

The experience of chronic stress plays an important role in the pathogenesis of major depressive disorder. Prolonged stress induces a state of chronically elevated glucocorticoid exposure in the brain, which is neurotoxic and can lead to the dysfunction of glutamatergic signaling. Since memory is highly dependent upon glutamatergic neurotransmission, patients with depression commonly display alterations in memory processing that bias the recollection of past events towards negative emotional information. Negative cognitive biases are believed to support the development and maintenance of depression, emphasizing the need for antidepressant treatments that can effectively combat these insidious symptoms. Memory for negative emotional events can be studied in animals using fear conditioning and extinction paradigms. Fear conditioning trains an animal to associate a neutral cue with an emotionally aversive experience, while extinction learning challenges this association by forming a new memory that identifies the cue as harmless. The aim of this dissertation was to investigate the antidepressant-like effects of ketamine on auditory fear conditioning and extinction in both healthy rats and those with chronic glucocorticoid exposure. The first experiment sought to dissociate the effects of ketamine on distinct stages of auditory fear conditioning and extinction by administering a subanesthetic dose of ketamine at one of three unique time points: before fear conditioning training, immediately after fear conditioning training, or before fear extinction training. Post-conditioning and pre-extinction ketamine attenuated the long-term expression of cue-elicited freezing, suggesting that the consolidation and recall of conditioned fear were impaired, respectively. Pre-conditioning ketamine did not disrupt the acquisition of fear conditioning, and none of the treatments examined affected the long-term expression of fear extinction. The second experiment aimed to build upon these findings by examining the effect of pre-extinction ketamine on conditioned fear and extinction behavior in a repeated exogenous corticosterone (CORT) animal model of depression. Repeated CORT treatment provoked a spontaneous recovery of conditioned freezing between extinction sessions and induced a reinstatement of freezing following a sub-conditioning retraining procedure. Ketamine prevented CORT-induced failures in long-term extinction expression, and also greatly reduced freezing during early phase extinction training. Collectively, the findings of this dissertation help establish ketamine as a powerful modulator of negatively-valenced memory and emotionally-driven behavior, and contribute to our understanding of its antidepressant-like qualities.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Lisa Kalynchuk for allowing me this incredible opportunity to learn and grow as a researcher. Your insightful advice on the development, execution, and analysis of my experiments has proved invaluable. It has been a privilege to be a member of your laboratory and to conduct meaningful research under your guidance.

I would also like to thank Dr. Jennifer Chlan and Dr. Lorin Elias for their valuable participation as members of my thesis committee. Your constructive feedback, direction, and encouragement throughout my studies has been greatly appreciated.

In addition, I extend sincere gratitude to all my lab mates, especially Wendie Marks, Erin Fenton, Katherina Lebedeva, Justin Botterill, and Dan Ferguson, who provided indispensable technical and intellectual support over the years. I not only regard you as valued colleagues, but also as genuine friends, and I am privileged to have worked with you.

Finally, to my mother Marilyn, my father William, and my brother Jeffrey, I owe the deepest thanks for your unwavering love and support throughout this journey. This thesis would not have been possible without you.

TABLE OF CONTENTS

PERMISSION TO USE.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS	iii
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	viii

CHAPTER 1

General Introduction

1. Thesis Outline	1
2. Diathesis, Stress, and the Role of HPA Axis Dysfunction in Depression	3
2.1. The Diathesis-Stress Model of Depression.....	3
2.2. The Role of the HPA Axis in the Regulation of Stress.....	5
2.3. HPA Axis Dysfunction in Depression	6
2.4. The Neurophysiological and Functional Consequences of Prolonged Stress Exposure	7
3. Glutamatergic System Dysregulation in the Pathophysiology of Depression	11
3.1. Introduction to the Glutamate Hypothesis of Depression.....	11
3.2. Evidence from Animal Research	13
3.3. Evidence from Human Research	16
4. The Use of Animal Models in the Study of Depression-Like Behaviors	19
4.1. Considerations of Validity	19
4.2. Animal Models of Depression	20
4.3. Measuring the Effect of Chronic CORT Exposure on Depression- Like Behaviors	22
4.3.1. <i>Anhedonia</i>	23
4.3.2. <i>Learned Helplessness</i>	23
4.3.3. <i>Negative Cognitive Biases in Attention, Processing, and Interpretation</i>	24
4.4. Summary: Using Repeated CORT Injection as a Model of Depression.....	29
5. Modeling Depression-Associated Memory Bias Through Fear Conditioning	29
5.1. Depression-Associated Negative Cognitive Bias in Memory	29
5.2. Fear Conditioning	31
5.2.1. <i>Behavior and cognition</i>	31
5.2.2. <i>Neurobiology</i>	34
5.3. The Effects of Stress and Antidepressants on Fear Conditioning.....	39
5.3.1. <i>Stress and fear conditioning</i>	39
5.3.2. <i>Antidepressants and fear conditioning</i>	40
5.4. Fear Extinction.....	42
5.4.1. <i>Behavior and cognition</i>	42
5.4.2. <i>Neurobiology</i>	43
5.5. The Effects of Stress and Antidepressants on Fear Extinction	48
5.5.1. <i>Stress and fear extinction</i>	48
5.5.2. <i>Antidepressants and fear extinction</i>	52
6. Ketamine as a Rapid-Acting Treatment for Depression	53
6.1. Limitations of Standard Antidepressant Treatments.....	53

6.2. Ketamine and the Treatment of Depression in Humans	53
6.3. The Neurobiology of Ketamine’s Antidepressant Effects	55
6.4. Ketamine in Animal Models of Depression.....	57
7. Objectives and Hypotheses.....	58

CHAPTER 2

Post-Conditioning and Pre-Extinction Ketamine Attenuates the Expression of Auditory Conditioned Fear

Abstract.....	62
1. Introduction.....	63
2. Materials and Methods.....	65
2.1. Animals	65
2.2. Experimental Design and Drug Injection Paradigm	66
2.3. Fear Conditioning	66
2.3.1. Apparatus	66
2.3.2. Conditioning	67
2.3.3. Novel context extinction.....	67
2.3.4. Behavioral measurement	68
2.4. Statistical Analysis.....	68
3. Results	69
3.1. Fear Conditioning Training Day	69
3.2. Fear Extinction Training Day	69
3.3. Fear Extinction Recall Day	71
4. Discussion	71
4.1. The Effect of Pre-Conditioning Ketamine on Auditory Fear Conditioning and Extinction	71
4.2. The Effect of Post-Conditioning Ketamine on Auditory Fear Conditioning and Extinction	76
4.3. The Effect of Pre-Extinction Ketamine on Auditory Fear Extinction	77
4.4. Clinical Implications.....	82
5. Conclusions	84

CHAPTER 3

Ketamine Prevents Repeated Corticosterone-Induced Reemergence of Extinguished Auditory Fear

Abstract.....	86
1. Introduction.....	87
2. Materials and Methods.....	90
2.1. Animals	90
2.2. Experimental Design and Drug Injection Paradigm	90
2.2.1. CORT	91
2.2.2. Ketamine	91
2.3. Fear Conditioning and Extinction	92
2.3.1. Apparatus	92
2.3.2. Conditioning	92
2.3.3. Novel context extinction.....	92

2.3.4. <i>Sub-conditioning retraining</i>	93
2.3.5. <i>Extinction retention test</i>	93
2.3.6. <i>Behavioral measurement</i>	94
2.3.7. <i>Body weight</i>	94
2.4. <i>Statistical Analysis</i>	94
3. Results	95
3.1. <i>Fear Conditioning Training Day</i>	95
3.2. <i>Fear Extinction Training Day</i>	95
3.3. <i>Fear Extinction Recall Day</i>	97
3.4. <i>Sub-Conditioning Retraining Day</i>	98
3.5. <i>Fear Extinction Retention Test</i>	101
3.6. <i>Body Weight</i>	104
4. Discussion	104
4.1. <i>The Effect of Repeated CORT on Auditory Fear Conditioning, Extinction, and Reinstatement in Animals Not Treated With Ketamine</i>	106
4.2. <i>The Effect of Subanesthetic Ketamine on Auditory Fear Extinction and Reinstatement in Repeated Vehicle- and CORT-Treated Animals</i>	111
4.3. <i>Clinical Implications</i>	120
5. Conclusions	120
 CHAPTER 4	
General Discussion	
1. Overview of Main Findings	122
2. The Effects of Ketamine on Fear Conditioning	123
3. The Delicate Balance of Fear Extinction	124
4. Depression-Like Behavior Induced by Chronic CORT Exposure	127
5. Antidepressant-Like Effects of Ketamine	131
6. Limitations	133
6.1. <i>Pathogenic Validity of Repeated Exogenous CORT Administration in Modeling Depression</i>	133
6.2. <i>Sex Differences in Depression-Like Behavior and Response to Ketamine</i>	134
7. Future Directions	136
7.1. <i>Retroactive Influence of Chronic CORT on Fear and Extinction Memory</i>	136
7.2. <i>Antidepressant-Like Effects of Ketamine Metabolites</i>	136
8. Conclusions	137
 REFERENCES	 139

LIST OF FIGURES

- Figure 2-1. Effect of pre-conditioning, post-conditioning, and pre-extinction ketamine on fear conditioning training
- Figure 2-2. Effect of pre-conditioning ketamine on extinction training and extinction recall testing
- Figure 2-3. Effect of post-conditioning ketamine on extinction training and extinction recall testing
- Figure 2-4. Effect of pre-extinction ketamine on extinction training and extinction recall testing
- Figure 3-1. Effect of CORT and ketamine on fear conditioning training
- Figure 3-2. Effect of CORT and ketamine on extinction training and extinction recall testing
- Figure 3-3. Effect of CORT and ketamine on extinction training and extinction recall testing, individual group comparisons
- Figure 3-4. Effect of CORT and ketamine on sub-conditioning retraining
- Figure 3-5. Effect of CORT and ketamine on extinction retention
- Figure 3-6. Effect of repeated vehicle and CORT administration on body weight over time
- Figure 4-1. Schematic representation of the possible underlying mechanisms by which CORT and ketamine modulate conditioned fear expression

LIST OF ABBREVIATIONS

5-HT _{2A}	5-hydroxytryptamine 2A
ACI	ambiguous cue interpretation
ACTH	adrenocorticotropin hormone
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APV	DL-2-amino-5-phosphonovaleric acid
BDNF	brain-derived neurotrophic factor
BDP-12	1-(quinoxolin-6-ylcarbonyl)piperidine
CA	cornu amonis
Ca ²⁺	calcium
cAMP	cyclic adenosine monophosphate
CE	central nucleus of the amygdala
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CORT	corticosterone
CR	conditioned response
CREB	cAMP response element-binding protein
CRF	corticotropin-releasing factor
CS	conditioned stimulus
ECT	electroconvulsive therapy
ERP	event-related potential
fMRI	functional magnetic resonance imaging
GABA	gamma-Aminobutyric acid
GFAP	glial fibrillary acidic protein
Gria1	glutamate ionotropic receptor AMPA type subunit 1
Grin2b	glutamate ionotropic receptor NMDA type subunit 2B
HNK	hydroxynorketamine
HPA	hypothalamic-pituitary-adrenal
IL	infralimbic cortex
ITC	intercalated cells of the amygdala
LA	lateral nucleus of the amygdala
LTD	long-term depression

LTP	long-term potentiation
MAOI	monoamine oxidase inhibitor
Mg ²⁺	magnesium
mPFC	medial prefrontal cortex
Na ⁺	sodium
NERI	norepinephrine reuptake inhibitor
NMDA	N-methyl-D-aspartate
PFC	prefrontal cortex
SNRI	serotonin and norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TrkB	tyrosine kinase B
US	unconditioned stimulus
vmPFC	ventromedial prefrontal cortex

CHAPTER 1

General Introduction

1. Thesis Outline

Depression is a serious and debilitating psychiatric disorder that is estimated to affect 350 million people worldwide. The World Health Organization has identified depression as the leading cause of global disability, making this disorder a source of substantial social, clinical, and economic burden (World Health Organization, 2012). The most salient symptoms of depression are chronically depressed mood and a profound loss of interest in once-pleasurable activities (anhedonia). However, depression may also present with disturbances in sleep or appetite, cognitive difficulties, feelings of guilt or worthlessness, and suicidal ideation (American Psychiatric Association, 2013). Supporting the development and maintenance of depression are symptoms referred to as cognitive biases: pathological modifications in attention, processing, interpretation, and memory that bias individuals towards focus on negative emotional content (Elliott, Zahn, Deakin, & Anderson, 2011; Harmer, Goodwin, & Cowen, 2009a; Robinson & Sahakian, 2008). These negative cognitive biases alter the way in which depressed individuals view their world, causing them to think and behave in an abnormally pessimistic manner that is detrimental to their wellbeing. If not successfully treated, depression can ultimately result in suicide (Lesage et al., 1994), a tragedy that claims the lives of over 842,000 people every year (GBD 2013 Mortality and Causes of Death Collaborators, 2015).

There is a clear need for fast-acting and effective antidepressant treatments, but many of today's commonly-prescribed antidepressant drugs leave considerable room for improvement in both of these regards. In a large open-label study of antidepressant efficacy, less than one-third of patients with depression achieved remission following up to four months of standard antidepressant drug treatment, and only 50% of patients remitted after six months of treatment and two unique drug therapies (Judd et al., 2002; Thase et al., 2005; Trivedi et al., 2006). Furthermore, a third of depressed patients remain resistant to treatment even after four antidepressant trials (Gaynes et al., 2009). The therapeutic delays and poor remission rates associated with standard antidepressant drugs illustrate significant limitations in current treatment strategies that must be addressed. Furthermore, both human and animal research has suggested that acute treatment with some antidepressants may actually worsen elements of negative cognitive biases (Browning, Reid, Cowen, Goodwin, & Harmer, 2007; Burghardt et al.,

2004; Burghardt, Sullivan, McEwen, Gorman, & LeDoux, 2013; Burhans, Smith-Bell, & Schreurs, 2013; Cassaday & Thur, 2015; Godsil et al., 2015; Lebrón-Milad, Tsareva, Ahmed, & Milad, 2013; Montezinho et al., 2010; Mørk et al., 2013; Ravinder, Burghardt, Brodsky, Bauer, & Chattarji, 2013), raising concerns that these drugs might have counter-productive effects in early stages of treatment.

Antidepressant drug development has largely been predicated upon the belief that symptoms of depression are primarily caused by disturbances in monoamine levels in the brain, but the considerable delay between antidepressant regulation of monoamine availability and therapeutic improvement casts doubt upon this hypothesis (Maeng & Zarate, 2007; Sanacora, Treccani, & Popoli, 2012). A wealth of evidence collected over the past two decades has lead researchers to identify pathological alterations in the excitatory glutamatergic system of the brain (and its downstream impairments of neuroplasticity) as a core factor in the neurobiology of depression, offering new targets for pharmacological intervention (Deutschenbaur et al., 2015; Krystal, Sanacora, & Duman, 2013; Pittenger, Sanacora, & Krystal, 2007; Pittenger & Duman, 2008; Sanacora et al., 2008; Sanacora et al., 2012). Furthermore, research has identified the experience of chronic stress as a significant mediating factor of both glutamatergic dysfunction and the onset of depression (Deutschenbaur et al., 2015; Pittenger & Duman, 2008; Sanacora et al., 2012; Willner, Scheel-Krüger, & Belzung, 2013). Amongst several novel antidepressants sought to address glutamatergic dysfunction in depression, the most promising thus far is ketamine, a glutamate receptor antagonist that is commonly used in anesthesia. Treatments with acute, subanesthetic doses of ketamine reliably produce rapid and long-lasting antidepressant effects in depressed patients, even those who are classified as treatment-resistant (Berman et al., 2000; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price, Nock, Charney, & Mathew, 2009; Rasmussen et al., 2013; Zarate et al., 2006). Instead of taking weeks or months to experience a therapeutic effect, depressed patients treated with a single dose of ketamine can achieve remission in as little as one day, and the effects of one treatment can last more than a week (Zarate et al., 2006). The antidepressant qualities of ketamine have been replicated in animal models of depression (Garcia et al., 2009; Jett et al., 2015; Koike, Iijima, & Chaki, 2013; Li et al., 2011; Perrine et al., 2014), but research on the efficacy of ketamine in relieving depression-associated negative cognitive biases remains limited.

This dissertation includes two studies that explore ketamine's ability to modulate the expression of negative emotional memory. The first study sought to dissociate the effects of ketamine on the acquisition, consolidation, and recall of auditory fear conditioning in rats. Furthermore, it addressed prior uncertainty regarding the influence of ketamine on the induction and recall of fear extinction. The second study aimed to expand upon these findings by examining the efficacy of ketamine in preventing long-term extinction retention failure induced by chronic exposure to the stress hormone corticosterone (CORT). Together, these studies help elucidate the relationship between chronic stress, glutamatergic neurotransmission, and memory for negative emotional information.

The remainder of this chapter is dedicated to a review of the etiology of human depression, the study of depression-like behaviors in chronic stress animal models, and the utility of ketamine as an antidepressant treatment. The discussion begins with an introduction to the role that chronic stress plays in the development of depressive symptomatology, and the neurophysiological and functional consequences of prolonged stress exposure. Following this is an overview of human and animal research supporting the stress-induced dysregulation of glutamatergic neurotransmission in the pathophysiology of depression. Next, the validity of chronic exogenous CORT administration as an animal model of depression is discussed. Fear conditioning and fear extinction are then explored as measures of negative emotional memory, and the effects of stress and antidepressant drugs on fear-motivated behavior are reviewed. The use of ketamine as a rapid-acting antidepressant is then discussed, along with the pharmacokinetic mechanisms which may underlie its antidepressant qualities. Concluding the chapter is an overview of the specific research questions that each experiment in this dissertation sought to address, and their respective hypotheses.

2. Diathesis, Stress, and the Role of HPA Axis Dysfunction in Depression

2.1. The Diathesis-Stress Model of Depression

There is no singular cause of depression; rather, the manifestation of a major depressive episode involves a complex interplay between genetics, personal history, and stress. Genetic diatheses are believed to account for 31-42% of variability in the development of depression, making it a highly heritable disorder (Sullivan, Neale, & Kendler, 2000). However, there remains a large contribution of individual-specific environmental effects (Sullivan et al., 2000), which

may include historical risk factors for depression such as childhood abuse (Kendler, Gardner, & Prescott, 2002; Kendler, Gardner, & Prescott, 2006) and early parental loss or separation (Slavich, Monroe, & Gotlib, 2011). The most salient and immediate factor preceding the onset of depression, however, is the recent experience of a stressful life event. Individuals who have recently experienced divorce, interpersonal loss (Farmer & McGuffin, 2003; Kessing, Agerbo, & Mortensen, 2003), severe humiliation (Farmer & McGuffin, 2003), or social rejection (Slavich, O'Donovan, Epel, & Kemeny, 2010) are at considerably greater risk for the development of a depressive episode. It is in part for this reason that stress is now widely believed to be a fundamental precipitating factor in the etiology of depression (Deutschenbaur et al., 2015; Monroe & Simons, 1991; Pittenger & Duman, 2008; Willner et al., 2013).

The severity of stress that precedes a depressive episode does not appear to be the same for all individuals (Dienes, Hammen, Henry, Cohen, & Daley 2006; Kendler et al., 2001). While some might interpret this as evidence against the contribution of stressful life events to the development of depression, this observation can be readily explained by the diathesis-stress model (Monroe & Simmons, 1991). Variation in one's genetic and historical (i.e., early-life risk factors) diatheses will likewise produce variation in the amount of stress required to elicit depression. Theoretically, a minor stressor could be sufficient to lead to a depressive episode in an individual with a strong diathesis, whereas an individual with a weak diathesis would necessitate a severe stressor for the same result. In support of this theory, a large twin study found that women at a low genetic risk for depression were far more likely to experience a severely stressful life event prior to the onset of depression than women at a high genetic risk (Kendler, Thornton, & Gardner, 2001). Similarly, lower levels of stress preceded bipolar depression in at-risk adults with early experience of adversity compared to adults without early experience of adversity (Dienes et al., 2006). The reason for why diatheses appear to lower the threshold of stress needed to develop depression is unsurprisingly complicated (see Willner et al., 2013 for review), but one essential element is that both genetic risk factors for depression (Gotlib, Joormann, Minor, & Hallmayer, 2008) and the experience of early life stressors (Essex et al., 2011; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Wilkinson & Goodyer, 2011) impair the brain's ability to regulate concentrations of potentially harmful stress hormones.

2.2. The Role of the HPA Axis in the Regulation of Stress

When an organism encounters an environmental stressor, a neuroendocrine system known as the hypothalamic-pituitary-adrenal (HPA) axis is activated in order to effectively adapt the body to these challenges towards homeostasis. The process is initiated by activation of the paraventricular nucleus of the hypothalamus through amygdalar inputs, which stimulates the release of corticotropin-releasing factor (CRF). The pituitary gland reacts to the presence of CRF by producing adrenocorticotropin hormone (ACTH), which then prompts the adrenal cortex to secrete glucocorticoids (cortisol in humans; CORT in rodents) into the bloodstream (Herman & Cullinan, 1997). Acute glucocorticoid release has beneficial effects on cognition that help an organism adapt to stressful events, but prolonged glucocorticoid exposure can have serious maladaptive physiological and behavioral consequences (de Kloet, Oitzl, & Joëls, 1999).

In the healthy brain, regulation of the HPA axis is accomplished through a number of internal excitatory and inhibitory feedback mechanisms that maintain homeostasis (de Kloet, Vreugdenhil, Oitzl, & Joëls, 1998; Willner et al., 2013). Relative levels of glucocorticoids are evaluated by the brain through the binding of these hormones to mineralocorticoid receptors and glucocorticoid receptors. Mineralocorticoid receptors are widely activated during basal levels of stress due to their high affinity for cortisol and CORT, and play an important role in maintaining stable neuronal firing through small calcium (Ca^{2+}) currents (de Kloet et al., 1998; Reul & de Kloet, 1985). Conversely, glucocorticoid receptors have a tenth the binding affinity of mineralocorticoid receptors, and as a result are only widely activated when cortisol or CORT levels are high (e.g., during times of stress; de Kloet et al., 1998). Glucocorticoid receptor activation in the hippocampus initiates negative feedback control over the HPA axis to inhibit further stress hormone release and protect the brain from excessive levels of stress (de Kloet et al., 1998; Herman & Cullinan, 1997; Jacobson & Sapolsky, 1991; Nestler et al., 2002; Willner et al., 2013). In addition to the hippocampus, the dorsomedial prefrontal cortex and the prelimbic cortex also serve a vital role in the inhibition of HPA axis activity (Jankord & Herman, 2008; Ulrich-Lai & Herman, 2009). The amygdala, however, has an excitatory effect on the HPA axis in response to glucocorticoid receptor activation, and stimulates further cortisol release in a positive feedback loop (Duvarci & Paré, 2007). The integrity of the hippocampus and other inhibitory structures is thus imperative in counteracting the amygdala and maintaining healthy HPA axis function, as damage to any of these structures could upset the fine balance of

homeostasis (Herman, Prewitt, & Cullinan, 1996; Jacobson & Sapolsky, 1991; Magariños, Somoza, & de Nicola, 1987; Nestler et al., 2002). For example, hippocampal lesioning has been shown to upregulate CRF mRNA and increase glucocorticoid release in rats and primates, presumably due to decreased inhibitory HPA axis control (Herman & Cullinan, 1997; Jacobson & Sapolsky, 1991).

2.3. HPA Axis Dysfunction in Depression

There is a large body of evidence supporting a strong relationship between HPA axis dysfunction and depression. Patients with depression exhibit elevated mean levels of plasma and urinary cortisol, and circadian cortisol secretion rhythmicity is likewise disturbed (Carroll, Curtis, & Mendels, 1976; Sachar, 1975). Contributing to this effect, the adrenal cortex of depressed individuals is hyperresponsive to ACTH, resulting in greater secretion of cortisol in response to this hormone than normal (Amsterdam, Maislin, Abelman, Berwish, & Winokur, 1986; Amsterdam, Maislin, Droba, & Winokur, 1987). The importance of elevated cortisol in the etiology of depression is underscored by the fact that Cushing's disease, a disorder caused by chronic excessive cortisol secretion, is highly comorbid with depression (Sonino & Fava, 2002). It is noteworthy that depressive symptomatology is ameliorated by correction of hypercortisolemia in the course of treatment for Cushing's disease (Starkman, Schteingart, & Schork, 1986).

A widely used tool in the study of depression's relationship with stress is the dexamethasone suppression test, which assesses the integrity of HPA axis inhibitory feedback control in response to glucocorticoid receptor activation. When dexamethasone binds to glucocorticoid receptors in healthy individuals, the potent steroid initiates inhibition of ACTH secretion, and in turn, downstream suppression of cortisol release (Holsboer, 2001). Studies have reliably demonstrated that depressed patients lack this dexamethasone-induced suppression of plasma cortisol levels, suggesting an impairment of HPA axis inhibition mechanisms (Amsterdam et al., 1986; Amsterdam et al., 1987; Carrol et al., 1976; Heuser et al., 1996; Holsboer, Lauer, Schreiber, & Krieg, 1995; Holsboer-Trachsler et al., 1994; Zobel, Yassouridis, Frieboes, & Holsboer, 1999; Zobel et al., 2000). Dexamethasone-induced cortisol suppression is normalized in depressed patients following successful antidepressant treatment (Heuser et al.,

1996; Holsboer-Trachsler et al., 1994), but remains impaired in treatment-resistant patients and those who relapse within a 6 month period (Zobel et al., 1999; Zobel et al., 2000).

Abnormal HPA axis functioning is also noted in individuals possessing significant risk factors for depression. Nondepressed individuals who have first-degree relatives with depression (high familial risk) exhibit impaired dexamethasone-CRH suppression of cortisol, though not to the same degree as those currently experiencing a major depressive episode (Holsboer et al., 1995). The experience of early life stressors is also associated with HPA axis hyperreactivity and an impaired ability to regulate stress in both children and adults (Essex et al., 2011; Francis & Meaney, 1999; Heim et al., 2008; Heim & Nemeroff, 2001; Wilkinson & Goodyer, 2011), an effect that is linked to the epigenetic modification of glucocorticoid receptors (Wilkinson & Goodyer, 2011). Experimental research has revealed similar HPA axis dysfunction in adult rats that have experienced maternal separation early in life (Rentesi et al., 2010). In line with the diathesis-stress model of depression, these genetic and early-life risk factors increase the odds of developing depression by impairing one's ability to cope with stress, exposing individuals to chronically elevated levels of cortisol, thereby reducing the threshold of stress required to trigger a depressive episode (Willner et al., 2013).

2.4. The Neurophysiological and Functional Consequences of Prolonged Stress Exposure

Although acute challenges of stress can normally be readily managed through HPA axis inhibition mechanisms, prolonged exposure to glucocorticoids is neurotoxic, and has profound negative effects on the morphology and functioning of the brain. Chronically elevated levels of glucocorticoids can arise from stressful life experiences, genetic and/or historical risk factors for depression, or a combination of both. Neurons in the hippocampus, prefrontal cortex (PFC), and amygdala are abundant in glucocorticoid receptors, making these structures highly vulnerable to the deleterious effects of chronic stress on morphological neuroplasticity (Feldman & Weidenfeld, 1999; Herman et al., 2003; Herman, Ostrander, Mueller, & Figueiredo, 2005; Mitra & Sapolsky, 2008; Morimoto et al., 1996).

In studies investigating the physiological and behavioral effects of chronic glucocorticoid exposure in rodents, elevated basal CORT levels can be achieved through long-term restraint stress or unpredictable stress paradigms (Ayensu et al., 1995; Bachis, Cruz, Nosheny, & Mochetti, 2008; Bielajew, Konkle, & Merali, 2002; Kitayama et al., 1989; Konkle et al., 2003;

Pitman, Ottenweller, & Natelson, 1988; Rodríguez Echandia, Gonzalez, Cabrera, & Fraccia, 1988; Song, Che, Min-Wei, Murakami, & Matsumoto, 2006; Ushijima, Morikawa, Higuchi, & Ohdo, 2006; Vogel & Jensh, 1988). Alternatively, behavioral stress manipulations can be circumvented entirely by systemically injecting exogenous CORT solutions into test subjects over a period of weeks. Prolonged exposure to unpredictable stress is associated with reduced hippocampal volume in rats (Delgado et al., 2011), which is believed to result from pathological alterations in neuronal morphology (Czeh & Lucassen, 2007; Tata & Anderson, 2010). Dendritic atrophy of CA3 and CA1 hippocampal pyramidal neurons occurs following chronic restraint stress (Magariños & McEwen, 1995a; Magariños & McEwen, 1995b; Watanabe, Gould, Daniels, Cameron, & McEwen, 1992a; Watanabe, Gould, & McEwen, 1992b), chronic unpredictable stress (Magariños & McEwen, 1995a; Magariños, McEwen, Flügge, & Fuchs, 1996; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000), or chronic exogenous CORT administration (Magariños, Orchinik, & McEwen, 1998; Morales-Medina, Sanchez, Flores, Dumont, & Quirion, 2009; Sousa et al., 2000; Watanabe et al., 1992a; Woolley, Gould, & McEwen, 1990). Treatment with a steroid synthesis blocker prevents dendritic atrophy in chronically stressed rats, suggesting that CORT secretion is an essential driving factor behind the neuronal damage (Magariños & McEwen, 1995b). Chronic stress also causes a profound loss of mossy fiber CA3 synapses, an observation that occurs independently of hippocampal volume reductions (Sousa et al., 2000; Tata, Marciano, & Anderson, 2006). This finding demonstrates that stress-induced damage to the hippocampus extends far beyond what is merely suggested by loss in structural volume (Tata et al., 2006). Impaired morphological plasticity, as evidenced by dendritic atrophy and reduced dendritic spine density, has likewise been found in the medial prefrontal cortex (mPFC) in response to chronic restraint stress (Cook & Wellman, 2004; Liston et al., 2006; Liu & Aghajanian, 2008; Radley et al., 2004), chronic unpredictable stress (Dias-Ferreira et al., 2009; Izquierdo, Wellman, & Holmes, 2006; Li et al., 2011; Liu & Aghajanian, 2008; Shansky & Morrison, 2009), and chronic exogenous CORT exposure (Liu & Aghajanian, 2008; Wellman, 2001).

Not all structures of the brain are affected in the same way by prolonged exposure to glucocorticoids, however. Some regions remain apparently unchanged in the face of chronic stress (Liston et al., 2006), but the amygdala undergoes alterations in morphological plasticity that pathologically intensifies its function (Drevets, 2003). Chronic daily restraint stress and

CORT treatment lead to long-lasting dendritic hypertrophy and increased dendritic spine density in the amygdala of rats (Mitra, Jadhav, McEwen, Vyas, & Chattarji 2005; Mitra & Sapolsky, 2008; Vyas, Bernal, & Chattarji, 2003; Vyas, Jadhav, & Chattarji, 2006; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002; Vyas, Pillai, & Chattarji, 2004).

The brains of human patients with depression exhibit similar alterations in morphology to those observed in chronically stressed rats. Magnetic resonance imaging reveals reductions of hippocampal volume in depressed individuals (Campbell, Marriott, Nahmias, & Macqueen, 2004; Cole et al., 2010; Colla et al., 2007; Lange & Irle, 2004; Sheline, Gado, & Kraemer, 2003; Sheline, Sanghavi, Mintun, & Gado, 1999; Videbech & Ravnkilde, 2004), with specific deformations in CA1, CA2, and CA3 subregions (Cole et al., 2010). Longer durations of untreated depression are correlated with greater hippocampal volume loss, as is the number of past depressive episodes (Cole et al., 2010; Colla et al., 2007; Sheline et al., 1999; Sheline et al., 2003). Antidepressant treatment reverses volume loss (Frodl et al., 2008; Sheline et al., 2003), but smaller hippocampal volumes and a history of depression predict poorer clinical outcomes (Frodl et al., 2008). The PFC has not been studied in humans as intensively as the hippocampus, but volume loss has been detected in the anterior cingulate cortex (Phillips, Drevets, Rauch, & Lane, 2003; Savitz & Drevets, 2009; van Tol et al., 2010). Similar to rats treated with chronic exogenous CORT, hypertrophy is also present in the amygdala of depressed humans (Bremner et al., 2000; Frodl et al., 2002; Lange & Irle, 2004), which is associated with greater levels of activity as measured by positron emission tomography (Drevets et al., 1992). Although these depression-associated alterations in brain morphology cannot be causally tied to chronic glucocorticoid exposure, it should be noted that patients with hypercortisolemia resulting from Cushing's Disease exhibit similar hippocampal atrophy, which is reversed following successful treatment and reduction of cortisol levels (Bourdeau et al., 2002; Heinz, Martinez, & Haengeli, 1977; Starkman et al., 1999).

The dendritic remodeling initiated by chronic stress presents a serious threat to HPA axis homeostasis. As previously discussed, the hippocampus and PFC serve a critical function in inhibiting HPA axis activity and glucocorticoid release (de Kloet et al., 1998; Willner et al., 2013). It is therefore likely that stress-induced dendritic atrophy and loss of synapses in these structures could lead to further impairment of negative feedback control (Nestler et al., 2002). Prolonged stress also leads to a loss of hippocampal glucocorticoid receptors, compromising the

means by which excessive glucocorticoid levels are detected by the brain in the first place (Raison & Miller, 2003). Meanwhile, neurons in the amygdala, which function to promote stress hormone secretion, are strengthened by chronic stress and become hyperreactive (Duvarci & Paré, 2007; Pittenger & Duman, 2008), biasing the HPA axis towards a system of positive feedback. A vicious cycle is thereby created in which the brain becomes increasingly incapable of managing stress and preventing continued neuronal damage caused by excessive glucocorticoid exposure (Willner et al., 2013). Since depression is consistently accompanied by increased cortisol levels and impaired stress response, it is believed that this process of stress-induced dendritic modification and HPA axis dysfunction plays a role in the development of the disorder (Holsboer, 2001; Nestler et al., 2002; Parker, Schatzberg, & Lyons, 2003; Sachar, 1976; Sachar & Baron, 1979; Sterner & Kalynchuk, 2010).

Some of the functional implications of chronic stress exposure can be seen through its effects on long-term potentiation (LTP) and long-term depression (LTD). LTP is a component of neuroplasticity that is characterized by a strengthening of synaptic efficacy between presynaptic and postsynaptic neurons that experience temporally coincident depolarization (Bliss & Collingridge, 1993). This activity-dependent potentiation of synaptic transmission is believed to play a fundamental role in learning and memory by forming new associative pathways in the brain (Malenka & Bear, 2004). Conversely, LTD is an opposing process that weakens synaptic efficacy, making it less likely for a presynaptic neuron to stimulate depolarization in a postsynaptic neuron (Bear & Malenka, 1994). Stress has the potential to modulate synaptic efficacy because the induction of LTP and LTD is regulated by extracellular stress hormone concentrations. Whereas LTP is favored when cortisol binding to glucocorticoid receptors is low, high levels of cortisol binding induce LTD instead (Coussens, Kerr, & Abraham, 1997; Diamond, Bennett, Fleshner, & Rose, 1992; Pavlides, Ogawa, Kimura, & McEwen, 1996; Rey, Carlier, Talmi, & Soumireu-Mourat, 1994). Accordingly, chronic behavioral stress has been found to impair hippocampal LTP in rodents while facilitating LTD (Foy, Stanton, Levine, & Thompson, 1987; Garcia, Vouimba, & Jaffard, 1997; Kim, Foy, & Thompson, 1996; Pavlides, Nivón, & McEwen, 2002; Shors, Seib, Levine, & Thompson, 1989; Xu, Anwyl, & Rowan, 1997). In addition, chronic stress impairs LTP in the hippocampal-PFC pathway, a neural system that is integral in suppressing amygdalar activity (Cerqueira, Mailliet, Almeida, Jay, & Sousa, 2007). Considering LTP's importance in learning and memory, it is not surprising that chronic

CORT exposure and depression is associated with an array of pathological alterations in cognition, particularly in processes that depend upon function of the hippocampus, PFC, and amygdala (Sterner & Kalynchuk, 2010).

3. Glutamatergic System Dysregulation in the Pathophysiology of Depression

3.1. Introduction to the Glutamate Hypothesis of Depression

While it is apparent that chronic excessive glucocorticoid exposure impairs the morphological plasticity of prefrontal and hippocampal neurons, the mechanisms through which this damage takes place have not yet been discussed. It is now widely believed that glucocorticoid-induced dysregulation of glutamatergic neurotransmission underlies the neuronal damage and impaired synaptic plasticity observed in depressed patients, a theory that is referred to as the glutamate hypothesis of depression (Deutschenbaur et al., 2015; Maeng & Zarate, 2007; Pittenger & Duman, 2008; Pittenger et al., 2007; Sanacora et al., 2012; Willner et al., 2013).

Glutamate is the most commonly utilized excitatory neurotransmitter in the brain, and plays a fundamental role in learning and memory by regulating mechanisms of neuroplasticity (Sanacora, Zarate, Krystal, & Manji, 2008). Glutamatergic neurotransmission occurs both through fast-acting ionotropic receptors, as well as slower-acting metabotropic receptors. Ionotropic glutamate receptors include the α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor, which functions as the primary mechanism of excitatory synaptic transmission at resting membrane potentials, and the N-methyl-D-aspartate (NMDA) receptor, which initiates LTP through voltage-dependent activation and increased AMPA receptor subunit membrane insertion (Deutschenbaur et al., 2015; Johansen, Cain, Ostroff, & LeDoux, 2011; Maeng & Zarate, 2007; Nowak, Bregestovski, Ascher, Herbet, & Prochiantz, 1984; Pape & Paré, 2010; Sah, Westbrook, & Lüthi, 2008).

In order to maintain homeostasis and normal neuronal functioning, synaptic glutamate is constantly and rapidly cleared by glutamate transporters located in astrocyte glial cells, thus preventing extracellular neurotransmitter accumulation (Pittenger et al., 2007). Successful and sustained glutamate clearance is imperative to neuronal survival, as high levels of extracellular glutamate can lead to excitotoxicity through stimulation of extrasynaptic NMDA receptors. Unlike synaptic NMDA receptors, which serve a critical function in healthy neurotransmission and promote mechanisms of neuroplasticity (Deutschenbaur et al., 2015; Pittenger et al., 2007),

extrasynaptic NMDA receptors initiate intracellular processes that impair neuroplasticity (Hardingham, Fukunaga, & Bading, 2002; Ivanov et al., 2006) and even promote cell death (Ankarcrona et al., 1995; Manev, Favaron, Guidotti, & Costa, 1989). Accordingly, glutamate-induced neurotoxicity and cell death is prevented by administration of NMDA receptor antagonists (Manev et al., 1989), which block extrasynaptic NMDA receptors from permitting Ca^{2+} influx and triggering anti-plasticity cascades (Hardingham et al., 2002). Extrasynaptic NMDA receptor activation impairs neuroplasticity by decreasing activation of the cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) transcription factor (Hardingham et al., 2002; Ivanov et al., 2006), which in turn downregulates the production of brain-derived neurotrophic factor (BDNF; Conti, Cryan, Dalvi, Lucki, & Blendy, 2002; Hardingham et al., 2002; Tao, Finkbeiner, Arnold, Shaywitz, & Greenberg, 1998). The BDNF protein plays an important role in promoting mechanisms of synaptic plasticity (Horch, Krüttgen, Portbury, & Katz, 1999; Yoshii & Constantine-Paton, 2007; Yoshii & Constantine-Paton, 2010) and is necessary for the induction of LTP (Kang, Welcher, Shelton, & Schuman, 1997; Korte, Staiger, Griesbeck, Thoenen, & Bonhoeffer, 1996; Patterson et al., 1996), so the loss of BDNF resulting from excessive glutamate exposure poses a significant threat to normal learning, memory, and processing in the brain (Heldt, Stanek, Chhatwal, & Ressler, 2007).

The glutamate hypothesis of depression postulates that the experience of chronic stress causes a sustained and excessive accumulation of glucocorticoids in the brain, which then bind to neurons expressing glucocorticoid receptors (especially those in the PFC and hippocampus) and initiate an enhanced presynaptic release of glutamate (Sanacora et al., 2012; Stein-Behrens et al., 1992; Stein-Behrens, Lin, & Sapolsky, 1994; Venero & Borrell, 1999). This increase in synaptic glutamate release, coupled with a stress-induced impairment of astrocyte-mediated synaptic glutamate clearance, may then lead to an accumulation of extracellular glutamate and the activation of extrasynaptic NMDA receptors. The resulting downregulation of CREB expression would in turn downregulate BDNF, and thus lead to an impairment of synaptic plasticity and mechanisms of cell survival (Pittenger & Duman, 2008; Pittenger et al., 2007). It is believed that the neuronal damage caused by excessive glutamate exposure in regions such as the PFC and hippocampus may underlie some of the cognitive and behavioral pathologies typically observed in depression (Deutschenbaur et al., 2015; Pittenger & Duman, 2008; Pittenger et al., 2007; Sanacora et al., 2012).

Paradoxically, high levels of extracellular glutamate might ultimately lead to an impairment of overall glutamatergic neurotransmission by damaging dendrites and synapses containing AMPA and NMDA receptors (Deutschenbaur et al., 2015; Pittenger et al., 2007). The functional and neurobiological effects of this damage would be compounded by the fact that a loss of synaptic NMDA receptor activation would also decrease the postsynaptic membrane insertion of AMPA receptors (Pittenger et al., 2007). Since both synaptic NMDA and AMPA receptor activation induces BDNF (Hardingham et al., 2002; Jourdi et al., 2009), impaired ionotropic glutamate receptor activity would exacerbate the downregulation of this important neurotrophin.

3.2. Evidence from Animal Research

The glutamate hypothesis of depression is supported by considerable evidence from animal research demonstrating a mediatory relationship between stress, a major precipitating factor of depression that causes neuronal damage, and extracellular glutamate accumulation, an inducer of depression-like neuronal damage. Acute and chronic behavioral stress increases extracellular glutamate levels in the PFC and hippocampus (Bagley & Moghaddam, 1997; de Vasconcellos-Bittencourt et al., 2011; Fontella et al., 2004; Lowy, Gault, & Yamamoto, 1993; Moghaddam, 1993; Moghaddam, Bolinao, Stein-Behrens, & Sapolsky, 1994; Musazzi et al., 2010; Reznikov et al., 2007; Satoh & Shimeki, 2010; Wang & Wang, 2009), two brain regions which also exhibit neuronal damage in chronic stress paradigms and human depression, as previously discussed. Glucocorticoid release underlies stress-induced increases of extracellular glutamate, as prior removal of the CORT-releasing adrenal gland abolishes the majority of excess glutamate accumulation in stressed animals (Lowy et al., 1993; Moghaddam et al., 1994). Furthermore, direct administration of CORT through injection or pellet implantation causes a dose-dependent increase of extracellular glutamate in the hippocampus (Stein-Behrens et al., 1992; Stein-Behrens et al., 1994; Venero & Borrell, 1999), with levels reaching as high as 4x above baseline at doses equivalent to behavioral stress (Stein-Behrens et al., 1994).

The extrasynaptic accumulation of glutamate following chronic stress suggests that astrocytes and excitatory amino acid transporters are unable to adequately clear the extracellular space of this neurotransmitter relative to the rate of presynaptic release. This effect might be the result of stress-induced increases in presynaptic glutamate release (Pittenger et al., 2007; Satoh

& Shimeki, 2010; Wang & Wang, 2009), but could also be exacerbated by the fact that chronic stress and CORT exposure impairs hippocampal glutamate uptake (Almeida et al., 2010; Banasr et al., 2010; de Vasconcellos-Bittencourt et al., 2011; Yang, Huang, & Hsu, 2005; Virgin et al., 1991). Hippocampal and cortical expression of glutamate transporters is reduced in a learned helplessness rodent model of depression, suggesting one means by which impaired glutamate uptake might occur (Zink, Vollmayr, Gebicke-Haerter, & Henn, 2010). In addition, chronic behavioral stress reduces the number and proliferation of astrocytes in the PFC (Banasr & Duman, 2008; Banasr et al., 2007; Czéh et al., 2007; Leventopoulos et al., 2007), as well as the number of astrocytes in the hippocampus (Liu et al., 2011). Supporting a role for disrupted glutamate clearance in the etiology of depression's behavioral symptomatology, blocking glutamate uptake in PFC glial cells produces anhedonia in rats (John et al., 2012). Conversely, reversal of stress-induced glial dysfunction with the antiglutamatergic drug riluzole is accompanied by a similar reversal of anhedonic behavior (Banasr et al., 2010). Treatment with riluzole also demonstrates antidepressant qualities in the forced swim and incentive disengagement tests of depression-like behavior (Gourley, Espita, Sanacora, & Taylor, 2012). Further illustrating a modulatory role for glutamate clearance in depression-like behavior, stimulation of glutamate uptake with ceftriaxone produces antidepressant-like behavioral effects in the tail suspension and forced swim tests (Mineur, Picciotto, & Sanacora, 2007).

Evidence suggests that stress-induced extracellular glutamate accumulation likely leads to the activation of extrasynaptic NMDA receptors, which in turn inhibits intracellular mechanisms of neuroplasticity and alters behavior. First, the hippocampal neuronal damage caused by chronic stress is prevented by NMDA receptor antagonism, implicating glutamatergic neurotransmission as a downstream mediating factor of the neurophysiological effects of glucocorticoid exposure (Duman, Li, Liu, Duric, & Aghajanian, 2012; Magariños & McEwen, 1995b). Additionally, stress-induced impairments in LTP and enhancements in LTD are dependent upon extrasynaptic NMDA receptor function, linking extracellular glutamate accumulation to functional modification of the brain (Kim et al., 1996; Yang et al., 2005). Finally, NMDA antagonists produce a variety of antidepressant-like behavioral effects in rodents, including reductions of depression-like behavior in tests for anhedonia (Carrier & Kabbaj, 2013; Garcia et al., 2009; Li et al., 2011; Papp & Moryl, 1993; Papp & Moryl, 1994; Papp & Moryl, 1996), behavioral despair (Autry et al., 2011; Carrier & Kabbaj, 2013;

Chaturvedi, Chandra, & Bapna, 1999; Gigliucci et al., 2013; Koike et al., 2013; Koike, Iijima, & Chaki, 2011; Li et al., 2010; Maeng et al., 2008; Moryl, Danysz, & Quack, 1993; Popik, Kos, Sowa-Kućma, & Nowak, 2008; Perrine et al., 2014; Przegaliński, Tatarczyńska, Dereń-Wesołek, & Chojnacka-Wojcik, 1997; Trullas & Skolnick, 1990; Yilmaz, Schulz, Aksoy, & Canbeyli, 2002), negative cognitive bias (Amann et al., 2009; Pietersen et al., 2006), and cognitive flexibility (Jett et al., 2015). The antidepressant-like properties of NMDA antagonists extend to the abolishment of depression-like behaviors induced by chronic stress paradigms (Chaturvedi et al., 1999; Garcia et al., 2009; Gigliucci et al., 2013; Jett et al., 2015; Koike et al., 2011; Koike et al., 2013; Li et al., 2011; Papp & Moryl, 1993; Papp & Moryl, 1994; Papp & Moryl, 1996; Perrine et al., 2014; Trullas & Skolnick, 1990), and are accompanied by the normalization of circulating CORT (Garcia et al., 2009), promotion of synaptic plasticity (Li et al., 2010), and reversal of stress-induced neuronal atrophy (Li et al., 2011). Although NMDA antagonists can bind to both synaptic and extrasynaptic glutamate receptors, they are more likely to bind extrasynaptically due to the greater presence of high affinity Grin2b NMDA receptor subunits in this area and the reduced binding competition with endogenous glutamate in extracellular space (Pittenger & Duman, 2008). Combined with the fact that synaptic NMDA receptors promote neuroplasticity while extrasynaptic NMDA receptors oppose it, it is likely that the antidepressant morphological and behavioral effects of NMDA antagonists occur primarily through blocking extrasynaptic NMDA receptors (Krystal et al., 2013; Pittenger & Duman, 2008). It is thus clear from the reviewed research that NMDA receptor activation is a significant mediating factor of stress-induced neuronal damage and behavioral disorder, positioning the glutamate system as a valuable target for antidepressant action.

In addition to morphological and functional neuronal deficits, chronic stress is associated with intracellular neurobiological changes that are consistent with extrasynaptic NMDA receptor activation. Exposure to chronic stress reduces hippocampal CREB transcriptional activity in rodents alongside increases in anhedonic behavior (Gourley, Kiraly, Howell, Olausson, & Taylor, 2008a; Grønli et al., 2006). Chronic stress also decreases CREB protein expression and CREB phosphorylation in the PFC (Liu et al., 2014; Zhu et al., 2016). Levels of CREB have been demonstrated to mediate depression-like behavior. For example, viral vector-enhanced hippocampal CREB expression produces an antidepressant response in tests for behavioral despair and learned helplessness (Chen, Shirayama, Shin, Neve, & Duman, 2001a). One study

has also demonstrated that chronic activation of glucocorticoid receptors with CORT inhibits CREB transcriptional activity in clonal neurons (Föcking, Hölker, & Trapp, 2003), which would directly impair CREB's downstream effects. Since CREB regulates transcription of the BDNF gene, it is unsurprising that chronic stress and CORT exposure decrease BDNF mRNA and protein expression in the hippocampus of rodents (Chen, Li, Zhao, & Yang, 2008; Dwivedi, Rizavi, & Pandey, 2006; Filho et al., 2015; Fu et al., 2014; Gourley et al., 2008a; Grønli et al., 2006; Hamani et al., 2012; Jacobsen & Mørk, 2006; Jin, Yu, Tian-Lan, & Quan, 2015; Li et al., 2012; Liang et al., 2012a; Liang et al., 2012b; Liu et al., 2014; Liu et al., 2015; Luoni, Macchi, Papp, Molteni, & Riya, 2014; Monsey et al., 2014; Nibuya, Takahashi, Russell, & Duman, 1999; Nitta, Fukumitsu, Kataoka, Nomoto, & Furukawa, 1997; Smith, Makino, Kvetnansky, & Post, 1995; Yan et al., 2015; Zhang et al., 2015a). BDNF expression within the PFC is likewise impaired as a result of chronic stress paradigms (Aboul-Fotouh, 2015; Chen et al., 2008; Filho et al., 2015; Fu et al., 2014; Guo et al., 2014; Jin et al., 2015; Liang, Lu, Cui, Wang, & Tu, 2012a; Liu et al., 2014; Liu et al., 2015; Ray et al., 2011; Yan et al., 2015; Zhang et al., 2015a; Zhu et al., 2016). While CREB mediates the transcription of a number of genes, its effects on BDNF are likely of the greatest relevance to depression, as mice with forebrain (Monteggia et al., 2007) or hippocampal (Adachi, Barrot, Autry, Theobald, & Monteggia, 2008; Taliaz, Stall, Dar, & Zangen, 2010) BDNF gene deletions exhibit a variety of depression-like behaviors. Conversely, central (Hoshaw, Malberg, & Lucki, 2005; Siuciak, Lewis, Wiegand, & Lindsay, 1997) or hippocampal (Gourley et al., 2008a; Shirayama, Chen, Nakagawa, Russell, & Duman, 2002;) BDNF infusions have antidepressant-like effects in rodent models of depression, even following chronic stress (Schmidt & Duman, 2010).

3.3. Evidence from Human Research

Although the majority of research investigating the glutamate hypothesis of depression has utilized rodent subjects, human patient research has replicated many of these findings and supports an association between depression and glutamatergic dysfunction. Blood serum glutamate content is increased in depressed patients compared to healthy controls (Altamura et al., 1993; Almaturo, Maes, Dai, & Meltzer, 1995; Kim, Schmid-Burgk, Claus, & Kornhuber, 1970; Küçükbrahimoğlu et al., 2009; Mauri et al., 1998; Mitani et al., 2006), and is positively correlated with depressive symptomatology (Mitani et al., 2006). Additionally, postmortem

analysis of brain tissue from individuals with depression has revealed increases in PFC glutamate levels (Hashimoto, Sawa, & Iyo, 2007), and both prefrontal (Jun et al., 2014; Lan et al., 2009) and hippocampal (Jun et al., 2014) glutamate levels are enhanced in patients with bipolar depression. Glial pathology has also been noted in depressed patients, including reductions in both the density and size of glial cells in various regions of the PFC (Miguel-Hidalgo et al., 2002; Ongür, Drevets, & Price, 1998; Rajkowska et al., 1999; Rajkowska, Halaris, & Selemon, 2001); reduced numbers of glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the PFC (Miguel-Hidalgo et al., 2010; Si, Miguel-Hidalgo, O'Dwyer, Stockmeier, & Rajkowska, 2004; Toro, Hallak, Dunham, & Deakin, 2006), hippocampus (Müller et al., 2001; Toro et al., 2006), and cerebellum (Fatemi et al., 2004); and reduced immunoreactivity of glutamate transporters (Choudary et al., 2005; Miguel-Hidalgo et al., 2010). Together, these disruptions of normal glial function could lead to a harmful accumulation of extracellular glutamate, triggering anti-neuroplasticity processes (Sanacora et al., 2012). In line with this theory, depressed individuals exhibit downstream pathological alterations in neurobiology that are consistent with extracellular glutamate accumulation and extrasynaptic NMDA receptor activation. Postmortem analyses have demonstrated reduced CREB in the PFC of depressed individuals (Dowlatshahi, MacQueen, Wang, & Young, 1998; Yamada, Yamamoto, Ozawa, Reiderer, & Saito, 2003), as well as reduced BDNF mRNA in the hippocampus and PFC of suicide victims (Dwivedi et al., 2003). Serum BDNF levels are also lower in patients with depression compared to healthy controls (Aydemir, Deveci, & Taneli, 2005; Gervasoni et al., 2005; Karege et al., 2002; Karege et al., 2005; Lee, Kim, Park, & Kim, 2007; Sen, Duman, & Sanacora, 2008; Shimizu et al., 2003), and are negatively correlated with the severity of depressive symptoms (Brunoni, Lopes, & Fregni, 2008; Deuschle, et al., 2013; Gervasoni et al., 2005; Karege et al., 2002; Kim et al., 2007b; Ricken et al., 2013; Shimizu et al., 2003). Although further human research is needed in this area, there is ample evidence to suggest that many of the same systems that are disordered in chronic stress animal models of depression are likewise disturbed in human depression.

If the glutamate hypothesis of depression is correct, then it would be expected that pharmacological intervention with any of the pathological processes described above would have antidepressant behavioral effects. Interestingly, virtually all classes of antidepressant treatments modulate the glutamatergic system and initiate neurobiological processes which oppose the effects of glutamate excitotoxicity. First, a variety of antidepressant drugs, including those from

the selective serotonin reuptake inhibitor (SSRI), monoamine oxidase inhibitor (MAOI), norepinephrine reuptake inhibitor (NERI), serotonin and norepinephrine reuptake inhibitor (SNRI), tricyclic, and melatonergic classes, have been shown to reduce depolarization-evoked presynaptic release of glutamate (Bonanno et al., 2005; Michael-Titus, Bains, Jeetle, & Whelpton, 2000; Musazzi et al., 2010; Reznikov et al., 2007), thus preventing stress-induced enhancements in extracellular glutamate accumulation (Musazzi et al., 2010; Reznikov et al., 2007). Second, the binding characteristics of the NMDA receptor are altered by imipramine, citalopram, and electroconvulsive therapy (ECT; Nowak, Trullas, Layer, Skolnick, & Paul, 1993; Paul, Nowak, Layer, Popik, & Skolnick, 1994; Skolnick, 1999), and NMDA receptor antagonists have powerful antidepressant effects in humans (Berman et al., 2000; Cusin, Hilton, Nierenberg, & Fava, 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price et al., 2009; Rasmussen et al., 2013; Zarate et al., 2006). Third, postmortem analysis of brain tissue from depressed patients has revealed that antemortem antidepressant treatment abolishes the reduced temporal cortex concentration of CREB that is otherwise present in untreated depressed individuals (Dowlatsahi et al., 1998). Furthermore, chronic administration of a wide variety of antidepressant drugs increases CREB mRNA and CREB protein in the hippocampus of rats (Nibuya, Nestler, & Duman, 1996), as well as CREB phosphorylation (Thome et al., 2000; Tiraboschi et al., 2004), CREB binding activity (Frechilla, Otano, & Del Rio, 1998), and CREB transcription (Thome et al., 2000) in the PFC and hippocampus. Finally, hippocampal (Chen et al., 2001b) and serum (Aydemir et al., 2005; Brunoni et al., 2008; Deuschle et al., 2013; Gervasoni et al., 2005; Ricken et al., 2013; Sen et al., 2008) BDNF concentrations are likewise increased in patients that have received antidepressant treatment compared to untreated patients with depression. Rodent research has also revealed increases in prefrontal and hippocampal BDNF mRNA resulting from several classes of antidepressant drugs and ECT (Coppell, Pei, & Zetterström, 2003; Dwivedi et al., 2006; Molteni et al., 2006; Nibuya, Morinobu, & Duman, 1995; Russo-Neustadt, Beard, & Cotman, 1999; Zetterström, Pei, & Grahame-Smith, 1998). It has also been found that the therapeutic actions of SSRI and tricyclic antidepressants are blocked by either whole-brain (Ibarguen-Vargas et al., 2009; Saarelainen et al., 2003), forebrain- (Monteggia et al., 2007), or hippocampal-specific (Adachi et al., 2008) BDNF gene knockouts in mice. It is noteworthy that antidepressant-induced upregulation of CREB and BDNF only occurs following chronic, but not acute, antidepressant

treatment, thus mirroring the time course of antidepressant behavioral effects (Coppell et al., 2003; Thome et al., 2000; Tiraboschi et al., 2004).

Although it is common for current antidepressant treatments to address glutamatergic dysfunction in one way or another, most do not target the brain's glutamatergic system directly, leading to a considerable delay in therapeutic effect. As a result, there is substantial room for improvement in antidepressant therapies, and drugs with a more targeted influence on the glutamatergic system may hold the future for faster-acting and more effective antidepressant treatments.

4. The Use of Animal Models in the Study of Depression-Like Behaviors

Human patient research has established a clear relationship between stress, HPA axis dysregulation, and depression, but the conclusions one can draw from these correlational studies alone are limited. Precise experimental manipulation of stress induction is required in order for causal inferences between glucocorticoid exposure and the development of depression to be made. Studies using animal models of depression thus fill an important void left by human patient research, and give researchers an opportunity to experimentally explore questions that could not be ethically addressed in humans. Some of these studies were discussed above in regards to the effects of prolonged stress on the brain, but animal models are also used to examine the stress-induced development of pathological depression-associated behaviors.

4.1. Considerations of Validity

The use of animal models in the study of a human psychiatric disorder such as depression introduces a number of inherent challenges. If the results from these studies are to produce meaningful insights into the etiology of depression and guide the development of novel therapeutics, then the animal models being used must bear remarkable resemblance to the human disorder on a number of levels. However, it is unlikely that any one animal model can successfully reconstruct every possible facet of a psychiatric illness, nor would it necessarily be realistic if it could. Depression is characterized by a significant degree of heterogeneity in symptoms among patients, some of which are contradictory (e.g., insomnia or hypersomnia), and others which are impossible to measure in non-human animals (e.g., suicidal ideation, feelings of worthlessness). Nevertheless, valuable animal models of depression can be developed within

these limits, provided they take into consideration a number of factors defining the validity of the experimental model. Belzung and Lemoine (2011) have developed a comprehensive set of criteria to evaluate the validity of psychiatric disorder animal models, the most important of which will be summarized below in specific reference to rodent studies of depression.

First, it is important that behavioral animal models have pathogenic validity: the processes leading to disease in rodents should be semantically similar to those believed to lead to depression in humans (i.e., recent or early life stress). These etiological factors must then in turn produce a physiological and behavioral pathology similar to the human disorder of interest, a criterion known as induction validity. There should also be a shared neurobiological mechanism underlying the disorder (mechanistic validity), such as the neurodegeneration observed in both humans and rodents following stress, in addition to similarity of biomarkers indicative of depression (biomarker validity; e.g., elevated glucocorticoid levels). Since pathological behavioral alterations are the primary feature of psychiatric disorders, face validity requires that animal models must feature behavioral symptoms that are analogous to those seen in humans with depression. Symptoms like anhedonia or cognitive impairment will manifest differently between humans and rodents, so it must be noted that the material similarity of these behaviors is less important to validity than equivalency in what they mean to the animals producing them. Finally, of prime importance to the development and testing of antidepressant treatments is the concept of predictive validity. Drugs and therapies that are effective in treating depression in humans should likewise be effective in reversing the symptoms of pathology produced in the animal model. Excellent predictive validity, in conjunction with the fulfillment of the accompanying validity criteria discussed above, can allow animal models to produce meaningful results in the evaluation, refinement, and development of antidepressant treatments (Belzung & Lemoine, 2011).

4.2. Animal Models of Depression

There are several different animal models available to researchers in the study of depression. Each model possesses a unique set of strengths and weaknesses in validity, and the choice of which model a researcher should use depends on the specific aims of the proposed study. Since the experience of prolonged stress appears to be a significant precipitating factor in the development of human depression, a number of models have utilized exposure to various

forms of chronic stress as a pathogenically valid method of producing depression-like physiological and behavioral pathology in animals. The induction of chronic stress in a study can take one of two general forms: experimenter-applied behavioral stress, or exogenous CORT administration. In the former category, the most commonly used paradigms include repeated restraint stress and chronic unpredictable stress. Repeated restraint stress involves daily immobilization of the rodent in a restraint apparatus for several hours, and is typically performed over period of 21 days. This method of applying chronic stress is successful at inducing the neuronal atrophy that is characteristic of depression (Beck & Luine, 2002; Conrad, Magariños, LeDoux, & McEwen, 1999; Cook & Wellman, 2004; Liston et al., 2006; Liu & Aghajanian, 2008; Magariños & McEwen, 1995a; Magariños & McEwen, 1995b; Radley et al., 2004; Watanabe et al., 1992a; Vyas et al., 2002; Vyas et al., 2003; Vyas et al., 2006), but produces inconsistent and sometimes contradictory alterations in depression-like behaviors (Dunn & Swiergiel, 2008; Platt & Stone, 1982), or fails to produce depressive symptomatology at all (Gregus, Wintink, Davis, & Kalynchuk, 2005; Lussier, Caruncho, & Kalynchuk, 2009; Perrot-Sinal, Gregus, Boudreau, & Kalynchuk, 2004). One explanation for these inconsistent behavioral effects is that is that rats can eventually habituate to the restraint procedure, causing a decrease in CORT release over subsequent sessions (Galea et al., 1997; Gregus et al., 2005; Grissom et al., 2007).

The chronic unpredictable stress model avoids the issue of procedure habituation by exposing rats to a variety of mild stressors, twice or several times a day, over a prolonged period of time (often 21 days). The stressors used in this protocol can include overnight food and water deprivation, alterations in colony room lighting (e.g., on during night, off during day, stroboscope during night), isolation, crowding, or abnormal home cage manipulations (e.g., tilt, rotation, bedding, odor), and are designed to be analogous to the daily life stressors that might precede the onset of depression. The chronic unpredictable stress model has excellent pathogenic and face validity and produces robust increases in depression-like behaviors (Willner, 1997; Willner, 2005), but its exceptional time and space requirements may exceed the available resources of many labs. One issue that is common amongst all experimenter-applied stress models is that the effects of chronic stress on behavior are liable to be influenced by individual variations in an animal's stress susceptibility and HPA axis response. While this variability is also present in random human populations, and may prove useful in the study of the differences

between at-risk and depression-resistant individuals, it is problematic if the researcher desires to study a homogenous sample of highly stressed animals with uniform depression-like behavior.

More precise control over stress exposure is made possible through chronic exogenous CORT administration models. Animals can be exposed to CORT daily through drinking water, pellet implantation, or subcutaneous injections over the course of 21 days. By exposing animals directly to CORT, these models bypass the subjective interpretation of a stressor, avoiding the possibility of stressor habituation and individual variability in stressor response. A daily injection paradigm was used in the present studies to ensure that every rat received the exact same dose of CORT by weight. As previously discussed, chronic CORT administration produces hippocampal (Magariños et al., 1998; Magariños et al., 1999; Morales-Medina et al., 2009; Sousa et al., 2000; Tata et al., 2006; Watanabe et al., 1992a; Woolley et al., 1990), prefrontal (Liu & Aghajanian, 2008; Morales-Medina et al., 2009; Seib & Wellman, 2003; Wellman, 2001), and amygdalar (Mitra & Sapolsky, 2008) alterations in neuronal morphology in rodents similar to that observed in human depression (Bremner et al., 2000; Campbell et al., 2004; Cole et al., 2010; Colla et al., 2007; Frodl et al., 2002; Lange & Irle, 2004; Sheline et al., 1999; Sheline et al., 2003; Videbeck & Ravnkilde, 2004). These pathological changes in neuroplasticity are believed to contribute to cognitive and behavioral symptoms of depression (Duman et al., 2012; Nestler et al., 2002; Pittenger & Duman, 2008; Sterner & Kalynchuk, 2010; Willner et al., 2013), providing the repeated exogenous CORT model with sound mechanistic validity.

4.3. Measuring the Effect of Chronic CORT Exposure on Depression-Like Behaviors

Depression is associated with a number of possible maladaptive alterations in cognition and behavior, which have become well-established through human research. However, practical and ethical constraints have restricted the extent of information one can glean regarding the etiology and treatment of these symptoms when using human patients. As a result, researchers have turned to the animal models described above in order to more extensively investigate cognitive and behavioral symptoms associated with depression. Numerous test protocols have been developed in order to study depression-like symptoms in animals through experimentally-induced changes in overt behavior. In the interest of face validity, the changes in animal behavior being measured should bear semantic similarity to the corresponding symptoms of human depression that the researchers are attempting to replicate. Through the use of the CORT

injection animal model of depression, in conjunction with behavioral test protocols, a wealth of evidence has been uncovered linking chronic stress to the development of depression-like behavior. The effects of chronic CORT on depression-like behaviors, the various tests used to assess behavioral pathology, and the face validity of these protocols will be discussed in detail below.

4.3.1. Anhedonia

Anhedonia, an inability to derive pleasure from normally enjoyable activities, is inferred in animal models of depression through an abnormally dampened response to natural rewards. The sucrose preference test takes advantage of the fact that rats possess an innate affinity for sweet substances. It measures a rat's consumption of a sucrose solution compared to plain water when both solutions are available *ad libitum* in identical bottles in the animal's home cage. Chronic CORT exposure decreases sucrose consumption (David et al., 2009; Gorzalka, Hanson, Harrington, Killam, & Campbell-Meiklejohn, 2003; Gourley et al., 2008a; Gupta, Radhakrishnan, & Kurhe, 2015; Wu et al., 2013; Yau et al., 2014), suggesting that the hedonic impact of this reward has been diminished. In addition to modified sucrose preference, rats undergoing chronic CORT exposure demonstrate anhedonia through decreased sexual activity (Gorzalka, Brotto, & Hong, 1999; Gorzalka & Hanson, 1998; Gorzalka, Hanson, & Hong, 2001), and an impaired value of food rewards (Gourley et al., 2008a; Gourley et al., 2008b). The stress-induced suppression of hedonic indulgence supports both the face validity of these anhedonia measures, as well the induction validity of the chronic exogenous CORT model.

4.3.2. Learned Helplessness

The forced swim test is a popular tool in the study of depression, and is believed to measure despair, resignation, and helplessness by presenting animals with the imminent threat of drowning (Porsolt, Anton, Blavet, & Jalfre, 1978; Porsolt, Le, & Jalfre, 1977). Animals are placed in a cylindrical tank of water from which escape is impossible, and their swimming behavior is recorded. Typically, rats will actively attempt to escape the tank for as long as possible, but premature passivity (i.e. immobility) is inferred to represent a cognitive state of learned helplessness. Chronic exposure to CORT reduces active coping and increases passive immobility in the forced swim test (Brummelte, Pawluski, & Galea, 2006; David et al., 2009;

Fenton et al., 2015; Gourley et al., 2008a; Gregus et al., 2005; Hill, Brotto, Lee, & Gorzalka, 2003; Kalynchuk, Gregus, Boudreau, & Perrot-Sinal, 2004; Koike et al., 2013; Marks, Fournier, & Kalynchuk, 2009; Murray, Smith, & Hutson, 2008; Wu et al., 2013) in a dose-dependent manner (Johnson, Fournier, & Kalynchuk, 2006; Zhao et al., 2008a; Zhao et al., 2008b). Consistent with the theory that prolonged stress is required for the development of depression, acute treatment of CORT is ineffective at increasing depression-like behavior in the forced swim test (Johnson et al., 2006; Stone & Lin, 2008; Zhao et al., 2008b).

Although the behavioral inferences made by the forced swim test have been questioned by some researchers (Borsini & Meli, 1988), the measure's greatest strength lies in its value as a predictive tool for effective antidepressant treatments. A wide array of known antidepressant therapies ameliorate depression-like behavior in the forced swim test (Porsolt et al., 1977; Porsolt et al., 1978), including imipramine (Fenton et al., 2015), ketamine (Koike et al., 2013), BDNF (Gourley et al., 2008a), spironolactone (mineralocorticoid receptor antagonist; Wu et al., 2013), metabotropic glutamate 2/3 receptor antagonists (Ago et al., 2013), sertraline (Ulloa et al., 2010), melatonin (Crupi et al., 2010), ECT (O'Donovan, Dalton, Harkin, & McLoughlin, 2014), piperine (Mao, Huang, Zhong, Xian, & Ip, 2014), peony glycosides (Mao, Huang, Ip, Xian, & Che, 2012), ginseng total saponins (Chen et al., 2014), and curcumin (Huang et al., 2011) in animals chronically injected with CORT. This excellent predictive validity makes the exogenous CORT model and forced swim test extremely valuable in the study and pursuit of novel therapeutics.

4.3.3. Negative Cognitive Biases in Attention, Processing, and Interpretation

The psychological effects of depression are not merely limited to subjective feelings of depressed mood, but extend to serious maladaptive alterations in cognition that cause individuals to perceive information and interact with their environment in an abnormally pessimistic manner. Depression is associated with pathological modifications in attention, processing, interpretation, and memory that bias individuals towards focus on exogenous and endogenous negative emotional content. Collectively referred to as a negative cognitive bias, these abnormalities in perception and memory are believed to support the development and maintenance of depression (Elliott et al., 2011; Harmer et al., 2009a; Robinson & Sahakian, 2008), emphasizing the need to

address these symptoms through psychological and pharmacological intervention (Clark, Chamberlain, & Sahkian, 2009; Elliott et al., 2011; Roiser, Elliott, & Sahakian, 2012).

The ways in which negative cognitive biases can manifest in depression are varied and numerous. Several affective biases are quantitatively measurable through cognitive testing, which has allowed researchers to identify many of the mental processes impaired by depression. Attention, the cognitive process responsible for selecting and maintaining focus on specific environmental stimuli, is pathologically altered in association with depression, causing profound changes in the way depressed individuals view their world. Compared to healthy controls, depressed individuals spend less time gazing at faces with happy expressions (Isaac, Vrijzen, Rinck, Speckens, & Becker, 2014) and instead exhibit an attentional bias for faces expressing sadness (Gotlib, Krasnoperova, Yue, & Joormann, 2004), an effect which extends to individuals with a high genetic predisposition to depression (Joorman, Talbot, & Gotlib, 2007). Additionally, depression is associated with decreased attention towards pictures depicting positive emotional scenes and increased attention towards negative emotional scenes, with an impaired ability to disengage from negative stimuli (Caseras, Garner, Bradley, & Mogg, 2007; Sears, Thomas, LeHuquet, & Johnson, 2010). An attentional bias for negative emotional words is likewise present alongside depression (Broomfield, Davies, MacMahon, Ali, & Cross, 2007; Gupta & Kar, 2012), which appears to remain even following successful treatment (Gupta & Kar, 2012). Attentional biases for negative emotional stimuli are pernicious, as they could help contribute to the development and reinforcement of unrealistically pessimistic perceptions of the world (Balut, Paulewicz, Szastok, Prochwicz, & Koster, 2013).

Electrophysiology and neuroimaging research have revealed depression-related alterations in the underlying neural processing of emotional stimuli, which could be responsible for affective attentional biases. Mood-congruent attentional biases for faces are accompanied by changes in event-related potentials (ERP), with depressed individuals exhibiting higher intensity scores, as well as greater P1 and P2 amplitudes in response to sad expressions compared to healthy controls (Dai & Feng, 2012). Amplitude and latency of the N170 component, an ERP indicator of facial emotion processing, is disturbed in patients with a first episode of major depression, and even more greatly disordered in patients with recurrent major depression (Chen et al., 2014). Functional magnetic resonance imaging (fMRI) of blood oxygen level-dependent activity has aided in the identification of several brain regions implicated in the depression-

associated abnormal processing of emotional stimuli (Stuhrmann, Suslow, & Dannlowski, 2011). Of particular interest is the amygdala (due to its hypertrophy in the depressed brain), which in depressed patients exhibits increased activity in response to negative facial emotional expressions, including both sad (Fu et al., 2004; Fu et al., 2008; Surguladze et al., 2005; Suslow et al., 2010; Victor, Furey, Fromm, Ohman, & Drevets, 2010) and fearful faces (Matthews, Strigo, Simmons, Yang, & Paulus, 2008; Peluso et al., 2009; Sheline et al., 2001; Zhong et al., 2011). Conversely, depression is accompanied by decreased amygdalar activation in response to faces expressing happiness (Suslow et al., 2010; Victor et al., 2010). Additional brain regions in depressed patients that display abnormal neural reactivity to facial emotional expressions include the PFC, parahippocampal gyrus, insula, putamen, and fusiform face area (Stuhrmann et al., 2011). Supporting the relationship between stress and depressive symptomatology, the recent experience of negative life stressors is significantly correlated with neural activation in regions of the orbitofrontal cortex, ventrolateral PFC, subgenual cingulate cortex, and nucleus accumbens of depressed individuals (but not healthy controls) when viewing negative emotional words (Hsu, Langenecker, Kennedy, Zubieta, & Heitzberg, 2010). It is thus well established that depressed individuals process exogenous sources of information differently than healthy individuals, with a general bias of hyperactivation in response to negative emotional stimuli and hypoactivation in response to positive emotional stimuli.

Antidepressant treatment has been found to normalize pathological neural responses to negative emotional stimuli in depressed individuals (Davidson, Irwin, Anderle, & Kalin, 2003; Fu et al., 2004; Sheline et al., 2001), affecting regions including the amygdala as well as several others (for review, see Harmer & Cowen, 2013). In addition, antidepressants increase previously-suppressed neural responses to positive emotional stimuli in several brain regions (Fu et al., 2007; Schaefer, Putnam, Benca, & Davidson, 2006). Interestingly, acute antidepressant treatments can positively bias cognitive emotional processing while simultaneously being insufficient to induce congruent changes in mood, which requires longer-term administration (Harmer, 2013; Harmer & Cowen, 2013). This suggests that affective states do not play a causal role in the pharmacological correction of negative cognitive biases (and perhaps the opposite is instead true). Therefore, identifying drugs that are particularly efficacious at addressing the underlying neuropathologies of cognitive affective biases is an endeavor of great value to the treatment of depression.

Not only is the perceptual experience of depressed individuals biased towards negativity through disordered attention, but also through a propensity to interpret ambiguous information in a pessimistic manner. When orally presented words containing both emotionally neutral and negative homophones (e.g., *gilt/guilt*, *teas/tease*), depressed individuals demonstrate negative interpretations of the ambiguous words more frequently than healthy controls (Mogg, Bradbury, & Bradley, 2006). Additionally, currently depressed individuals are more likely to interpret ambiguous facial expressions as exhibiting negative emotions, and this perceptual bias is associated with an increased likelihood of relapse (Bouhuys, Geerts, & Gordijn, 1999). Negative interpretational biases have also been found to extend to girls at genetic risk for depression following the presentation of both emotionally ambiguous words and narratives (Dearing & Gotlib, 2009).

Another form of negative cognitive bias is evident in the way that depressed individuals perceive their future. When asked to make predictions about the likelihood of various desirable and undesirable life events occurring in the next 30 days, individuals with high levels of depressive symptoms are strongly pessimistic in their judgements (Strunk & Adler, 2009; Strunk, Lopez, & DeRubeis, 2006). In addition, the pessimistic predictions made by depressed individuals are unrealistic and largely inaccurate, as they do not coincide with the actual life experiences reported 30 days later by the same participants (Strunk et al., 2006). It seems possible that the unrealistically pessimistic expectations observed in depression could in part develop from attentional, interpretational, and memory biases that cause individuals to perceive their world in an exaggeratedly negative light. If cognition is constantly biased towards negative emotional information, then one might have little reason to expect positive outcomes in the future when they have paid little attention to them in the past.

Although largely unexplored until relatively recently, the study of negative cognitive biases in animals and the development of respective evaluative measures has offered researchers the opportunity to explore yet another facet of depression through animal models. Supporting the translational value of such research, the neural circuits implicated in the processing of negative cognitive biases in humans are also present in rodents (Cryan & Holmes, 2005). In order to detect cognitive affective biases in animals, researchers have developed a modified ambiguous cue interpretation (ACI) protocol inspired by similar tests used with human patients. In ACI tests, rats are trained to press a lever to receive a food reward when prompted by a tone of a

specific frequency. In addition, the same rats are also trained to press a separate lever to avoid an aversive electric footshock when prompted by another tone of a different frequency. The dependent variable is the choice of lever press an animal makes when presented with a new, ambiguous tone of intermediate kHz frequency that is halfway between reward and punishment tone. Pressing the reward lever in response to the ambiguous tone cue is inferred to reflect an animal's positive interpretation of the stimulus and optimistic expectation of future outcome. Conversely, pressing the punishment avoidance lever is inferred to be indicative of a negative cognitive bias, as the animal has interpreted the intermediate tone cue to be predictive of an emotionally negative outcome.

As with other forms of depression-like behavior, a negative cognitive bias can be induced in animals through the experience of stress. Rats that have been exposed to repeated restraint stress (Rygula, Papciak, & Popik, 2013), chronic unpredictable stress (Harding, Paul, & Mendl, 2004), and psychosocial stress (Papciak, Popik, Fuchs, & Rygula, 2013) exhibit pessimistic behavior in ACI testing compared to nonstressed animals. Using a genetic animal model of depression, rats selectively bred for helpless behavior in the forced swim test also respond negatively towards an ambiguous tone cue (Enkel et al., 2010). Supporting the diathesis-stress theory of depression, a natural variance exists in the degree of pessimistic responding in the ACI test, and inherently pessimistic rats are more vulnerable to stress-induced anhedonia than inherently optimistic rats (Rygula et al., 2013). One week of treatment with the antidepressant fluoxetine is capable of mitigating negative ACI responding in rats (Anderson, Munafò, & Robinson, 2013), providing the measure with some early potential for predictive validity. Since animal research investigating this measure is still in its infancy, only one study to date has examined the effect of CORT injection on ACI behavior. However, CORT injection prior to ACI testing in rats has yielded results consistent with studies using multiple forms of exogenous stress exposure, causing a bias towards pessimistic lever pressing (Enkel et al., 2010). Although the ACI measure of negative cognitive bias has generated reliable stress-induced effects on behavior, and possesses strong face validity, further research is needed in order to establish good predictive validity and replicate findings in the CORT animal model of depression.

4.4. Summary: Using Repeated CORT Injection as a Model of Depression

The repeated CORT injection model of depression offers an excellent platform from which one can study depression-like behaviors as well as the efficacy and underlying neurobiology of antidepressant treatments. By exposing animals to an elevated level of circulating glucocorticoids, a state believed to strongly contribute to the development of human depression, this model is capable of producing depression-like alterations in neuronal morphology and behavior in rodents, thus fulfilling the requirements of pathogenic, induction, and mechanistic validity. The various depression-like behaviors produced by chronic CORT exposure have been evaluated through valid testing protocols including the sucrose preference test, forced swim test, and ambiguous cue interpretation test. In addition, these behaviors are preventable through antidepressant treatment, supporting the model with valuable predictive validity. In addition to fulfilling the major criteria of animal model validity outlined by Belzung and Lemoine (2011), exogenous CORT administration offers advantages over other models of depression through precise manipulation of stress exposure, avoiding the undesired effects of stressor habituation or individual variance in stressor response. It is for these reasons that a repeated CORT injection paradigm was selected as a model of depression in the experiments performed in my thesis research.

5. Modeling Depression-Associated Memory Bias Through Fear Conditioning

5.1. Depression-Associated Negative Cognitive Bias in Memory

In addition to the cognitive affective biases of attention, processing, and interpretation described above, individuals with depression also experience maladaptive alterations in memory that skew the recollection of past events towards negative emotional information. One facet of depression-associated memory bias is evident in the process of rumination, which includes a pathological fixation on mentally rehearsing distressing memories. Rumination is a significant mediating factor on the development, severity, and chronicity of depression (Nolen-Hoeksema, 2000). Not only do depressed individuals selectively attend to negative memories, but their ability to recall specific positive memories appears to be compromised, while the ability to recall negative memories is strengthened. When prompted to recall autobiographical memories related to positive, negative, or neutral cue words, depressed individuals recall positive events with limited detail and an increased response delay, but recall negative events with enhanced ability

compared to healthy controls (Gupta & Kar, 2012). A negative autobiographical memory bias is also present in individuals with currently remitted depression, but not those who have never been depressed (Gupta & Kar, 2012).

Affective memory bias in humans has been most heavily studied through tests assessing the explicit recall of incidentally encoded emotionally valenced trait adjectives. Similar to findings with autobiographical memory, depressed individuals demonstrate impaired recall of positively valenced words (Harmer et al., 2009a), instead exhibiting a memory bias for negatively valenced information in both adults (Balut et al., 2013; Gerritsen et al., 2012; Hsu et al., 2010; Liu et al., 2012; Mogg et al., 2006; Taylor & John, 2004), as well as children and adolescents (Neschat-Doost, Taghavi, Moradi, Yule, & Dalgeish, 1998). The emotional context of information also affects memory, as recall for neutral words is enhanced in depressed individuals when the words are incidentally encoded while reading emotionally negative narratives, compared to positive or neutral narratives (Nitschke, Heller, Etienne, & Miller, 2004). Although a negative memory bias for words has been demonstrated to be reversible through a single dose of the antidepressant reboxetine (Harmer et al., 2009b), individuals with remitted depression generally continue to experience maladaptive recall of negatively valenced stimuli (Romero, Sanchez, & Vasquez, 2014). The potential harm of negative cognitive biases is amplified in older patients, as these biases mediate global memory performance, which worsens in correlation with increased depressive symptom severity (Crane, Bogner, Bron, & Gallo, 2007).

The various forms of negative cognitive bias do not operate in isolation, but rather possess the ability to influence the expression of one other. Attentional biases for negative stimuli not only coexist among memory biases in depression (Gupta & Kar, 2012), but also mediate the pathological enhancements in negative emotional memory described above (Balut et al., 2013). When trained to overcome the tendency to attend to negative stimuli, individuals with depression no longer exhibit a memory bias for negatively valenced words (Balut et al., 2013). Pessimistic stimulus interpretation also influences memory bias, as depressed individuals display enhanced memory for orally presented ambiguous homophones (e.g., dye/die; Mogg et al., 2006). However, there are several other psychological and neurological factors that appear to underlie and contribute to the development of depression-associated memory bias. First, the degree to which memory is biased towards negative information increases in conjunction with

the severity of depression and symptoms of anhedonia (Liu et al., 2012). Negative memory bias is also associated with pathological neural activity and morphology in regions of the brain that are well-known to be susceptible to the effects of stress and depression. Electroencephalography of depressed individuals has revealed abnormal right PFC activity in association with emotional context word memory bias, a region heavily implicated in the production of negative emotions (Nitschke et al., 2004). Hippocampal atrophy and amygdalar hypertrophy are distinctive features of the depressed brain, and both the relative and absolute size of these structures are related to the expression of negative memory bias. Small hippocampal volume, large amygdalar volume, and a high ratio of amygdalar to hippocampal volume, are all associated with negatively biased memory (Gerritsen et al., 2012). The recent experience of stressful life events, a significant risk factor for depression (Farmer & McGuffin, 2003; Kessing et al., 2003; Slavich et al., 2010), is also positively associated with negative memory bias in depressed patients (Hsu et al., 2010). Furthermore, administration of cortisol in healthy individuals causes an enhancement in memory for negatively valenced words (Tops et al., 2003), illustrating a clear relationship between stress and negatively biased memory.

5.2. Fear Conditioning

5.2.1. *Behavior and cognition*

Although the prevalence of negative emotional memory biases in depressed individuals has been well-established through human patient research, the use of animal models in this field of study offers valuable opportunities to further evaluate the causative role of stress in memory bias, as well as the efficacy of antidepressants in alleviating these harmful symptoms. To study the effects of stress and antidepressant treatment on memory for emotionally valenced stimuli in animals, researchers have utilized various forms of Pavlovian fear conditioning. Fear is an adaptive emotional response that is triggered by the perception of an environmental threat, which initiates a cascade of changes in neurotransmission, behavior, and bodily function in order to prepare an organism for avoiding danger and minimizing physical harm. Some stimuli naturally evoke fear responses in rodents, such as the smell of a predator or physical pain, but the remarkable plasticity of the brain allows new stimuli-fear response associations to be formed, supporting dynamic adaptation to similarly dynamic environments (Kim & Jung, 2006; Pape & Paré, 2010).

Fear conditioning takes advantage of this flexible learning process by repeatedly pairing an emotionally neutral conditioned stimulus (CS; e.g., tone cue, visual contextual cues) with an innately aversive and negatively valenced unconditioned stimulus (US; e.g., footshock), causing the formation of a predictive association between the two events (Johansen et al., 2011; Kim & Jung, 2006; Rodrigues, LeDoux, & Sapolsky, 2009; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2013). Following successful fear conditioning, presentation of the CS alone will elicit defensive fear responses, also known as conditioned responses (CRs), which are then measured to evaluate the degree of fear learning. Measures of CRs in rats can include ultrasonic distress vocalization, heart rate, respiration rate, blood pressure, potentiated startle, and pain sensitivity, but by far the most commonly utilized CR in fear conditioning research is freezing behavior (Kim & Jung, 2006). Freezing, defined as the absence of all non-respiratory movement, is an inborn rodent defense mechanism used to avoid predatory detection, and is automatically triggered in response to perceived threats (Blanchard & Blanchard, 1969; VanElzakker et al., 2013). The percentage of time spent freezing during CS presentation is thus frequently used as a measure of fear conditioning, with greater percentages of freezing suggesting stronger CS-US association learning.

Researchers utilize a variety of different fear conditioning protocols depending on their specific needs, and often the most important distinction between testing procedures involves the nature of the conditioned stimuli. In contextual fear conditioning, a rat simply receives a series of footshocks while inside an operant chamber, which causes a fearful association to be formed between the aversive shock and the various ambient visual, auditory, and olfactory cues of the training environment. The animal will then exhibit freezing behavior if it is later placed within the same context that fear conditioning was acquired in, but not if it is exposed to a novel context with different persistent environmental cues. Auditory fear conditioning differs by repeatedly pairing a footshock with the termination of a discrete tone cue, thus effectively forming a predictive association between the onset of the tone and an emotionally unpleasant experience. Following auditory fear conditioning, the rat will freeze, even in a novel context, if presented with the same tone cue used during training. However, since conditioning to operant chamber contextual cues will unavoidably take place in tandem with conditioning to tone cues during auditory fear training, the rat will also freeze if placed again within the training environment. For this reason, tests of freezing to tone cues typically occur in a modified operant chamber with

novel ambient visual, auditory, and olfactory cues so that only auditory fear conditioning is being measured. Delay fear conditioning allows researchers to assess both contextual and auditory fear conditioning by following training with two distinct freezing tests that dissociate each form of learning (i.e., freezing to tone in novel context, and freezing without tone in training context). Both contextual and auditory fear conditioning paradigms are of value to researchers, as they recruit distinct neural circuits and can be differentially affected by treatments like chronic stress. Although successful contextual and auditory fear learning share a necessity for intact amygdala functioning, only contextual fear conditioning requires intact function of the hippocampus (LeDoux, 2000).

Although the induction of fear conditioning is relatively simple from a procedural standpoint, the cognitive systems underlying this phenomenon are complex, requiring the recruitment of a series of unique memory processes that must operate without error in order for CU-US associations to have long-lasting effects on behavior. The first stage of fear conditioning is acquisition, which begins immediately following the first CS-US pairing and consists of the automatically learned association between these two stimuli (Abel & Lattal, 2001; Johansen et al., 2011; Rodrigues et al., 2009). Memories of the CS-US pairing are extremely labile and sensitive to interference (e.g., drugs) during the period shortly following acquisition, and thus the long-term survival of these memories is contingent upon their ability to be converted into stable engrams (Abel & Lattal, 2001; Dudai, 2004; Rodrigues et al., 2009). Consolidation is the process responsible for the post-acquisition formation of long-term memories, which are largely resistant to pharmacological and behavioral disruption, and begins within minutes of fear conditioning (Duncan, 1949; McGaugh, 1966). Evaluation of the learned fear association typically takes place on a later date through presentation of the CS in the absence of US reinforcement, which initiates the process of long-term memory retrieval (Rodrigues et al., 2009). Expression, a related but distinct process, is responsible for the behavioral output (i.e., freezing, other CRs) resulting from the retrieval of fear memories.

All four of these memory processes are critical to successful fear conditioning, and interference at any stage through pharmacological treatment, brain lesions, or transient neuronal inactivation can dramatically alter the expression of CRs, both temporarily or permanently. This intrinsic feature of fear learning is highly exploitable by researchers, as precise manipulation of the time course of interference procedures can help uncover the structures and neurobiological

processes underpinning each stage of fear learning, as well as reveal which stages of memory a particular drug might affect. For example, if a drug administered immediately following fear conditioning training is later associated with lower levels of freezing during retrieval testing, it has likely impaired memory consolidation. Alternatively, if a drug reduces freezing when administered immediately prior to retrieval testing, but is ineffective when administered at earlier stages of fear conditioning, it likely interferes with the retrieval or expression of fear memory. However, it can sometimes be difficult to pinpoint the specific process of memory a drug might influence if the pharmacological treatment has long-lasting effects, as processing at multiple stages could potentially be altered.

5.2.2. Neurobiology

An immense wealth of experimental research has culminated in the identification of the primary structures, nuclei, neural pathways, and neurobiological processes recruited during each stage of fear conditioning. Central to the induction of fear learning and the expression of fear-motivated behavior is the amygdala, which integrates CS and US input, and initiates CR output. Early research revealed that large pre- and post-training amygdaloid lesions not only impaired fear conditioning (Blanchard & Blanchard, 1972; Kim, Rison, & Fanselow, 1993), but also prevented the expression of unconditioned fear-motivated behavior (Blanchard & Blanchard, 1972; Hitchcock, Sananes, & Davis, 1989; Kim & Davis, 1993), suggesting a convergence of both conditioned and unconditioned stimuli processing in this structure (Kim & Jung, 2006). However, in order to fully comprehend the nuanced functioning of the amygdala in fear conditioning, it is necessary to distinguish between the dissociable amygdalar subsystem nuclei responsible for distinct stages of fear learning and behavioral response. Namely, the lateral (LA) and central (CE) amygdalar nuclei are of particular importance to the processing of fear associations and fear-motivated behavior.

The LA functions as the primary site of synaptic convergence for thalamic and cortical projections carrying a wide array of sensory information, including auditory, visual, and olfactory conditioned stimuli, as well as somatosensory (i.e. electrical shock) unconditioned stimuli (Dityatev & Bolshakov, 2005; Johansen et al., 2011; Kim & Jung, 2006; Maren, 1999a; Pape & Paré, 2010; Rodrigues et al., 2009; Sah et al., 2008). The LA also receives synaptic input from the hippocampus, which transmits information regarding diffuse ambient stimuli essential

for contextual fear learning (Ji & Maren, 2007; LeDoux, 2000; Maren, 1999a; Phillips & LeDoux, 1992; Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000). Since the LA is the primary sensory gateway for incoming fear-relevant stimuli, lesioning of this nucleus or its afferent sensory projections prevents both the induction of fear learning and the expression of fear-motivated behavior, as observed in a wide variety of experimental protocols using several distinct forms of conditioned stimuli (Campeau & Davis, 1995; Cousens & Otto, 1998; Kim et al., 1993; Kim & Fanselow, 1992; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Lee, Walker, & Davis, 1996; Maren, 1998; Maren, Aharonov, & Fanselow, 1996a; Maren, Aharonov, & Fanselow, 1997; Phillips & LeDoux, 1992; Romanski & LeDoux, 1992; Rosen et al., 1992; Sananes & Davis, 1992). Even if the amygdala is intact when recall testing occurs, transient neurotransmitter receptor inactivation of the LA during training results in impaired contextual and auditory fear learning, presumably because the CU-US association was initially unable to be formed in the LA (Bauer, Schafe, & LeDoux, 2002; Campeau, Miserendino, & Davis, 1992; Fanselow & Kim, 1994; Fendt, 2001; Gewirtz & Davis, 1997; Helmstetter & Bellgowan, 1994; Lee & Kim, 1998; Maren et al., 1996b; Miserendino, Sananes, Melia, & Davis, 1990; Muller, Corodimas, Fridel, & LeDoux, 1997; Rodrigues, Schafe, & LeDoux, 2001; Walker & Davis, 2000). Further demonstrating the LA's role in associative processing, rats with LA lesions are still capable of freezing in a unique conditioning paradigm that recruits amygdala-independent circuits (Maren, 1999b), suggesting that damage to the LA does not impair the ability to physically express fear-motivated behavior, but rather interferes with the upstream transmission of CS information in most auditory and contextual conditioning procedures. After being processed in the LA, signals initiated by unconditioned and/or conditioned stimuli are forwarded to the CE, which forms a common divergent output of hypothalamic, mesencephalic, and brain stem efferent projections that mediate defensive fear responses (i.e. CRs in fear conditioning; Johansen et al., 2011; Kim & Jung, 2006; LeDoux, 2000; Maren, 1999a; Pape & Paré, 2010; Rodrigues et al., 2009; Sah et al., 2008). Accordingly, lesioning of the CE abolishes the expression of conditioned fear (Hitchcock & Davis, 1986; Kim & Davis, 1993), while electrical stimulation initiates a CR-like physiological response in rats (Iwata, Chida, & LeDoux, 1987; Kapp, Gallagher, Underwood, McNall, & Whitehorn, 1982). The CE's role in fear conditioning is limited to fear expression, as temporary inactivation of the CE during training has no effect on

freezing during subsequent recall testing, and thus the encoding and consolidation of fear memory (Fanselow & Kim, 1994).

Although the amygdala is the common locus of associative processing for a wide range of fear conditioning paradigms, there are other structures in the brain that play a significant role in various aspects of fear conditioning. As briefly mentioned above, the hippocampus projects contextual information to the LA, and thus the integrity of this structure is essential to the induction and expression of contextual fear conditioning (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992; Maren et al., 1997; Phillips & LeDoux, 1992). However, auditory fear conditioning is hippocampus-independent, as cue-induced freezing is unaffected by hippocampal lesioning (Maren et al., 1997; Phillips & LeDoux, 1992). The mPFC is an important structure in the mediation of conditioned responding, as it possesses the ability to inhibit amygdalar output through efferent projections to the LA. Further discussion of the mPFC's inhibitory influence on freezing can be found in the later section on fear extinction. Lastly, of particular relevance to the expression of fear conditioning is the periaqueductal gray, which serves a critical function in the freezing response (LeDoux et al., 1988).

Lesion studies have helped identify the LA as the point of synaptic convergence for US and CS signal input, but the research discussed thus far offers little insight into how associative fear learning occurs on a neurobiological level. Underlying the induction of conditioned fear is a form of synaptic plasticity referred to as Hebbian LTP, an activity-dependent strengthening of synaptic signaling that is initiated when presynaptic glutamate release coincides with postsynaptic depolarization (Johansen et al., 2011; Pape & Paré, 2010). One implication of Hebbian plasticity is that if a neuron receives a strong presynaptic input from one afferent connection, and at the same time receives a weak presynaptic input from a second afferent connection, membrane depolarization caused by the strong input is sufficient to enhance synaptic efficacy at both synapses (Johansen et al., 2011). Applying this model to fear conditioning training, a single LA pyramidal neuron may receive excitatory input from a cell transmitting shock information, as well as from a second cell transmitting auditory cue information (Romanski, Clugnet, Bordi, & LeDoux, 1993), but only the shock signal input is strong enough to cause postsynaptic depolarization and ultimately initiate a freezing response. However, the temporal pairing of the auditory presynaptic input and the shock-induced postsynaptic depolarization causes the auditory-LA synapse to strengthen, allowing presentation of the

auditory cue alone to initiate freezing (Johansen et al., 2010; Johansen et al., 2011; LeDoux, 2000; Pape & Paré, 2010; Sah et al., 2008). Experimentally replicating this associative learning mechanism, optically-activated LA pyramidal neuron depolarization, when temporally paired with auditory cue presentation, causes rats to develop cue-elicited freezing in the absence of aversive conditioning (Johansen et al., 2010). The Hebbian model of associative learning is further supported by a wealth of *in vivo* and *in vitro* research demonstrating the enhanced efficacy of LA pyramidal neuron synapses following fear conditioning (Clem & Huganir, 2010; Hong et al., 2009; Hong et al., 2011; Kim et al., 2007a; McKernan & Shinnick-Gallagher, 1997; Rumpel, LeDoux, Zador, & Malinow, 2005; Schroeder & Shinnick-Gallagher, 2005).

Neurotransmission between afferent sensory projections (e.g., cortical and thalamic auditory inputs) and LA pyramidal neurons occurs through the presynaptic release of glutamate, which then binds to two types of coexpressed ionotropic glutamate receptors in the postsynaptic membrane (Mahanty & Sah, 1999). The AMPA receptor allows sodium and potassium ions to pass through the postsynaptic membrane when bound by glutamate, and functions as the primary mechanism of excitatory synaptic transmission in glutamatergic neurons at resting membrane potential (Deutschenbaur et al., 2015; Johansen et al., 2011; Maeng & Zarate, 2007; Sah et al., 2008). Conversely, the NMDA receptor demonstrates voltage-dependent glutamate activation, as extracellular binding of magnesium (Mg^{2+}) at resting membrane potential precludes the passage of ions through the channel pore. Cell membrane depolarization, induced by sufficient AMPA receptor ion transmission, frees the NMDA receptor of its Mg^{2+} block, allowing Ca^{2+} to flow into the cell (Deutschenbaur et al., 2015; Johansen et al., 2011; Maeng & Zarate, 2007; Nowak et al., 1984; Pape & Paré, 2010; Sah et al., 2008). This increase of intracellular Ca^{2+} plays an important role in LTP, initiating a signaling cascade that upregulates postsynaptic membrane insertion of AMPA subunit *Gria1*, thereby enhancing synaptic efficacy and the ease with which presynaptic glutamate release may stimulate the pyramidal neuron (Johansen et al., 2011; Malinow & Malenka, 2002; Pape & Paré, 2010; Sah et al., 2008).

The unique circumstances by which NMDA receptors are activated causes them to act as coincidence detectors of pre- and postsynaptic activity, which combined with their ability to mediate LTP (and thus strengthen synapses between CS inputs and LA pyramidal neurons), makes NMDA receptors a critical component of associative fear learning (Johansen et al., 2011; Pape & Paré, 2010). Accordingly, infusion of the NMDA receptor antagonist DL-2-amino-5-

phosphonovaleric acid (APV) into the LA (but not the CE; Fanselow & Kim, 1994) during training prevents the acquisition of fear conditioning (Bauer et al., 2002; Campeau et al., 1992; Fanselow & Kim, 1994; Fendt, 2001; Gewirtz & Davis, 1997; Lee, Choi, Brown, & Kim, 2001; Lee & Kim, 1998; Maren, Aharonov, Stote, & Fanselow, 1996b; Miserendino et al., 1990; Walker & Davis, 2000). A number of studies have demonstrated that LA infusion of APV prior to recall testing also impairs the expression of conditioned fear in successfully trained rats (Fendt, 2001; Lee et al., 2001; Lee & Kim, 1998; Maren et al., 1996b), but the evidence supporting the necessity of NMDA functioning during fear recall has been equivocal (Bauer et al., 2002; Campeau et al., 1992; Gewirtz & Davis, 1997; Miserendino et al., 1990; Walker & Davis, 2000). Likewise, the role of NMDA receptors in memory consolidation is unclear, as immediate post-training infusion of APV does not impair subsequent recall test freezing (Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991; Maren et al., 1996b), whereas reversible post-training genetic suppression of hippocampal NMDA receptor function has proved effective in interfering with contextual fear conditioning (Shimizu, Tang, Rampon, & Tsien, 2000). Additionally, human patient research suggests that emotional memory consolidation may be impaired by peritraumatic administration of the NMDA receptor antagonist ketamine (McGhee, Maani, Garza, Gaylord, & Black, 2008). Further investigation into the function of NMDA receptor activity during fear memory consolidation and recall is needed in order to help reconcile these seemingly conflicting results.

Since NMDA receptors cannot be activated without prior AMPA-facilitated membrane depolarization, and ultimately exert their influence on LTP in part through enhanced GluR1 subunit membrane insertion, AMPA receptors share an essential role in the induction and expression of Pavlovian fear conditioning. Fear conditioning training reliably enhances the surface expression of AMPA receptor subunits in LA pyramidal neuron synapses (Kim et al., 2007a; Nedelescu et al., 2010; Rumpel et al., 2005; Yeh, Mao, Lin, & Gean, 2006). Furthermore, not only is AMPA receptor synaptic incorporation dependent upon NMDA receptor activation (Yeh et al., 2006), but interference with this cell membrane insertion process also impairs fear conditioning (Rumpel et al., 2005). Additionally, intra-amygdalar infusion of the AMPA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) blocks the expression of conditioned fear (Kim et al., 1993), while the AMPA receptor agonist 1-(quinoxolin-6-ylcarbonyl)piperidine (BDP-12) facilitates fear acquisition (Rogan, Stäubli, & LeDoux, 1997).

5.3. The Effects of Stress and Antidepressants on Fear Conditioning

5.3.1. *Stress and fear conditioning*

The experience of chronic stress causes a number of morphological and functional alterations in the brain that have the potential to profoundly influence the neural processes underlying conditioned fear. Chronic behavioral stress or exogenous CORT exposure induces long-lasting dendritic hypertrophy and increased dendritic spine density in the LA of rats (Mitra et al., 2005; Mitra & Sapolsky, 2008; Vyas et al., 2002; Vyas et al., 2003; Vyas et al., 2004; Vyas et al., 2006). In addition to these morphological changes, stress and CORT attenuates GABAergic inhibition of LA neurons (Duvarci & Paré, 2007; Rodríguez Manzanares, Isoardi, Carrer, & Molina, 2005), and enhances LA neuron excitability (Duvarci & Paré, 2007; Garcia, Paquereau, Vouimba, & Jaffard, 1998; Kavushansky & Richter-Levin, 2006; Kavushansky, Vouimba, Cohen, & Richter-Levin, 2006; Rodríguez Manzanares et al., 2005). By enabling the induction of LTP at thalamic-LA synapses, stress enhances the ability for amygdalar neurons to form the associative connections that are pivotal to fear conditioning (Roosendaal, McEwen, & Chattarji, 2009; Tully, Li, Tsvetkov, & Bolshakov, 2007).

Glucocorticoids play an essential role in associative fear learning, as a variety of experimental manipulations that inhibit glucocorticoid receptor activation (i.e., adrenalectomy, amygdalar glucocorticoid receptor antagonism, and viral vector genes) likewise impair the consolidation of both auditory and contextual fear conditioning (Conrad, Mauldin-Jourdain, & Hobbs, 2001; Cordero, Kruyt, Merino, & Sandi, 2002; Donley, Schulkin, & Rosen, 2005; Jin, Lu, Yang, Ma, & Li, 2007; Kolber et al., 2008; Pugh, Fleshner, & Rudy, 1997a; Pugh, Tremblay, Fleshner, & Rudy, 1997b; Rodrigues & Sapolsky, 2009; Roosendaal & McGaugh, 1997). Not only are glucocorticoids necessary for fear conditioning, but their physiological concentrations also mediate how strongly fear is learned. Post-training levels of CORT and cortisol are correlated with the degree of conditioned responding in rodents (Cordero, Merino, & Sandi, 1998; Cordero, Venero, Kruyt, & Sandi, 2003b) and humans (Zorawski, Blanding, Kuhn, & LaBar, 2006; Zorawski, Cook, Kuhn, & LaBar, 2005), respectively. By experimentally increasing glucocorticoid levels through either behavioral stress or CORT injection, further mediating effects on fear conditioning have been observed. Acute stress treatments have the ability to enhance both the consolidation and long-term recall of conditioned fear (Cordero et al., 2003b; Corodimas, LeDoux, Gold, & Schulkin, 1994; Hui et al., 2004; Hui, Hui, Roosendaal,

McGaugh, & Weinberger, 2006; Izquierdo, Barros, Medina, & Izquierdo, 2002; Kohda et al., 2007; Rau & Fanselow, 2009; Zorawski & Killcross, 2002), the effects of which are blocked by glucocorticoid receptor antagonists (Kohda et al., 2007). However, the most profound effects of glucocorticoids on fear-motivated behavior are seen in chronic stress animal models of depression. Chronic behavioral stress or chronic CORT injection prior to training reliably increases long-term conditioned freezing to contextual cues (Conrad et al., 1999; Conrad et al., 2004; Cordero, Kruyt, & Sandi, 2003a; Marks, Fenton, Guskjolen, & Kalynchuk, 2015; Sandi, Merino, Cordero, Touyarot, & Venero, 2001; Skórzewska et al., 2006; Thompson, Erickson, Schulkin, & Rosen, 2004), and enhancements in auditory fear conditioning have also been demonstrated in these paradigms (Bisaz & Sandi, 2010; Conrad et al., 1999; Farrell, Sayed, Underwood, & Wellman, 2010; Marks et al., 2015; Monsey et al., 2014; Zhang & Rozencranz, 2013). Prolonged glucocorticoid exposure increases conditioned responding in a dose-dependent manner (Marks et al., 2015), demonstrating a clear mediatory relationship between stress and memory for negatively-valenced stimuli.

5.3.2. Antidepressants and fear conditioning

A wide array of antidepressants have been evaluated for their efficacy in suppressing the encoding and recall of negative emotional memories induced through fear conditioning. Studies on rodents have revealed that acute antidepressant treatment prior to fear memory recall can reduce context-elicited freezing, including the drugs citalopram (Hashimoto, Inoue, Muraki, & Koyama, 2009; Inoue et al., 2004; Kitaichi et al., 2014), desipramine (Santos, Martinez, & Brandão, 2006), diazepam (Miyamoto, Tsuji, Takeda, Nawa, & Matsumiya, 2000), escitalopram (Montezinho et al., 2010), flesinoxan (Li, Inoue, Hashimoto, & Koyama, 2001), fluoxetine (Santos et al., 2006), fluvoxamine (Li et al., 2001; Miyamoto et al., 2000; Miyamoto et al., 2004), milnacipran (Miyamoto et al., 2004), mirtazapine (An et al., 2013; An et al., 2015), risperidone (Miyamoto et al., 2004), and venlafaxine (Slipczuk et al., 2013). Fewer studies have examined chronic antidepressant treatment regimens, but chronic citalopram (Burghardt et al., 2004; Hashimoto et al., 2009) and tianeptine (Burghardt et al., 2004) treatment has been shown to impair contextual fear acquisition, while chronic flesinoxan, and fluvoxamine treatment has demonstrated efficacy in reducing auditory fear memory recall (Li et al., 2001). Very little research has investigated the effects of antidepressants on negative emotional memory in

chronically stressed animals, but recent research suggests that fluoxetine is effective in reducing tone cue-elicited freezing in rats that have received chronic daily CORT injection (Marks, 2014, unpublished results). Finally, in a single prolonged stress animal model of post-traumatic stress disorder (PTSD), chronic paroxetine treatment has proved efficacious in suppressing stress-induced enhancements in contextual freezing (Takahashi, Morinobu, Iwamoto, & Yamawaki, 2006).

However, not all antidepressants that have been investigated are effective at reducing conditioned fear, and some carry significant limitations as well. Acute treatments of paroxetine and tianeptine fail to reduce conditioned responding in stressed and nonstressed rats, respectively, exhibiting a delayed therapeutic effect (Burghardt et al., 2004; Takahashi et al., 2006). Chronic tianeptine treatment has also failed to prevent a chronic stress-induced enhancement of auditory and contextual fear conditioning (Conrad et al., 1999). Additionally, the antidepressant effects of acute mirtazapine on contextual fear conditioning are short-lived, lasting less than a week (An et al., 2015). Surprisingly, a number of antidepressants actually enhance fear conditioning under certain conditions. When administered prior to training, acute escitalopram (Montezinho et al., 2010), fluvoxamine (Cassaday & Thur, 2015), sertraline (Cassaday & Thur, 2015), and vortioxetine (Mørk et al., 2013) enhance the acquisition of contextual fear conditioning, and acute citalopram (Burghardt et al., 2004) and fluoxetine (Burhans et al., 2013; Ravinder et al., 2013) enhance the acquisition of auditory fear conditioning. Although further investigation into this matter is needed, these results suggest that it might be possible for someone who is in the early stages of antidepressant treatment to experience an enhancement in memory for negative emotional information: an obviously undesirable and counter-productive effect. Based on the animal research conducted thus far, there appears to be considerable room for improvement in the efficacy of antidepressant drugs on suppressing measures of fear conditioning. An ideal antidepressant drug should produce an immediate and long-lasting reduction of conditioned responding, with no drug-induced enhancements of fear memory at any point during treatment.

5.4. Fear Extinction

5.4.1. Behavior and cognition

Associative fear conditioning is rapidly induced through the pairing of a neutral stimulus with an inherently aversive emotional experience, but these fearful associations and their respective behavioral responses can later be challenged through a form of novel learning known as extinction. Extinction takes place by repeatedly presenting the CS in the absence of US reinforcement, which causes the organism to learn that the CS is no longer predictive of an unpleasant experience, resulting in a reduction of CS-elicited fear-motivated behavior (e.g., freezing; Johansen et al., 2011; Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). The process of extinction begins immediately with the first unreinforced presentation of the CS, and continues to strengthen over the course of the extinction training trial (Cammarota et al., 2004).

Similar to fear conditioning, fear extinction recruits its own distinct acquisition, consolidation, and retrieval stages that must operate without interference in order to successfully modify conditioned behavioral responding (Quirk & Mueller, 2008). The acquisition of fear extinction is assessed by examining within-session CS-elicited conditioned responding during extinction training, while manipulations of the consolidation process are studied alongside memory retrieval through the expression of conditioned fear during a subsequent (typically next-day) extinction recall test (Pape & Paré, 2010). Increases in between-session conditioned fear can be caused by a disruption of extinction consolidation (Quirk & Mueller, 2008), but also occur naturally with the passage of time due to the gradual decay of extinction memory, a phenomenon known as spontaneous conditioned fear recovery (Cruz, López, & Porter, 2014; Pape & Paré, 2010). In addition, extinction memories are poorly expressed in contexts incongruent with that in which extinction training occurred (unlike auditory fear conditioning, which is expressed in both the training context as well as novel environments), leading to a process referred to as conditioned fear renewal (Bouton, 2004; Bouton & Bolles, 1979a; Bouton & Bolles, 1979b; Bouton & King, 1983; Bouton, Westbrook, Corcoran, & Maren, 2006). Further demonstrating the fragile nature of extinction memory, conditioned fear can be rapidly reinstated in extinction trained animals through presentation of an unpaired US (Laurent & Westbrook, 2010; McAllister & McAllister, 2006; Rescorla & Heth, 1975; Shen, Igarashi, Imamura, Matsuki, & Nomura, 2013), a sub-conditioning retraining procedure that pairs the CS with a

weak US (e.g., mild footshock) that is normally incapable of inducing fear conditioning in naive animals (Deschaux et al., 2013; Deschaux, Motanis, Spennato, Moreau, & Garcia, 2011a; Deschaux, Spennato, Moreau, & Garcia, 2011b; Laurent & Westbrook, 2010; Zheng et al., 2013), or simply exposure to acute stress (Deschaux et al., 2013; Zheng et al., 2013). The ability for conditional fear responding to reemerge in previously fear-extinguished animals, even in the absence of full reconditioning procedures, suggests that extinction learning is not simply a process of forgetting or erasing previously conditioned fear associations. Conversely, fear extinction largely consists of the formation of a new memory that competes with and inhibits the CS-US association to reduce the expression of fear-motivated behavior (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). However, while a considerable body of evidence supports fear extinction as a distinct form of novel learning, recent studies have demonstrated that the process of extinction also partially involves of the depotentiation and synaptic weakening of fear conditioning circuits (Clem & Huganir, 2010; Dalton, Wang, Floresco, & Phillips, 2008; Kim et al., 2007a).

Fear extinction is a valuable tool in the study of negative cognitive biases because it allows researchers to investigate the way an animal recalls memory for an emotionally-relevant interpretable stimulus (i.e., the CS). Since fear conditioning memories and fear extinction memories for the same CS co-exist in the brain, an extinction-trained animal that is presented with a CS can interpret it either pessimistically (i.e., the CS is predictive of a footshock) or optimistically (i.e., the CS is not predictive of a footshock), depending upon the relative strength of these memory associations (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). Both internal and external factors can modulate an animal's behavioral response to an ambiguous CS (as can be seen in the renewal, recovery, and reinstatement of conditioned fear), which allows researchers the opportunity to test how exposure to various hormones or drugs alter the competing influence of conditioning and extinction memories.

5.4.2. Neurobiology

The induction and recall of conditioned fear extinction recruits a complex system of neural circuitry involving communication between the mPFC, hippocampus, and amygdala. The ventromedial prefrontal cortex (vmPFC) was first suggested to be implicated in auditory fear extinction when it was observed that lesions of this structure impaired rats' long-term recall of

extinction memories while leaving the initial acquisition of extinction intact (Morgan, Romanski, & LeDoux, 1993), a finding that has since been replicated (Fernandez, 2003; Lebrón, Milad, & Quirk, 2004; Morgan, Shulkin, & LeDoux, 2003; Quirk, Russo, Barron, & Lebron, 2000). Specifically, the infralimbic cortex (IL) was later identified as the crucial region of the vmPFC involved in extinction, as selective lesioning (Quirk et al., 2000) or chemical inactivation (Sierra-Mercado, Corcoran, Lebrón-Milad, & Quirk, 2006) of this structure impairs between-session auditory fear extinction recall, but not the acquisition of fear conditioning nor the within-session short-term acquisition of extinction. Supporting a role in the consolidation of extinction memory, IL LTP (Farinelli, Deschaux, Hugues, Thevenet, & Garcia, 2006; Herry & Garcia, 2002; Hugues, Chessel, Lena, Marsault, & Garcia, 2006) and neuronal bursting (Burgos-Robles, Vidal-Gonzalez, Santini, & Quirk, 2007) is induced shortly following extinction training, the presence or disruption of which is correlated with the success or failure of long-term extinction retention, respectively. Experimental manipulation of IL activity further demonstrates its involvement in extinction behavior acquisition, as electrical stimulation of IL neurons reduces CS-elicited freezing in the absence of extinction training (Milad & Quirk, 2002; Milad, Vidal-Gonzalez, & Quirk, 2004; Vidal-Gonzalez, Vidal-Gonzalez, Rauch, & Quirk, 2006), but only when stimulation and CS presentation co-occur with a high degree of precision (Milad et al., 2004).

The IL is also directly implicated in the process of recalling extinction memories. Presentation of an extinguished auditory CS cue elicits temporally coincident firing of IL neurons during extinction recall testing, and the degree of IL neuron firing is correlated with the successful suppression of freezing behavior (Milad & Quirk, 2002; Wilber et al., 2011). Additionally, the retention of extinction memory is highly dependent upon the excitability and synaptic strength of mPFC neurons. Extinction training reverses a fear conditioning-induced depression of IL neuron excitability, but the return of IL depression after 17 days is associated with a corresponding recovery of CS-elicited freezing, implicating the IL in the long-term maintenance of extinction memories (Cruz et al., 2014; Herry & Mons, 2004). Furthermore, facilitation of LTP in the mPFC through high frequency stimulation prevents the reinstatement of conditioned fear following a sub-conditioning retraining procedure in extinction-trained rats (Deschaux et al., 2011a; Zheng et al., 2013). Thus, the mPFC can be viewed as a critical component of fear extinction, and since enhancement of mPFC function likewise strengthens

extinction memory, facilitation of this structure's activity may prove to be a useful target for treating disorders characterized by extinction failure.

It is believed that the mPFC mediates conditioned fear responding through descending excitatory IL projections to the inhibitory, GABA receptor-expressing intercalated (ITC) neurons of the amygdala (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzaker et al., 2013). Accordingly, IL activation stimulates ITC neurons (Beretta, Pantazopoulos, Caldera, Pantazopoulos, & Paré, 2005), which in turn project to the CE (Paré & Smith, 1993) to inhibit conditioned responding (Jüngling et al., 2008; Likhtik, Popa, Apergis-Schoute, Fidacaro, & Paré, 2008; Royer & Paré, 2002). This circuitry forms a system in which IL activity restricts the ability of LA stimulation (typically initiated by CS) to initiate CE output of fear-motivated behavior (Quirk, Likhtik, Pelletier, & Paré, 2003). ITC neurons also receive glutamatergic projections from the LA in addition to the IL, and undergo NMDA receptor-dependent LTP with repeated upstream stimulation of IL neurons (Royer & Paré, 2002). Thus, it seems possible that extinction utilizes a similar process of synaptic plasticity to that which occurs in the induction of fear conditioning: specifically, through NMDA receptor coincidence detection between LA and IL inputs on ITC neurons that subsequently strengthens the ability for the conditioned stimuli to produce a no-freezing response (Quirk & Mueller, 2008).

The expression of extinction memory is highly context-specific, as conditioned fear behavior is renewed when a CS is presented in an environment that is incongruent from that in which extinction training occurred (Bouton, 2004; Bouton & Bolles, 1979a; Bouton & Bolles, 1979b; Bouton et al., 2006; Bouton & King, 1983). The context specificity of extinction necessitates a locus for processing contextual information and gating the output of emotional responses accordingly, a role to which the hippocampus has been suggested to fulfill. Extinction memory recall is associated with increased expression of c-Fos, a protein biomarker of recent neuronal activity, in several regions of the hippocampus (Knapska & Maren, 2009). Lesioning (Ji & Maren, 2007) or chemical inactivation (Corcoran, Desmond, Frey, & Maren, 2005; Corcoran & Maren, 2001; Corcoran & Maren, 2004; Hobin, Ji, & Maren, 2006) of the hippocampus prior to extinction memory retrieval abolishes the context-dependent expression of fear extinction. When hippocampal inactivation occurs prior to extinction training, extinction retrieval is impaired and conditioned responding is high, possibly due to a disruption of contextual encoding during the acquisition of extinction memory (Corcoran et al., 2005). It has been suggested that

the hippocampus may regulate fear output by transmitting contextual information to the mPFC (Quirk & Mueller, 2008), since LTP develops between the hippocampal-mPFC pathway shortly following extinction training, and interference with this process impairs extinction memory recall (Farinelli et al., 2006; Garcia, Spennato, Nilsson-Todd, Moreau, & Deschaux, 2008b; Hugues et al., 2006; Hugues & Garcia, 2007). It has also been demonstrated in fear extinguished rats that the hippocampus regulates the firing of prelimbic cortical neurons that are implicated in fear-motivated behavior (Sotres-Bayon, Sierra-Mercado, Pardilla-Delgado, & Quirk, 2012), as well as neurons in the LA that demonstrate context-specific activity (Maren & Hobin, 2007). Similar to the IL of the mPFC, the hippocampus could be a useful target for pharmacological treatments aimed at enhancing extinction, due to its involvement in regulating conditioned fear.

A number of neurobiological processes have been identified to underlie conditioned fear extinction. As previously discussed, the NMDA receptor serves a vital function in the induction and expression of conditioned fear, but it is also heavily involved in extinction memory. Systemic NMDA receptor antagonism impairs the consolidation of extinction memory, while sparing the initial acquisition of fear inhibition (Liu et al., 2009; Santini, Muller, & Quirk, 2001; Suzuki et al., 2004). However, selective inactivation of the NMDA receptor subunit Grin2b through systemic administration of ifendprodil impairs extinction acquisition (Sotres-Bayon, Bush, & LeDoux, 2007) in addition to long-term consolidation (Sotres-Bayon, Diaz-Mataix, Bush, & LeDoux, 2009). Thus far, extinction-related NMDA receptor signaling has been localized to two regions of the brain: the amygdala, and the mPFC. Local amygdalar NMDA antagonism impairs the acquisition of extinction (Falls, Miserendino, & Davis, 1992), even when only targeting LA Grin2b subunits (Laurent, Marchand, & Westbrook, 2008; Laurent & Westbrook, 2008; Sotres-Bayon et al., 2007). Additionally, synaptic plasticity in ITC neurons is dependent upon NMDA receptor function (Royer & Paré, 2002). However, while NMDA receptors in the LA do not appear to serve a critical role in the consolidation of extinction memory, NMDA receptor activity is necessary for extinction consolidation in the vmPFC (Laurent & Westbrook, 2008; Sotres-Bayon et al., 2009). Specifically, NMDA receptors are required for the IL neuronal bursting that is associated with successful extinction consolidation (Burgos-Robles et al., 2007).

Another important neurobiological component underlying extinction learning is BDNF, a protein which binds to the Tyrosine kinase B (TrkB) receptor to promote elements of synaptic

plasticity, including synaptogenesis (Horch et al., 1999; Yoshii & Constantine-Paton, 2010) and dendritogenesis (Yoshii & Constantine-Paton, 2007). The BDNF protein's ability to strengthen synapses, even in the absence of electrical stimulation, makes it a powerful modulator of memory and behavior (Autry et al., 2011; Liu et al., 2012; Messaoudi, Ying, Kanhema, Croll, & Bramham, 2002; Peters, Dieppa-Perea, Melendez, & Quirk, 2010). While fear conditioning impairs hippocampal BDNF transcription (Rasmusson, Shi, & Duman, 2002), fear extinction is associated with an upregulation of BDNF in fear suppression pathways (Bredy et al., 2007; Chhatwal, Stanek-Rattiner, Davis, & Ressler, 2006; Rosas-Vidal, Do-Monte, Sotres-Bayon, & Quirk, 2014). Following fear extinction, the presence of BDNF protein is increased in the ventral hippocampus (Rosas-Vidal et al., 2014), suggesting a role in the consolidation of extinction memory. BDNF also appears to mediate extinction memory retrieval, as hippocampal BDNF levels are correlated with the degree of successful long-term fear extinction recall (Peters et al., 2010). Projection of hippocampal BDNF to the IL plays a fundamental role in the BDNF-induced suppression of conditioned fear expression (Peters et al., 2010), and the presence of BDNF within the hippocampus even enhances the excitability of extinction-critical IL neurons (Rosas-Vidal et al., 2014). Furthermore, extinction training initiates epigenetic regulation of BDNF gene expression in the IL, increasing histone H4 acetylation of BDNF promoter regions to result in an upregulation of prefrontal BDNF mRNA (Bredy et al., 2007). Accordingly, administration of histone deacetylase inhibitors before extinction training augments this process, and produces a corresponding enhancement in extinction memory (Bredy et al., 2007). BDNF levels within the amygdala also appear to be implicated in fear extinction, since an upregulation of BDNF mRNA takes place in the LA following extinction training (Chhatwal et al., 2006).

Experimental manipulation of BDNF activity has demonstrated a clear mediatory role of the neurotrophin in the successful suppression of conditioned fear. For example, systemic administration of a TrkB agonist can facilitate extinction retrieval by preventing the renewal of conditioned fear in novel contexts (Baker-Andresen, Flavell, Li, & Bredy, 2013). Interestingly, the infusion of BDNF protein into the hippocampus (Peters et al., 2010) or IL (Peters et al., 2010; Rosas-Vidal et al., 2014) also induces a long-term reduction in the expression of conditioned fear, even in animals that have never received extinction training (Peters et al., 2010). Conversely, lentiviral inactivation of TrkB in the LA (Chhatwal et al., 2006), lentiviral deletion of the BDNF gene in the hippocampus (Heldt et al., 2007), or infusion of an anti-BDNF

antibody in the IL (Rosas-Vidal et al., 2014) impairs the long-term expression of extinction behavior. Thus, BDNF in hippocampal-mPFC-amygdala circuitry is both necessary and sufficient for fear extinction, and likely exerts its behavioral influence by supporting neural activity in pathways that are fundamental to extinction learning (Heldt et al., 2007; Peters et al., 2010; Rosas-Vidal et al., 2014).

The prevailing theory of fear extinction has been that extinction learning consists of the formation of a new memory that competes with the original conditioned fear association to inhibit fear-motivated behavioral output, as opposed to the fear memory being erased outright (Quirk & Mueller, 2008). However, while an immense body of research supports this view of extinction memory, it has become apparent that the process of fear extinction simultaneously involves a reversal of conditioning-induced enhancements of synaptic efficacy. As previously discussed, fear conditioning training enhances the surface expression of AMPA receptor subunits in LA pyramidal neuron synapses (Kim et al., 2007a; Nedelescu et al., 2010; Rumpel et al., 2005; Yeh et al., 2006), a process that is required for successful fear conditioning (Rumpel et al., 2005). Conversely, extinction decreases the surface expression of AMPA receptor subunits in LA neurons of previously conditioned animals through a process of endocytosis (Clem & Huganir, 2010; Kim et al., 2007a). Disruption of AMPA receptor endocytosis through administration of a GluR2-derived peptide accordingly impairs the acquisition and recall of fear extinction (Dalton et al., 2008; Kim et al., 2007a). Additionally, extinction reverses LTP in both cortical-LA (Hong et al., 2009) and thalamic-LA synapses (Hong et al., 2011; Kim et al., 2007a) that are essential to conditioned fear. However, the reversal of conditioning-induced LTP and AMPA receptor membrane insertion that occurs as a result of extinction should not be confused with memory erasure, which does not occur under normal extinction conditions, as conditioned fear can still be spontaneously recovered, or readily renewed or reinstated in the absence of re-conditioning (Pape & Pare, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013).

5.5. The Effects of Stress and Antidepressants on Fear Extinction

5.5.1. *Stress and fear extinction*

Not only does the experience of stress facilitate fear conditioning, but it also induces several pathological alterations in the morphology and neurobiology of the brain that impair neuroplasticity and threaten the underlying processes of successful fear memory extinction. As

previously discussed, the morphological integrity of the PFC is essential to extinction memory retrieval, as damage to this structure is associated with a reemergence of conditioned responding (Fernandez, 2003; Lebrón et al., 2004; Morgan et al., 1993; Morgan et al., 2003; Quirk et al., 2000; Sierra-Mercado et al., 2006). Posing a considerable threat to the healthy functioning of the PFC, both chronic behavioral stress and chronic exogenous CORT exposure have been found to induce significant dendritic atrophy and impairment of dendritic spine density in this region (Cook & Wellman, 2004; Dias-Ferreira et al., 2009; Izquierdo et al., 2006; Li et al., 2011; Liston et al., 2006; Liu & Aghajanian, 2008; Radley et al., 2004; Shansky & Morrison, 2009; Wellman, 2001). In addition, the hippocampus plays a vital role in ensuring that extinction memory is recalled in the appropriate context in which it was encoded (Corcoran et al., 2005; Corcoran & Maren, 2001; Corcoran & Maren, 2004; Hobin et al., 2006; Ji & Maren, 2005), a function that is potentially endangered by the volume reduction, dendritic atrophy, and mossy fiber synapse loss that accompanies chronic stress exposure (Delgado et al., 2011; Magariños et al., 1996; Magariños et al., 1998; Magariños et al., 1999; Magariños & McEwen, 1995a; Magariños & McEwen, 1995b; Morales-Medina et al., 2009; Sousa et al., 2000; Tata et al., 2006; Watanabe et al., 1992a; Watanabe et al., 1992b; Woolley et al., 1990). Interestingly, patients with PTSD, a disorder characterized by extinction memory failure, exhibit reductions in hippocampal volume as revealed by magnetic resonance imaging (O'Doherty, Chitty, Saddiqui, Bennett, & Lagopoulos, 2015).

Ultimately, it is neuronal activity within the PFC and hippocampus that underlies the suppression of fear-motivated behavior, so changes in LTP mediated by NMDA receptor activation in these regions could have dramatic effects on fear extinction. NMDA receptors in the PFC are critical to the successful acquisition and consolidation of extinction memory (Burgos-Robles et al., 2007; Laurent & Westbrook, 2008; Sotres-Bayon et al., 2009). Stress-induced damage to PFC dendrites and synapses could impair glutamatergic activation of local NMDA receptors, which would in turn impair LTP (Deutschenbaur et al., 2015; Pittenger et al., 2007).

In addition to the aforementioned pathological alterations of neuronal morphology, chronic stress induces changes in neurotrophin expression within extinction circuits that could impair the ability to successfully suppress conditioned fear. BDNF activity within the hippocampal-mPFC pathway is essential to the long-term expression of fear extinction, and past

research has demonstrated that interference with this mechanism results in the reemergence of conditioned fear responding (Heldt et al., 2007; Peters et al., 2010; Rosas-Vidal et al., 2014). Significantly undermining the integrity of fear extinction circuits, chronic stress and CORT exposure impairs BDNF expression in both the hippocampus and PFC (Aboul-Fotouh, 2015; Chen et al., 2008; Dwivedi et al., 2006; Filho et al., 2015; Fu et al., 2014; Gourley et al., 2008a; Grønli et al., 2006; Guo et al., 2014; Hamani et al., 2012; Jacobsen & Mørk, 2006; Jin et al., 2015; Li et al., 2012; Liang et al., 2012a; Liang et al., 2012b; Liu et al., 2014; Liu et al., 2015; Luoni et al., 2014; Monsey et al., 2014; Nibuya et al., 1999; Nitta et al., 1997; Ray et al., 2011; Smith et al., 1995; Yan et al., 2015; Zhang et al., 2015a; Zhu et al., 2016). Since BDNF is an inducer of LTP (Kang et al., 1997; Korte et al., 1996; Patterson et al., 1996), a stress-induced downregulation of this important neurotrophin could produce impairments in neuronal activity as well. Accordingly, chronic stress impairs LTP in both the hippocampus and hippocampal-PFC pathway of rodents (Cerqueira et al., 2007; Foy et al., 1987; Garcia et al., 1997; Kim et al., 1996; Pavlides et al., 2002; Shors et al., 1989; Xu et al., 1997). This carries significant consequences for long-term extinction retention, as hippocampal and prefrontal LTP exerts powerful influence over the prevention of conditioned fear reinstatement (Deschaux, Koumar, Canini, Moreau, & Garcia, 2015; Herry & Garcia, 2002; Zheng et al., 2013). Conversely, the suppression of LTP in the hippocampal-PFC pathway (as occurs as result of chronic stress) impairs extinction consolidation, promoting the spontaneous recovery of conditioned fear (Garcia et al., 2008b). Interestingly, the mere exposure of rats to conditioned tone and contextual cues also causes a downregulation of hippocampal BDNF mRNA, further impairing plasticity (Rasmusson et al., 2002).

Although impairment of hippocampal and prefrontal plasticity (and by extension, inhibitory control over the amygdala) would be sufficient to compromise fear extinction, this effect is likely exacerbated by the hypertrophy, reduced GABAergic inhibition, and enhanced excitability of the amygdala that results from stress and glucocorticoid exposure (Duvarci & Paré, 2007; Garcia et al., 1998; Kavushansky et al., 2006; Kavushansky & Richter-Levin, 2006; Mitra et al., 2005; Mitra & Sapolsky, 2008; Rodríguez Manzanares et al., 2005; Vyas et al., 2002; Vyas et al., 2003; Vyas et al., 2004; Vyas et al., 2006). Stress-induced enhancements in initial fear learning and fear memory retrieval could also pose an additional challenge to extinction, since stronger fear memories would have to be overcome compared to nonstressed

animals. Stress hormones in the amygdala also appear to mediate extinction learning, as intra-amygdalar infusion of CRF prior to extinction training impairs the long-term expression of extinction behavior (Abiri et al., 2014). Collectively, these studies suggest that stress produces a harmful combination of morphological, neurobiological, and functional alterations that impair the neural systems responsible for inhibiting fear, while simultaneously strengthening those that promote fear-motivated behavior.

Consistent with what can be predicted by studies on the neurophysiological consequences of stress exposure, behavioral animal research has confirmed that chronic stress compromises the ability to effectively extinguish fear memories and maintain suppression of conditioned fear. Several studies have reported that chronic behavioral stress or CORT exposure increases freezing during auditory cue extinction training and slows the rate at which conditioned responding is suppressed (Dagnino-Subiabre et al., 2012; Farrell et al., 2010; Gourley, Kedves, Olausson, & Taylor, 2009; Hoffman, Lorson, Nanabria, Olive, & Conrad, 2014; Zhang & Rozencranz, 2013). However, an initial impairment of extinction memory acquisition is not always observed (Baran, Armstrong, Niren, Hanna, & Conrad, 2009; Gourley et al., 2009; Miracle, Brace, Huyck, Singler, & Wellman, 2006; Wilber et al., 2011), and the effects of stress on fear extinction may not be apparent until subsequent tests for extinction memory recall are conducted. Research has reliably demonstrated that chronic behavioral stress impairs the recall of extinction memories, as evidenced by enhanced between-session freezing (i.e., spontaneous fear recovery) despite successful fear extinction by the end of the initial extinction training period (Baran et al., 2009; Farrell et al., 2010; Hoffman et al., 2014; Miracle et al., 2006; Wilber et al., 2011). Interestingly, stressed rats exhibiting poor extinction recall also display dendritic atrophy in IL neurons (Izquierdo et al., 2006), and fail to express an increase of CS-elicited IL neuronal activity that is normally observed in nonstressed animals (Wilber et al., 2011). Since IL activity is correlated with successful suppression of freezing behavior (Milad & Quirk, 2002), and the depression of IL neuron excitability is accompanied by spontaneous fear recovery (Cruz et al., 2014), this deficit of IL neuronal firing in stressed animals is a likely culprit for extinction memory recall failure. Although the effect of chronic exogenous CORT exposure on fear extinction recall has not yet received extensive investigation, one study has demonstrated a deficit in contextual extinction memory recall as a result of this treatment (Gourley et al., 2009). The results of this study also revealed a CORT-induced cortical reduction of Grin2b expression (Gourley et al.,

2009). Activation of the Grin2b NMDA receptor subunit is essential to extinction memory acquisition and consolidation (Sotres-Bayon et al., 2007; Sotres-Bayon et al., 2009). Accordingly, it was found that vmPFC levels of Grin2b were correlated with conditioned responding throughout extinction (Gourley et al., 2009).

There is evidence that stress and depression can also influence fear extinction in humans. For example, high scores for depressive symptoms predict enhanced fear conditioning and impaired fear extinction in adolescents (Den, Graham, Newall, & Richardson, 2015). Additionally, the experience of acute stress prior to extinction training impairs next-day extinction memory retrieval in adults, despite having no effects on initial extinction acquisition (Raio, Brignoni-Perez, Goldman, & Phelps, 2014). Further supporting a role for stress in the impairment of extinction consolidation, post-extinction stress exposure enhances both spontaneous fear recovery and trained fear reinstatement in men (Hamacher-Dang, Merz, & Wolf, 2015).

5.5.2. Antidepressants and fear extinction

Research on the efficacy of antidepressants in facilitating components of fear extinction has produced mixed results. In nonstressed rodents, chronic fluoxetine has been demonstrated to reduce conditioned responding during extinction acquisition (Camp et al., 2012; Lebrón-Milad et al., 2013), and impair or outright prevent the spontaneous recovery (Deschaux et al., 2011b; Deschaux et al., 2013; Karpova et al., 2011; Lebrón-Milad et al., 2013; Popova et al., 2014), contextual renewal (Karpova et al., 2011), sub-conditioning reinstatement (i.e., pairing the CS with a weak shock that is otherwise incapable of inducing fear conditioning in naive animals; Deschaux et al., 2013; Karpova et al., 2011; Spennato, Zerbib, Mondadori, & Garcia, 2008), or stress-induced reinstatement (Deschaux et al., 2013) of previously extinguished fear-motivated behavior. Acute and chronic treatment with venlafaxine has also been found to prevent spontaneous fear recovery and sub-conditioning reinstatement, respectively (Yang et al., 2012). In non-depressed humans, chronic pretreatment with escitalopram can accelerate the acquisition of fear extinction, but extinction memory retention was not investigated (Bui et al., 2013). However, chronic antidepressant treatment with either citalopram or tianeptine has surprisingly been found to impair the acquisition of fear extinction and downregulate expression of amygdalar Grin2b in rats (Burghardt, Sigurdsson, Gorman, McEwen, & LeDoux, 2013).

Likewise, acute fluoxetine or tianeptine treatment impairs both extinction acquisition and recall in rats (Godsil et al., 2015; Lebrón-Milad et al., 2013). These findings raise concerns that treatment with these drugs might be counter-productive to challenging negative emotional memory biases. Further research into the pharmacological facilitation of extinction is needed, as no studies have yet investigated the ability for antidepressants to prevent chronic stress-induced fear recovery. A fast-acting drug that can both accelerate the suppression of conditioned responding and support the long-term maintenance of extinction memory in chronically-stressed animals could prove valuable in the treatment of depression-associated negative cognitive biases.

6. Ketamine as a Rapid-Acting Treatment for Depression

6.1. Limitations of Standard Antidepressant Treatments

Most antidepressants prescribed today exert their influence on mood and behavior by regulating the availability of monoamines (e.g., serotonin, norepinephrine) in the brain. However, despite having rapid effects on extracellular monoamine availability, achieving remission with these drugs often takes several weeks or months of treatment and more than one unique drug treatment (Judd et al., 2002; Thase et al., 2005; Trivedi et al., 2006). These findings suggest that the antidepressant properties of monoamine-regulating drugs are the result of downstream neurobiological alterations in the brain, and not that of direct modulation of the monoaminergic system itself (Heninger, Delgado, & Charney, 1996; Maeng & Zarate, 2007; Sanacora et al., 2008; Sanacora et al., 2012). Since a third of depressed patients remain resistant to treatment even after trialling four unique antidepressant drugs (Gaynes et al., 2009), there is a clear need for new strategies in the pharmacological treatment of depression.

6.2. Ketamine and the Treatment of Depression in Humans

A wealth of research has implicated disordered glutamatergic neurotransmission in the pathophysiology of depression (Deutschenbaur et al., 2015; Krystal et al., 2013; Pittenger & Duman, 2008; Pittenger et al., 2007; Sanacora et al., 2008; Sanacora et al., 2012), making the glutamatergic system a potential target for novel antidepressant treatments. One drug that has received particular attention due to its rapid-acting antidepressant effects is ketamine. Ketamine is primarily a noncompetitive antagonist of the NMDA receptor that blocks receptor activation by binding to the phencyclidine site of the complex's cation channel (Sanacora et al., 2008). In

addition to being an NMDA receptor antagonist, ketamine can also block peripheral sodium (Na^+) currents (Benoit, Carratù, Dubois, & Mitolo-Chieppa, 1986), and block the nicotinic acetylcholine receptor (Scheller et al., 1996). Furthermore, ketamine acts as a partial agonist of the D_2 dopamine receptor (Kapur & Seeman, 2002), and as a weak agonist of the 5-hydroxytryptamine 2A (5-HT_{2A}) receptor (Kapur & Seeman, 2002), sigma receptor (Oye, Paulsen, & Maurset, 1992; Smith et al., 1987), delta opioid receptor (Hirota et al., 1999), kappa opioid receptor (Hirota et al., 1999; Hustveit, Maurset, & Oye, 1995), and mu opioid receptor (Gupta, Devi, & Gomes, 2011; Hirota et al., 1999).

In randomized, double-blind clinical trials of patients with major depression, acute subanesthetic infusions of ketamine have demonstrated robust antidepressant effects compared to placebo (Berman et al., 2000; Diazgranados et al., 2010; Zarate et al., 2006), a finding which has since been reliably replicated in several open-label studies (Cusin et al., 2012; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price et al., 2009; Rasmussen et al., 2013). Reductions in depression symptom severity are apparent within 2 hours of a single ketamine infusion, and full remission can be achieved in some patients in as little as 24 hours (Diazgranados et al., 2010; Murrough et al., 2013; Zarate et al., 2006). Patients who respond positively to ketamine treatment can experience antidepressant effects lasting more than 2 weeks from a single dose (Aan Het Rot, Zarate, Charney, & Matthew, 2012), and serial ketamine treatment can maintain remission for over 6 months (Cusin et al., 2012). When combined with ECT, ketamine produces a greater reduction in depressive symptoms than ECT alone (Kranaster, Krammerer-Ciernioch, Hoyer, & Sartorius, 2011; Okamoto et al., 2010; Wang, Liu, Patterson, Paule, & Slikker, 2013). Ketamine has even demonstrated remarkable efficacy in treating patients who have previously proved to be unresponsive to standard antidepressant drugs and ECT (Cusin et al., 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price et al., 2009; Zarate et al., 2006). While standard antidepressant drugs can take 8 weeks to achieve a response rate of 65% in non-treatment-resistant samples (Entsuah, Huang, & Thase, 2001; Thase et al., 2005), a single dose of subanesthetic ketamine produces over a 70% response rate in treatment-resistant patients after a mere 24 hours (Zarate et al., 2006).

6.3. The Neurobiology of Ketamine's Antidepressant Effects

The robust and fast-acting antidepressant effects of ketamine are without a doubt impressive. According to Machado-Vieira and colleagues, “There has never been a report of any other somatic or pharmacological intervention that consistently and reproducibly results in such a dramatically rapid and prolonged response—well beyond the half-life on the drug—with a single administration” (Machado-Vieira, Salvadore, DiazGranados, & Zarate, 2009). Research has begun to shed light on the complex mechanisms through which ketamine might exert these unique antidepressant qualities. First, ketamine stimulates presynaptic glutamate release by reducing the activity of inhibitory GABAergic interneurons in the PFC and hippocampus (Chowdhury et al., 2012; Homayoun & Moghaddam, 2007; Moghaddam, Adams, Verma, & Daly, 1997; Stone et al., 2012). At first glance, this glutamatergic efflux may appear to be counter-productive to the treatment of depression, since high levels of extracellular glutamate are neurotoxic and associated with depression itself (Hashimoto et al., 2007; Jun et al., 2014; Lan et al., 2009). However, glutamate-induced neurotoxicity and cell death is dependent upon the activation of extrasynaptic NMDA receptors, which are blocked by ketamine (Hardingham et al., 2002; Manev et al., 1989; Ivanov et al., 2006). Furthermore, ketamine likely binds preferentially to extrasynaptic NMDA receptors as opposed to synaptic NMDA receptors, due to the greater presence of high-affinity Grin2b NMDA receptor subunits and reduced relative concentration of endogenous glutamate in extrasynaptic regions of the neuron (Pittenger & Duman, 2008). Thus, by preferentially blocking the activation of extrasynaptic NMDA receptors while simultaneously stimulating presynaptic glutamate release, ketamine enhances glutamatergic neurotransmission through synaptic AMPA and NMDA receptors without the risk of glutamate excitotoxicity. Accordingly, administration of ketamine has been shown to potentiate excitatory field potentials in the hippocampus (Autry et al., 2011; Nosyreva et al., 2013) and PFC (Li et al., 2011). The facilitation of glutamatergic neurotransmission is an essential underlying component of ketamine's antidepressant behavioral effects, which are blocked by the co-administration of an AMPA receptor antagonist (Autry et al., 2011; Koike et al., 2011).

However, while this transient burst of glutamatergic throughput plays an important role in ketamine's antidepressant qualities, the fact that a clinical response is maintained well beyond the drug's 180 minute half-life suggests that there are further downstream mechanisms at work (Clements, Nimmo, & Grant, 1982). Ketamine's second major therapeutic mechanism of action

appears to involve a positive modulation of neuroplasticity that causes a sustained reversal of stress-induced neuronal damage. Notably, ketamine has been shown to modulate the expression of the BDNF protein, which plays an important role in promoting synaptic plasticity (Horch et al., 1999; Yoshii & Constantine-Paton, 2007; Yoshii & Constantine-Paton, 2010) and LTP (Kang et al., 1997; Korte et al., 1996; Patterson et al., 1996). While chronic stress induces a long-lasting inhibition of hippocampal and prefrontal BDNF transcription and synthesis (Aboul-Fotouh, 2015; Chen et al., 2008; Dwivedi et al., 2006; Filho et al., 2015; Fu et al., 2014; Gourley et al., 2008; Grønli et al., 2006; Guo et al., 2014; Hamani et al., 2012; Jacobsen & Mørk, 2006; Jin et al., 2015; Li et al., 2012; Liang et al., 2012a; Liang et al., 2012b; Liu et al., 2014; Liu et al., 2015; Luoni et al., 2014; Monsey et al., 2014; Nibuya et al., 1999; Nitta et al., 1997; Ray et al., 2011; Smith et al., 1995; Yan et al., 2015; Zhang et al., 2015a; Zhu et al., 2016), administration of a subanesthetic dose of ketamine reverses this effect by inducing an increase of BDNF protein expression in the hippocampus and prefrontal cortex within 30 minutes (Autry et al., 2011; Garcia et al., 2008a; Zhang et al., 2015a; Zhang et al., 2015b). A single dose of ketamine can attenuate stress-induced reductions in hippocampal and prefrontal BDNF for at least 8 days (Zhang et al., 2015a), resembling the time course of its long-lasting antidepressant effects in rodent models of depression (Autry et al., 2011; Jett et al., 2015; Li et al., 2011; Maeng et al., 2008). This upregulation of BDNF is likely the result of ketamine's temporary facilitation of glutamatergic neurotransmission, since both synaptic AMPA and NMDA receptor activation induces BDNF (Hardingham et al., 2002; Jourdi et al., 2009). BDNF induction plays a necessary role in the antidepressant-like behavioral effects of ketamine, which are abolished when ketamine is administered to animals with BDNF gene mutation or knockout (Autry et al., 2011; Liu et al., 2012), or TrkB receptor knockout (Autry et al., 2011). Consistent with increased BDNF activity, subanesthetic ketamine also upregulates a number of synaptic proteins within an hour of administration, including synapsin I, PSD-95, and AMPA subunit Gria1, reversing stress-induced impairments in the expression of these proteins (Li et al., 2010; Li et al., 2011; Zhang et al., 2015a). The ultimate neurophysiological result of ketamine's neurotrophin and synaptic protein upregulation is an enhancement of PFC dendritic spine density and maturity that occurs within 24 hours of infusion, reversing damage caused by chronic stress (Li et al., 2010; Li et al., 2011; Monsey et al., 2014). It is likely this facilitation of neuroplasticity that allows

ketamine to maintain an antidepressant response long after NMDA receptor antagonism has ceased and the drug has been cleared from the body.

6.4. Ketamine in Animal Models of Depression

The rapid antidepressant-like effects of ketamine have been reliably reproduced in rodent models of depression and tests of depression-like behavior. Within an hour of acute subanesthetic ketamine injection, nonstressed rodents exhibit reduced immobility in the forced swim test (Autry et al., 2011; Carrier & Kabbaj, 2013; Gigliucci et al., 2013; Maeng et al., 2008; Nosyreva et al., 2013), reduced escape failures and latency to escape in a learned helplessness paradigm (Autry et al., 2011; Maeng et al., 2008), and increased sucrose solution consumption in the sucrose preference test of anhedonia (Carrier & Kabbaj, 2013). Furthermore, ketamine abolishes enhancements in depression-like behavior that result from chronic behavioral stress or exogenous CORT injection. Chronic stress impairs cognitive flexibility in the attentional set-shifting task (Jett et al., 2015), promotes passivity in the shock-probe defensive burying task (Jett et al., 2015), increases immobility in the forced swim test (Autry et al., 2011; Gigliucci et al., 2013; Jett et al., 2015; Koike et al., 2013; Perrine et al., 2014), and evokes anhedonic behavior in the sucrose preference test (Autry et al., 2011; Garcia et al., 2009; Li et al., 2011), all of which are normalized following a single infusion of ketamine. Similar to humans, antidepressant-like effects in rodents are maintained for at least a week after a single injection of ketamine (Autry et al., 2011; Jett et al., 2015; Li et al., 2011; Maeng et al., 2008).

The potential for ketamine to alleviate depression-associated negative cognitive biases has not yet received extensive investigation. Given the fundamental role that NMDA receptors play in the acquisition (Bauer et al., 2002; Campeau et al., 1992; Fanselow & Kim, 1994; Fendt, 2001; Gewirtz & Davis, 1997; Lee & Kim, 1998; Lee et al., 2001; Maren et al., 1996b; Miserendino et al., 1990; Walker & Davis, 2000), consolidation (Shimizu et al., 2000), and recall (Fendt, 2001; Lee et al., 2001; Lee & Kim, 1998; Maren et al., 1996b) of conditioned fear, it seems likely that the NMDA receptor antagonist ketamine could influence memory processing of negative emotional information. Early rodent studies have demonstrated that a subanesthetic dose of ketamine, administered 30 minutes prior to training, neutralizes conditioned freezing to auditory cues during recall testing, and reduces neuronal activity in the LA as indicated by c-Fos expression (Pietersen et al., 2006; Pietersen et al., 2007). Ketamine also impairs the long-term

recall of contextual fear memory when administered 15 minutes before contextual fear conditioning training (Calzavara et al., 2009). Interestingly, chronic treatment with ketamine, terminated 48 hours before both training and recall testing, similarly reduces context-elicited freezing during fear recall assessment in mice, suggesting long-term effects on memory processing that are independent of transient NMDA receptor antagonism (Amann et al., 2009). Some research has also suggested that ketamine can even mediate the retrieval of fear memory after successful memory consolidation has taken place. Mice treated with subanesthetic ketamine injections for 14 days following contextual fear conditioning display reduced freezing to contextual cues compared to controls (Zhang et al., 2015b). In humans, a single subanesthetic infusion of ketamine has demonstrated efficacy in reducing symptoms of PTSD, a disorder characterized by pathological recurrent fear memory retrieval (Feder et al., 2014).

7. Objectives and Hypotheses

Although animal research does support a modulatory role for ketamine in negative emotional memory, there is still some uncertainty regarding exactly which stages of memory processing ketamine influences. Ketamine's long-lasting neurophysiological effects could theoretically alter fear memory acquisition, consolidation, recall, or any combination of the three from a single pre-conditioning injection, so further research is needed in order to elucidate the specific cognitive processes that are influenced by this drug. Furthermore, no study to my knowledge has yet explored the potential for ketamine to relieve pathological enhancements in fear memory caused by chronic stress. Since a number of standard antidepressants are either ineffective or counter-productive in challenging negative cognitive biases with acute treatment (Browning et al., 2007; Burghardt et al., 2004; Burghardt et al., 2013; Burhans et al., 2013; Cassaday & Thur, 2015; Godsil et al., 2015; Lebrón-Milad et al., 2013; Montezinho et al., 2010; Mørk et al., 2013; Ravinder et al., 2013; Takahashi et al., 2006), a drug which can rapidly suppress negative emotional memory in conjunction with other potent antidepressant effects could prove incredibly valuable in the treatment of depression.

One issue that has not yet been examined in any capacity is the effect that ketamine might have on the extinction, recovery, and reinstatement of conditioned fear. This is an important topic to address, since acute and chronic treatment with some antidepressants actually impairs the acquisition and recall of fear extinction memory (Burghardt et al., 2013), a result that could

be counter-productive to the goal of challenging negative cognitive biases in depression. However, ketamine produces a number of neurobiological changes and functional alterations in the brain that could theoretically facilitate fear extinction, at least in chronically stressed animals. Chronic stress exposure impairs neuroplasticity and LTP in the hippocampus and PFC (Cerqueira et al., 2007; Cook & Wellman, 2004; Delgado et al., 2011; Dias-Ferreira et al., 2009; Foy et al., 1987; Garcia et al., 1997; Izquierdo et al., 2006; Kim et al., 1996; Li et al., 2011; Liston et al., 2006; Liu & Aghajanian, 2008; Magariños et al., 1996; Magariños et al., 1998; Magariños et al., 1999; Magariños & McEwen, 1995a; Magariños & McEwen, 1995b; Morales-Medina et al., 2009; Palvides et al., 2002; Radley et al., 2004; Shansky & Morrison, 2009; Shors et al., 1989; Sousa et al., 2000; Tata et al., 2006; Watanabe et al., 1992a; Watanabe et al., 1992b; Wellman, 2001; Woolley et al., 1990; Xu et al., 1997), compromising the ability for these structures to inhibit the amygdala and resulting fear output during extinction training and recall (Baran et al., 2009; Dagnino-Subiabre et al., 2012; Farrell et al., 2010; Gourley et al., 2009; Hoffman et al., 2014; Izquierdo; Miracle et al., 2006; Wilber et al., 2011; Zhang & Rozencranz, 2013). Conversely, ketamine enhances neuronal activity and mechanisms of neuroplasticity in the PFC and hippocampus of stressed animals (Chowdhury et al., 2012; Li et al., 2011; Moghaddam et al., 1997; Stone et al., 2012; Zhang et al., 2015a; Zhang et al., 2015b), which could allow these structures to regain inhibitory control over the amygdala, and thus successfully suppress fear-motivated behavior. However, NMDA receptor function is required for the acquisition and consolidation of extinction memory (Falls et al., 1992; Laurent et al., 2008; Laurent & Westbrook, 2008; Liu et al., 2009; Santini et al., 2001; Sotres-Bayon et al., 2007; Sotres-Bayon et al., 2009; Suzuki et al., 2004), so it is also possible that the NMDA receptor antagonist ketamine could impair these processes if enough of the drug is still present in the brain at this time. Resolving this uncertainty is a necessary step in evaluating the efficacy of subanesthetic ketamine infusions in challenging stress-induced negative cognitive biases.

The aim of this dissertation is to address these notable gaps in the literature by further characterizing the effects of ketamine on negative emotional memory in both healthy animals and those with chronic CORT exposure. The first experiment sought to dissociate the effects of ketamine on the acquisition, consolidation, and recall of auditory fear conditioning in rats. Furthermore, it was also designed to resolve prior uncertainty regarding the influence of ketamine on the induction and recall of fear extinction. To accomplish this, rats were fear

conditioned and extinguished, and a subanesthetic dose of ketamine was administered at one of three different time points: before fear conditioning training, immediately after fear conditioning training, or before fear extinction training. Fear-motivated behavior in the form of defensive freezing was recorded to measure the effects of ketamine on behavior throughout the conditioning and extinction trials. I hypothesized that ketamine would impair the long-term expression of freezing when administered at any of the three time points, but would not adversely affect extinction behavior.

The second experiment aimed to expand upon the findings of the first by examining the efficacy of ketamine in preventing chronic CORT-induced alterations in auditory fear conditioning and extinction behavior. Of particular interest was the effect of CORT and ketamine on the long-term retention of extinction behavior following a sub-conditioning reinstatement challenge. Rats were fear conditioned, extinction trained, and then submitted to a sub-conditioning retraining procedure that is designed to reinstate conditioned fear in previously trained, but not conditioning-naive rats. I hypothesized that chronic CORT treatment would impair the long-term retention of fear extinction between extinction training sessions and following sub-conditioning retraining. Furthermore, I hypothesized that a pre-extinction subanesthetic dose ketamine would reduce freezing during extinction training in both CORT-treated and healthy rats, and prevent CORT-induced deficits in extinction retention.

CHAPTER 2

Post-Conditioning and Pre-Extinction Ketamine Attenuates the Expression of Auditory Conditioned Fear

Brian D. Kulyk¹ and Lisa E. Kalynchuk²

¹ Department of Psychology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

² Department of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Abstract

Negative cognitive biases in memory skew an individual's recollection of past experiences towards negative emotional content, and contribute significantly to the burden of psychiatric disorders such as PTSD and major depressive disorder. Pharmacotherapeutic agents which can inhibit the recall of unpleasant memories are thus of significant value to the treatment of these disorders. By using fear conditioning tasks to assess the recall of negatively-valenced memory in rodents, past research has revealed that a single subanesthetic dose of the NMDA receptor antagonist ketamine can markedly suppress the long-term expression of context- and cue-elicited freezing behavior. However, these studies were unable to discern whether pre-conditioning ketamine treatment primarily interferes with the acquisition, consolidation, or recall of conditioned fear memory, and ketamine's influence on fear extinction has remained unexamined. The aim of the present study was to dissociate the effects of ketamine on distinct stages of auditory fear conditioning and extinction in order to gain a better understanding of the underlying cognitive processes that the NMDA receptor antagonist might modulate. Rats underwent auditory fear conditioning, and after a 9 day rest period were extinction trained and tested for fear recall. A subanesthetic 10 mg/kg dose of ketamine was systemically administered to rats at one of three different time points: before fear conditioning training, immediately after fear conditioning training, or before fear extinction training. Fear conditioning was unimpaired in rats that received pre-conditioning ketamine, but post-conditioning and pre-extinction ketamine treatment attenuated freezing during fear recall assessment. None of the ketamine treatment protocols examined had any effect on the final acquisition or long-term recall of fear extinction behavior. The findings of the present study suggest that ketamine likely exerts its influence on fear-motivated behavior by interfering with the consolidation and recall of auditory fear memory.

1. Introduction

Memory for negative emotional information is strongly modulated by the presence of stress hormones (Rodrigues et al., 2009), and is thus highly susceptible to pathological modification in stress-related psychiatric disorders. While it is well-known that PTSD is characterized by the recurrent involuntary recollection of traumatic events, individuals with depression also experience maladaptive alterations in memory that skew the recollection of past events towards negative emotional information (Balut et al., 2013; Gerritsen et al., 2012; Gupta & Kar, 2012; Harmer et al., 2009a; Hsu et al., 2010; Liu et al., 2012; Mogg et al., 2014; Taylor & John, 2004). These negative cognitive biases are believed to support the development and maintenance of depression (Elliott et al., 2011; Harmer et al., 2009a; Nolen-Hoeksema, 2000; Robinson & Sahakian, 2008), emphasizing the need for antidepressant treatments to effectively combat these insidious symptoms (Clark, Chamberlain, & Sahakian, 2009; Elliott et al., 2011; Roiser et al., 2012).

To experimentally investigate memory for negatively valenced stimuli in animals, researchers often use classical fear conditioning, a behavioral paradigm that promotes a learned association between an emotionally neutral conditioned stimulus (e.g., tone cue) and an innately aversive and negatively valenced unconditioned stimulus (e.g., footshock). Following successful fear conditioning, presentation of the conditioned stimulus alone will elicit fear-motivated responses such as defensive freezing behavior. Fear learning is rapidly acquired and generally very robust, but under proper circumstances can later be challenged through a form of novel learning known as extinction. Extinction learning occurs when a conditioned stimulus is repeatedly presented in the absence of unconditioned stimulus reinforcement, and consists of the formation of a new memory that competes with the original fear memory to suppress fear-motivated behavior (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). Together, fear conditioning and extinction paradigms have proved to be valuable assets in the study of pharmacotherapeutic agents used to treat psychiatric disorders and their effects on negatively-valenced memory.

SSRIs are often prescribed to treat depression and PTSD, but several common SSRIs have surprisingly been shown to enhance fear memory (Burghardt et al., 2004; Burhans et al., 2013; Cassaday & Thur, 2015; Montezinho et al., 2010; Ravinder et al., 2013) and impair extinction (Burghardt et al., 2013) in animal models of conditioned fear. This apparent

facilitation of negatively-biased memory might be counter-productive in the treatment of depression and PTSD, so the identification of drugs that instead inhibit fear memory could prove to be of great value to patients affected by these disorders. One promising drug is ketamine, a noncompetitive antagonist of the NMDA receptor that blocks receptor activation by binding to the phencyclidine site of the complex's cation channel (Sanacora et al., 2008). Subanesthetic infusions of ketamine produce rapid and robust antidepressant effects in patients with treatment-resistant depression (Aan Het Rot et al., 2012; Berman et al., 2000; Cusin et al., 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price et al., 2009; Rasmussen et al., 2013; Zarate et al., 2006), and also reduce symptom severity in patients with PTSD (Feder et al., 2014). Ketamine is well-equipped to mediate memory for negatively valenced stimuli, since the NMDA receptors it inactivates (specifically, those in the lateral amygdala) play an important role in the acquisition (Bauer et al., 2002; Campeau et al., 1992; Fanselow & Kim, 1994; Fendt, 2001; Gewirtz & Davis, 1997; Lee et al., 2001; Lee & Kim, 1998; Maren et al., 1996b; Miserendino et al., 1990; Walker & Davis, 2000), consolidation (Shimizu et al., 2000), and recall (Fendt, 2001; Lee et al., 2001; Lee & Kim, 1998; Maren et al., 1996b) of conditioned fear. Accordingly, infusion of a subanesthetic dose of ketamine prior to fear training impairs both auditory (Pietersen et al., 2006) and contextual (Calzavara et al., 2009) fear conditioning in rats.

While the results of these early studies support the hypothesis that ketamine can impair fear conditioning, they cannot identify the precise stages of memory that ketamine might influence. The effects of ketamine on the neurobiology of the brain extend far beyond transient NMDA receptor antagonism, and involve a complex downstream signaling cascade that upregulates the presence of BDNF in brain regions intimately involved in the processing of fear memory (Autry et al., 2011; Duman et al., 2012; Zhang et al., 2015a; Zhang et al., 2015b). The BDNF protein is an important regulator of neuroplasticity and synaptic transmission (Black, 1999; Jourdi et al., 2009), so this long-lasting impact on neuronal functioning means that a single infusion of ketamine might influence any combination of the acquisition, consolidation, and recall processes of fear memory. Although one study has suggested that repeated administration of ketamine can impair contextual fear recall in mice after undisturbed training (Zhang et al., 2015b), further research is needed to more fully characterize the effects that acute treatment with ketamine has on every stage of auditory fear learning.

To our knowledge, the short- and long-term effects of ketamine on fear extinction has not yet been examined in any capacity. Similar to fear conditioning, the acquisition (Falls et al., 1992; Laurent et al., 2008; Laurent & Westbrook, 2008; Sotres-Bayon et al., 2007) and consolidation (Laurent & Westbrook, 2008; Liu et al., 2009; Santini et al., 2001; Sotres-Bayon et al., 2009; Suzuki et al., 2004) of extinction memory relies heavily upon NMDA receptor activation, so it is possible that ketamine might impair these cognitive processes at a high enough dose and with sufficient temporal proximity to extinction training. However, since the endogenous promotion of prefrontal and hippocampal BDNF production plays a critical role in the long-term retention of fear extinction (Bredy et al., 2007; Heldt et al., 2007; Rosas-Vidal et al., 2014), a ketamine-induced upregulation of BDNF could potentially facilitate fear suppression instead. Resolving this uncertainty is an important step in characterizing the effects of ketamine on negative emotional memory, since either a facilitation or impairment of extinction memory could have significant clinical implications on the treatment of depression and PTSD with ketamine.

The aim of the present study was to dissociate the effects of ketamine on distinct stages of auditory fear conditioning and extinction in order to gain a better understanding of the underlying cognitive processes that the NMDA receptor antagonist might modulate. A subanesthetic dose of ketamine was administered to rats at one of three time points: before fear conditioning training, immediately after fear conditioning training, or before fear extinction training. Defensive freezing behavior was analyzed throughout the conditioning and extinction trials, and then compared to a non-drug-treated control group. We hypothesized that ketamine would impair the long-term expression of freezing when administered at any of the three time points, but would not adversely affect extinction behavior.

2. Materials and Methods

2.1. Animals

Thirty-three adult male Long-Evans rats (Charles River Laboratories; Montreal, Quebec, Canada), weighing 200-250 g upon arrival, were used in the study. Rats were housed individually in clear plastic cages with free access to food and water. The thermally-controlled colony room maintained a 12 hr/12 hr light/dark cycle. All procedures were approved by the

University of Saskatchewan Animal Research Ethics Board, and conducted in accordance with the Canadian Council on Animal Care.

2.2. Experimental Design and Drug Injection Paradigm

Prior to any fear conditioning procedures, rats were assigned to one of four weight-matched experimental conditions: untreated control ($n = 9$), pre-conditioning ketamine injection ($n = 8$), post-conditioning ketamine injection ($n = 8$), and pre-extinction ketamine injection ($n = 8$).

Ketamine hydrochloride (Vetalar; Bioniche Animal Health Canada Inc.; Belleville, Ontario, Canada) was suspended in saline and injected intraperitoneally at a dose of 10 mg/kg, and a volume of 1 ml/kg. This dose was selected as it has been shown to be efficacious in impairing fear conditioning when administered before training (Amann et al., 2009; Calzavara et al., 2009; Li et al., 2011). In the case of pre-conditioning and pre-extinction ketamine groups, rats received injections 60 minutes prior to fear conditioning training and extinction training, respectively. This delay between drug injection and behavioral testing was implemented to avoid the potentially confounding transient effects of ketamine on spontaneous locomotor activity. Since general locomotor activity in rats is normalized within 30 minutes of a 10 mg/kg dose of ketamine (Koike et al., 2011; Koike et al., 2013), any ketamine-associated changes in freezing beyond this time point cannot be attributed to alterations in baseline movement. Rats in the post-conditioning ketamine group received injections immediately following the conclusion of fear conditioning training. Administration of all injections took place in a procedure room separate from the animal colony and fear conditioning apparatus.

2.3. Fear Conditioning

Fear conditioning and extinction followed a modified version of a protocol previously used by Burghardt et al. (2013).

2.3.1. Apparatus

All behavioral testing procedures took place in standard operant chambers (VFC-008; Med Associates Inc., St. Albans, Vermont, United States) contained within sound-attenuating cubicles. Each operant chamber was constructed of aluminum walls and a clear plastic door

through which a video camera could record the rat's behavior in both visible and near-infrared light. An overhead white or near-infrared lamp illuminated the chamber through a clear plastic roof. A high-frequency speaker (ENV-224BM) mounted high within the chamber wall delivered auditory cues. The floor of the chamber consisted of a grid of stainless steel rods that delivered aversive footshocks through a shock generator (ENV-414S). Video Freeze Software (Med Associates Inc.) controlled the delivery of tone and shock stimuli, and recorded video from the camera at 30 frames per second.

2.3.2. Conditioning

Handling of all rats took place for 7 consecutive days before any behavioral procedures began to habituate the animals to the researcher. Following the conclusion of handling, rats were then habituated to the conditioning context of the operant chamber for 8 minutes (day 1). On day 2, each rat was placed inside the operant chamber again and given 180 s to habituate before fear conditioning began. Conditioning consisted of 2 tone cues (2 kHz, 20 s, 90 dB) that co-terminated with an aversive footshock (0.7 mA, 500 ms). Each tone-shock pairing was followed by a 120 s inter-trial interval. Upon completion of fear conditioning, rats were transported back to the colony room, and the operant chamber was cleaned with 0.4% glacial acetic acid before training for the next subject began.

2.3.3. Novel context extinction

Since the rats would form fearful associations with the contextual cues of the training environment in addition to the discrete tone cues, the context during extinction was altered in a number of ways so that only auditory-cued conditioned fear would be measured and extinguished during this phase. During transit to and from the conditioning room, the rats' cages were covered with dark curtain, and an alternative route was followed. The fluorescent lighting of the conditioning room was replaced with a red overhead lamp. Vanilla-scented deodorizer provided novel olfactory cues in the extinction context. Most importantly, the operant chambers were modified in shape and appearance with a colorful curved wall insert, and the metal grid floor was replaced with a smooth white plastic cover. A striped ceiling cover panel altered the lighting pattern within the chamber, and only near-infrared lighting was used. Contextual auditory cues were obscured with white noise played through the chamber's speaker (65 dB).

After each extinction session, the chamber was cleaned with 75% ethanol so that any residual scent of the cleaner would be unfamiliar.

Rats rested for 9 consecutive days before extinction training began (day 12). Rats were placed into the novel extinction context and given 180 s to habituate. Extinction consisted of 10 tone cues (2 kHz, 20 s, 90 dB) presented in the absence of footshock. Each tone was followed by a 120 s inter-trial interval. Upon completion of extinction training, rats were transported back to the colony room. On day 13, long-term retention of extinction memory was evaluated by presenting an additional 10 tone cues, using the same extinction protocol as described above. Once extinction retention testing concluded, animals were transported back to the colony room and sacrificed the following day.

2.3.4. Behavioral measurement

Fear conditioning was measured by freezing, defined as the absence of any movement not required for respiration. Using Video Freeze Software (Med Associates Inc.; St. Albans, Vermont, United States), movement was calculated as a change in pixel composition between adjacent video frames. To account for individual variation in baseline breathing movement, a motion threshold was set for each rat that defined the absolute limit beyond which movement would be registered as non-respiratory in nature. Immobility was required to last for at least 1 s before a freezing episode would be recorded in the data output. The percentage of time spent freezing during discrete time points (e.g. habituation, tones) was then calculated by Video Freeze Software and used as the dependent variable in analysis.

2.4. Statistical Analysis

Freezing behavior during pre-conditioning and pre-extinction habituation was analyzed using one-way ANOVAs to reveal any potential differences in baseline freezing between groups. A one-way ANOVA was also used to identify possible group differences in freezing behavior during fear conditioning training, as enhanced or impaired fear acquisition could affect the interpretation of subsequent measures. The treatment effects of ketamine on cue-elicited freezing during extinction training and extinction recall were analyzed using independent samples t-tests, which compared the average level of freezing for each treatment group (i.e. pre-conditioning ketamine, post-conditioning ketamine, and pre-extinction ketamine) to that of the control group

(i.e. no injection) in separate analyses. Animals that exhibited levels of freezing exceeding 1.5 standard deviations of the mean during extinction training or recall were considered outliers and excluded from statistical analysis.

3. Results

3.1. Fear Conditioning Training Day

Ketamine treatment did not affect freezing during the 180 s habituation period prior to training [$F(3, 21) = 1.656, p = 0.207$] (Fig. 2-1A). Average freezing during tone cue presentation was also not significantly affected by ketamine in the fear conditioning training phase [$F(3, 21) = 0.681, p = 0.573$] (Fig. 2-1B).

3.2. Fear Extinction Training Day

No significant group differences in freezing were found during the 180 s habituation period prior to extinction training, indicating that ketamine treatment did not affect behavior in a novel context [$F(3, 21) = 2.593, p = 0.080$] (Fig. 2-2A; Fig. 2-3A; Fig. 2-4A).

The long-term expression of conditioned fear was investigated by analyzing the average level of freezing across the first five tone cues of extinction training (early phase extinction training; T1-T5). Rats in the pre-conditioning ketamine group exhibited average levels of cue-elicited freezing similar to that of rats in the control group [$t(10) = 0.890, p = 0.413$] (Fig. 2-2B). However, rats in the post-conditioning ketamine [$t(11) = 2.550, p = 0.027$] (Fig. 2-3B) and pre-extinction ketamine [$t(12) = 2.926, p = 0.013$] (Fig. 2-4B) groups froze significantly less across the first five tones of extinction training than rats in the control group. Average freezing across the last five tone cues of extinction training (late phase extinction training; T6-T10) was analyzed to assess the relative level of extinction behavior acquisition during the latter half of the session. Each ketamine treatment group froze comparably to the control group in response to these final tones [pre-conditioning ketamine: $t(10) = -0.856, p = 0.412$; post-conditioning ketamine: $t(11) = -0.690, p = 0.505$; pre-extinction ketamine: $t(12) = -0.417, p = 0.684$] (Fig. 2-2C; Fig. 2-3C; Fig. 2-4C).

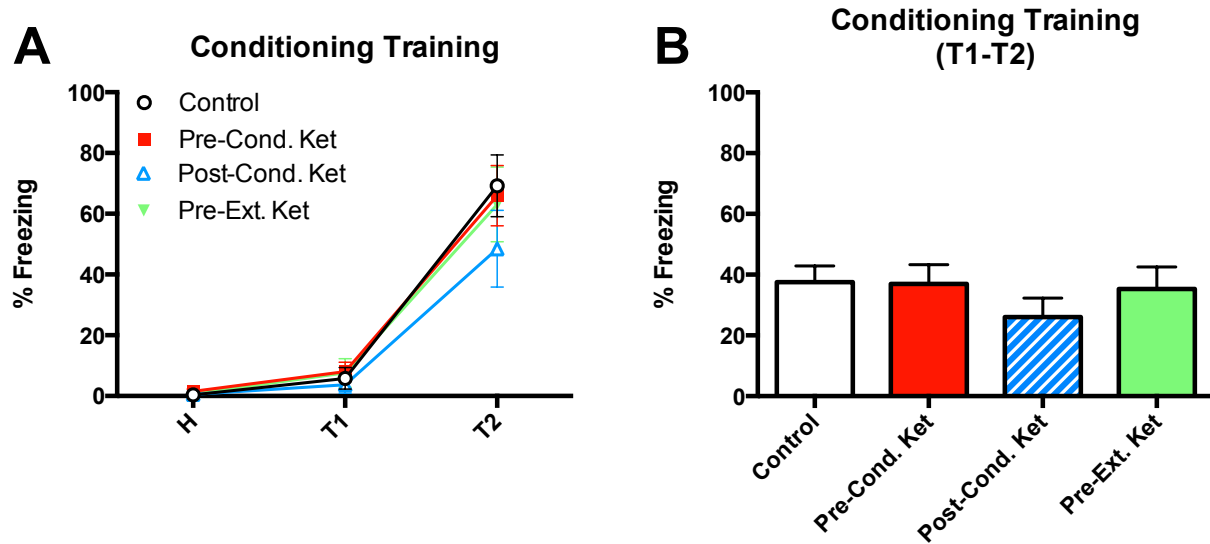


Figure 2-1. (A) Percentage of time spent freezing during fear conditioning training. None of the treatments examined significantly affected freezing during habituation. (B) None of the treatments examined significantly affected the acquisition of fear conditioning.

3.3. Fear Extinction Recall Day

No significant group differences in freezing were found during the 180 s habituation period prior to extinction recall testing [$F(3, 21) = 1.171, p = 0.344$] (Fig. 2-2D; Fig. 2-3D; Fig. 2-4D).

Average freezing across first five tones of extinction recall testing (early phase extinction recall; T1-T5) was analyzed to determine if ketamine treatment affected the long-term expression of extinction behavior. No significant differences in freezing were found between the ketamine treatment groups and the control group [pre-conditioning ketamine: $t(10) = 0.177, p = 0.863$; post-conditioning ketamine: $t(11) = 1.676, p = 0.122$; pre-extinction ketamine: $t(12) = 0.300, p = 0.770$] (Fig. 2-2E; Fig. 2-3E; Fig. 2-4E). Across the last five tones of extinction recall testing (late phase extinction recall; T6-T10), rats in each ketamine treatment group exhibited similar levels of freezing to those of the control group [pre-conditioning ketamine: $t(10) = 0.770, p = 0.459$; post-conditioning ketamine: $t(11) = 0.378, p = 0.712$; pre-extinction ketamine: $t(12) = 0.252, p = 0.805$] (Fig. 2-2F; Fig. 2-3F; Fig. 2-4F).

4. Discussion

The aim of this study was to characterize the effects of subanesthetic ketamine on fear conditioning and extinction when administered during various stages of behavioral testing. We observed that administration of a 10 mg/kg dose of ketamine an hour before fear conditioning training was insufficient to disrupt the expression of conditioned freezing behavior 10 days later. However, when administered either immediately following fear conditioning training or one hour prior to extinction training, ketamine impaired the long-term expression of cue-elicited freezing compared to untreated controls. None of the ketamine treatments examined affected the long-term retention of extinction behavior. Our results suggest that the time point at which ketamine is administered relative to fear conditioning and recall testing has a significant effect on the expression of fear-motivated behavior. These findings are discussed in further detail below.

4.1. The Effect of Pre-Conditioning Ketamine on Auditory Fear Conditioning and Extinction

In the present study, treatment with 10 mg/kg ketamine one hour before fear conditioning training did not impair the acquisition of cue-elicited freezing during associative learning.

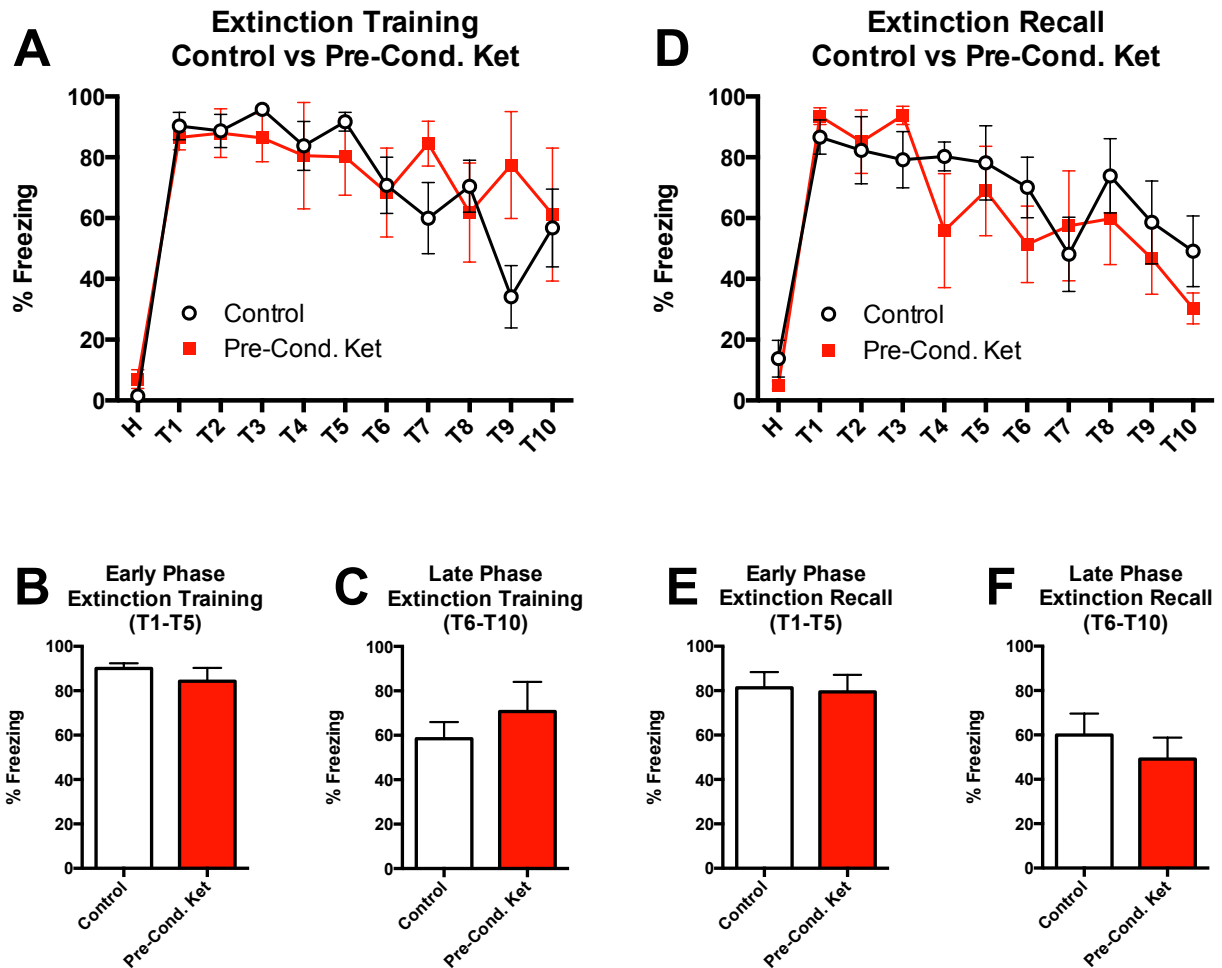


Figure 2-2. (A) Percentage of time spent freezing during extinction training in control vs. pre-conditioning ketamine conditions. Pre-conditioning ketamine did not significantly affect freezing during habituation. (B) Pre-conditioning ketamine did not significantly impact freezing across the first five tones of extinction training. (C) Pre-conditioning ketamine did not significantly impact freezing across the last five tones of extinction training. (D) Percentage of time spent freezing during extinction recall testing. Pre-conditioning ketamine did not significantly affect freezing during habituation. (E) Pre-conditioning ketamine did not significantly impact freezing across the first five tones of extinction recall testing. (F) Pre-conditioning ketamine did not significantly impact freezing across the last five tones of extinction recall testing.

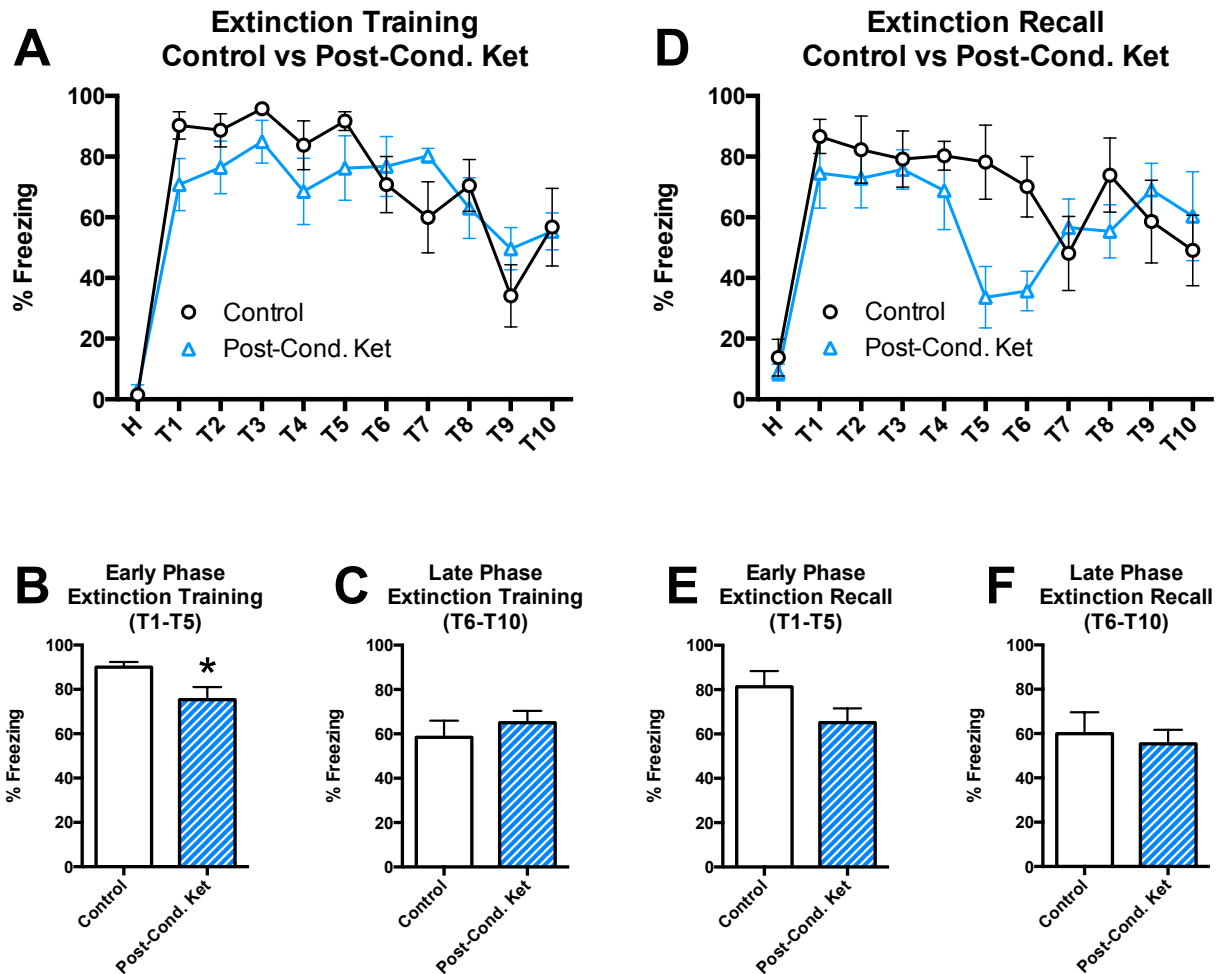


Figure 2-3. (A) Percentage of time spent freezing during extinction training in control vs. post-conditioning ketamine conditions. Post-conditioning ketamine did not significantly affect freezing during habituation. (B) Post-conditioning ketamine significantly reduced freezing across the first five tones of extinction training. (C) Post-conditioning ketamine did not significantly impact freezing across the last five tones of extinction training. (D) Percentage of time spent freezing during extinction recall testing. Post-conditioning ketamine did not significantly affect freezing during habituation. (E) Post-conditioning ketamine did not significantly impact freezing across the first five tones of extinction recall testing. (F) Post-conditioning ketamine did not significantly impact freezing across the last five tones of extinction recall testing.

* ($p < 0.05$)

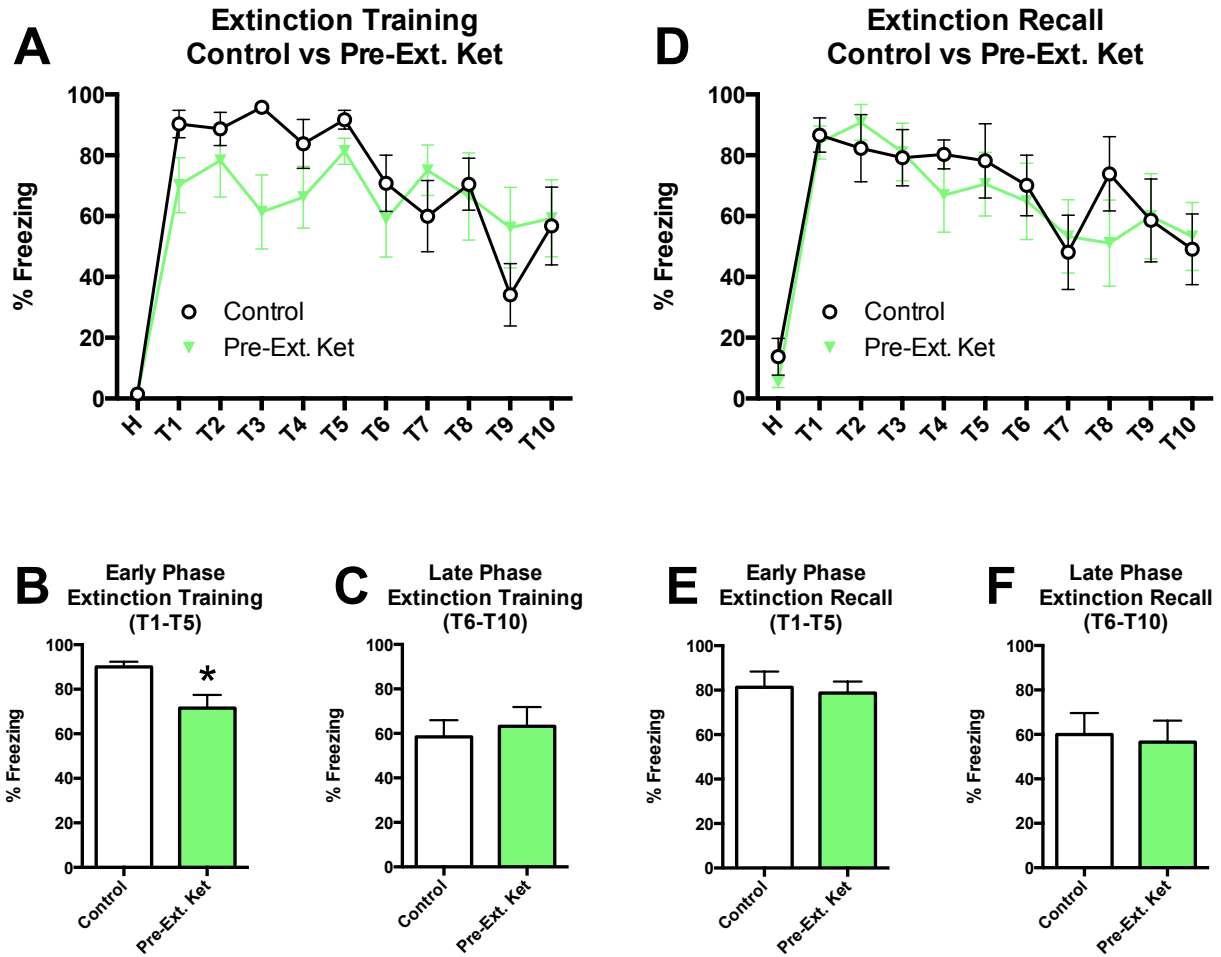


Figure 2-4. (A) Percentage of time spent freezing during extinction training in control vs. pre-extinction conditions. Pre-extinction ketamine did not significantly affect freezing during habituation. (B) Pre-extinction ketamine significantly reduced freezing across the first five tones of extinction training. (C) Pre-extinction ketamine did not significantly impact freezing across the last five tones of extinction training. (D) Percentage of time spent freezing during extinction recall testing. Pre-extinction ketamine did not significantly affect freezing during habituation. (E) Pre-extinction ketamine did not significantly impact freezing across the first five tones of extinction recall testing. (F) Pre-extinction ketamine did not significantly impact freezing across the last five tones of extinction recall testing.
* ($p < 0.05$)

Although NMDA receptor activation plays an essential role in the acquisition of conditioned fear, the significant delay between ketamine administration and behavioral training in our study is likely responsible for the lack of learning impairment that is typically observed when NMDA receptor antagonists are administered only 5-10 minutes before conditioning (Bauer et al., 2002; Campeau et al., 1992; Fanselow & Kim, 1994; Fendt, 2001; Gewirtz & Davis, 1997; Lee et al., 2001; Lee & Kim, 1998; Maren et al., 1996b; Miserendino et al., 1990; Walker & Davis, 2000). Ketamine has a short half-life of approximately 1.3 hours in rats (Veilleux-Lemieux, Castel, Carrier, Beaudry, & Vachon, 2013), so ketamine binding to NMDA receptors would be significantly reduced by the time animals began training in our conditioning paradigm. Furthermore, ketamine likely preferentially inactivates extrasynaptic NMDA receptors, which promote synaptic long-term depression, instead of inactivating synaptic NMDA receptors, which promote the LTP that underlies associative learning (Papouin & Oliet, 2014; Pittenger & Duman, 2008). To our knowledge, no other study has examined the effect of ketamine on auditory fear conditioning acquisition with the same dose and administration schedule used in the present investigation; however, one other lab has reported that chronic daily administration of 2.5 mg/kg ketamine did not affect the acquisition of contextual fear conditioning when administered 48 hours prior to training (Amann et al., 2009).

Although previous research has demonstrated that pre-conditioning subanesthetic ketamine injection suppresses the long-term expression of contextual (Calzavara et al., 2009) and auditory fear conditioning (Pietersen et al., 2006), we found no such treatment effect in the present study. While these findings may at first appear contradictory, previous studies have separated ketamine administration and recall testing by only 24 hours, whereas animals in the current investigation underwent recall assessment 10 days following ketamine treatment. This considerable delay between drug treatment and behavioral assessment opens the possibility for the long-term cognitive and behavioral effects of ketamine to wear off during this time period. Ketamine has been shown to induce antidepressant-like behavior in the forced swim test and sucrose preference test for at least 7 days (Autry et al., 2011; Li et al., 2011), but the ability for ketamine to affect rodent behavior beyond a week in any measure, let alone fear conditioning, has never before been explored. Thus, while subanesthetic ketamine has demonstrated efficacy in impairing the long-term expression of conditioned fear behavior for at least 24 hours (Calzavara et al., 2009; Pietersen et al., 2006), the results of the present study indicate that this effect lasts

fewer than 10 days. Furthermore, our findings suggest that the pre-conditioning ketamine-induced impairment of conditioned freezing that has been observed in past studies is not the result of impaired fear induction, but rather impaired fear recall. If pre-conditioning ketamine were to disrupt either the acquisition or consolidation of conditioned fear, then its resulting reduction of long-term fear expression should be permanent and not dependent upon the amount of time separating training and recall testing.

The present study is the first to our knowledge to investigate the effects of ketamine on the acquisition and retention of auditory fear extinction. We observed that pre-conditioning ketamine treatment did not affect the expression of freezing at the end of extinction training, suggesting that both pre-conditioning ketamine and control animals achieved a similar degree of extinction acquisition. The next-day expression of extinction was also unaffected by ketamine, as all animals displayed high levels of freezing at the beginning of extinction recall testing, regardless of treatment. This lack of treatment effect is unsurprising given the previously discussed absence of ketamine interference with conditioning recall, and is again likely the result of the long delay between ketamine administration and extinction training/testing.

4.2. The Effect of Post-Conditioning Ketamine on Auditory Fear Conditioning and Extinction

As anticipated, animals in the post-conditioning ketamine group exhibited similar levels of freezing acquisition during fear conditioning training to that of untreated controls, since no drug treatment had taken place yet at that time. However, immediate post-conditioning administration of 10 mg/kg ketamine was found to attenuate the long-term expression of auditory fear 10 days later, as evidenced by a 16% reduction in cue-elicited freezing across the first half of extinction training compared to control.

This ketamine-induced reduction in conditioned responding cannot be ascribed to a generalized increase in locomotor activity, as both post-conditioning ketamine and control animals displayed equivalent levels of freezing during the 180 s habituation period prior to extinction training. Furthermore, since pre-conditioning ketamine administration (which took place less than 70 minutes earlier than post-conditioning administration) did not affect conditioned responding after a 10 day delay, it is highly unlikely that reduced freezing behavior observed in the pre-conditioning ketamine group is the result of impaired memory recall. If ketamine were capable of impairing the cognitive process of fear recall after 10 days, it would be

expected that both pre-conditioning and post-conditioning ketamine groups would exhibit reductions in freezing behavior. Rather, the most parsimonious explanation for these findings is that post-conditioning ketamine treatment impaired the consolidation of conditioned fear memory, leading to a weakened expression of cue-elicited freezing during recall assessment. It is plausible that ketamine could impair fear memory consolidation, as previous research has demonstrated that temporary post-conditioning genetic suppression of hippocampal NMDA receptor function causes deficits in subsequent fear retrieval testing (Shimizu et al., 2000). Although post-conditioning administration of the NMDA receptor antagonist APV has failed to interfere with fear memory consolidation in the past, APV must bind competitively with endogenous glutamate to inhibit receptor activity, and thus might induce different behavioral effects than the non-competitive NMDA receptor antagonist ketamine (Kim et al., 1991; Maren et al., 1996b).

Immediate post-conditioning ketamine treatment did not affect the final acquisition of extinction training, and also had no influence on the next-day expression of extinction. Once again, we believe that the long delay between ketamine administration and extinction training/testing likely precluded the ability for drug treatment to influence new learning and memory recall 10 days later.

4.3. The Effect of Pre-Extinction Ketamine on Auditory Fear Extinction

Animals in both the pre-extinction ketamine group and control group exhibited similar levels of freezing acquisition during fear conditioning training, which was expected since no drug treatment had taken place yet at that time. Ten days later, we observed that treatment with 10 mg/kg ketamine one hour before extinction training attenuated the long-term expression of auditory fear, as evidenced by a 21% reduction in cue-elicited freezing across the first half of extinction training compared to control. A similar attenuation of conditioned responding has previously been found when chronic low-dose ketamine treatment precedes the assessment of contextual fear memory recall (Amann et al., 2009; Zhang et al., 2015b).

Once again, our observed ketamine-induced reduction in conditioned responding cannot be ascribed to a generalized increase in locomotor activity, as both pre-extinction ketamine and control animals displayed equivalent levels of freezing during the 180 s habituation period prior to extinction training. Furthermore, previous research has established that spontaneous locomotor

activity in the open field test is normalized within 40 minutes following a 16 mg/kg injection of ketamine in rats (Imre et al., 2006), and as little as 30 minutes following a 10 mg/kg injection (Koike et al., 2011; Koike et al., 2013). Since we maintained an hour delay between drug administration and extinction training, ketamine-treated animals in the present study had ample time for locomotor normalization, and exhibited behavior that was indistinguishable from control animals before extinction training began. It is also unlikely that ketamine induced perceptual distortions that impaired the perception of conditioned stimuli, as previous research has shown that 10 mg/kg ketamine does not affect auditory evoked potentials in rats (de Bruin, Ellenbroek, Cools, Coenen, & van Luijelaar, 1999).

One potential explanation for the attenuation of cue-elicited freezing is that ketamine impaired the cognitive process of memory recall by inactivating NMDA receptors in the amygdala. The lateral amygdala in particular plays a crucial role in integrating fear-relevant stimuli, and mediates the induction and expression of conditioned fear (Pape & Paré, 2010). Infusion of the NMDA receptor antagonist APV into the lateral amygdala prior to fear recall testing has been shown to attenuate conditioned responding, so it is possible that a systemic injection of ketamine in a high enough dose and close enough temporal proximity to behavioral testing could elicit a similar effect. However, we believe that transient NMDA receptor inactivation itself likely plays a minor and non-essential role in the present study's observed effects of pre-extinction ketamine on the expression of conditioned fear. Past studies that impaired conditioned fear recall through NMDA receptor antagonism administered APV only 5 minutes prior to behavioral testing, ensuring that APV binding to NMDA receptors would be high at the time of conditioned stimulus presentation. Conversely, animals in the present study received ketamine at least one hour before extinction training, which considering ketamine's short 1.3 hour half-life (Veilleux-Lemieux et al., 2013), likely reduced binding to NMDA receptors significantly at the time of behavioral assessment. Furthermore, previous research has demonstrated that ketamine can induce a similar attenuation of conditioned fear recall long after NMDA receptor antagonism has ceased. Chronic administration of 5 mg/kg ketamine, terminated 48 hours before recall testing, was shown to significantly reduce freezing in a contextual fear conditioning paradigm (Amann et al., 2009). Thus, while transient NMDA receptor antagonism at the time of fear recall may play a role in ketamine's suppression of conditioned freezing, it is likely an insufficient explanation for our observed results.

Instead, we propose that ketamine likely facilitates the suppression of conditioned fear by stimulating enhancements in neurotransmission and synaptic plasticity that mirror those underlying the natural process of fear extinction. As previously mentioned, the amygdala is responsible for forming a predictive association between conditioned and unconditioned fear stimuli, and acts as a gateway for the expression of conditioned responding (e.g. freezing; Pape & Paré, 2010). However, amygdalar output of conditioned fear can be suppressed through descending projections from the mPFC, which stimulate inhibitory GABAergic ITC neurons in the amygdala (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). The hippocampus also mediates activity within fear memory circuitry (Maren & Hobin, 2009; Sotres-Bayon et al., 2012), and communicates with the mPFC to facilitate extinction memory recall and suppress conditioned fear in a contextually appropriate manner (Farinelli et al., 2006; Garcia et al., 2008b; Hugues et al., 2006; Hugues & Garcia, 2007; Peters et al., 2010; Quirk & Mueller, 2008). Together, the mPFC and hippocampus can be viewed as the fundamental structures underlying the induction and expression of fear extinction, so pharmacological manipulation of activity within these structures has the potential to induce significant alterations in cognition and behavior (Burgos-Robles et al., 2007; Corcoran et al., 2005; Fernandez, 2003; Lebrón et al., 2004; Morgan et al., 1993; Morgan et al., 2003; Quirk et al., 2000; Sotres-Bayon et al., 2009).

Past research has illustrated a clear relationship between prefrontal and hippocampal neuronal activity and the successful suppression of conditioned fear. During the consolidation and recall of extinction memory, activity within the IL mPFC is positively correlated with the degree to which extinction behavior is successfully expressed (Burgos-Robles et al., 2007; Milad & Quirk, 2002; Wilber et al., 20011). Extinction recall is also associated with increased expression of c-Fos, a protein biomarker of recent neuronal activity, within the IL, hippocampus (CA1, CA3, dentate gyrus), and ITC neurons of the amygdala (Knapska & Maren, 2009). Interestingly, experimentally applied electrical stimulation of IL neurons can successfully inhibit fear expression and induce extinction-like behavior in the absence of extinction training (Milad et al., 2004; Milad & Quirk, 2002; Vidal-Gonzalez et al., 2006). Ketamine possesses the potential to similarly modulate conditioned fear suppression because it stimulates presynaptic excitatory neurotransmitter release (i.e. glutamate) in the PFC and hippocampus, namely by reducing the activity of inhibitory GABAergic interneurons in these regions (Chowdhury et al., 2012; Homayoun & Moghaddam, 2007; Moghaddam et al., 1997; Stone et al., 2012).

Accordingly, subanesthetic ketamine has been found to increase c-Fos expression in the mPFC (including the IL) and hippocampus (CA1, CA3, dentate gyrus) at rest (Imre, Fokkema, Boer, & Ter Horst, 2006). It is possible that an enhancement of activity in these regions facilitates the downstream inhibition of amygdalar fear output, as ketamine has also demonstrated efficacy in preventing conditioning-induced enhancement of c-Fos expression in the amygdala, while simultaneously inhibiting the expression of conditioned fear (Pietersen et al., 2006). Based on these findings, an immediate enhancement of presynaptic glutamate release in the mPFC and hippocampus might be one of the mechanisms underlying ketamine's immediate suppression of conditioned responding.

A transient ketamine-induced burst of glutamatergic neurotransmission could also promote long-term changes in synaptic plasticity, since activation of the AMPA glutamate receptor stimulates BDNF release in the brain (Jourdi et al., 2009). BDNF is a growth factor that mediates synaptic plasticity by promoting dendritic protein synthesis, dendritogenesis, synaptogenesis, and the induction of LTP (Horch et al., 1999; Jourdi et al., 2009; Kang et al., 1997; Korte et al., 1996; Patterson et al., 1996; Takei et al., 2004; Yoshii & Constantine-Paton, 2007; Yoshii & Constantine-Paton, 2010). The BDNF protein's ability to strengthen synapses, even in the absence of electrical stimulation, makes it a powerful modulator of memory and behavior (Autry et al., 2011; Liu et al., 2012; Messaoudi et al., 2002; Peters et al., 2010). While fear conditioning impairs hippocampal BDNF transcription (Rasmusson et al., 2002), fear extinction is associated with an upregulation of BDNF in fear suppression pathways (Bredy et al., 2007; Chhatwal et al., 2006; Rosas-Vidal et al., 2014). Following fear extinction, the presence of BDNF protein is increased in the ventral hippocampus (Rosas-Vidal et al., 2014), and hippocampal BDNF levels are correlated with the degree of successful fear extinction recall (Peters et al., 2010). Hippocampal BDNF is projected to the IL to suppress conditioned fear expression (Peters et al., 2010), and the presence of BDNF within the hippocampus even enhances the excitability of extinction-critical IL neurons (Rosas-Vidal et al., 2014). Furthermore, extinction training initiates epigenetic regulation of BDNF gene expression in the IL, increasing histone H4 acetylation of BDNF promoter regions to result in an upregulation of prefrontal BDNF mRNA (Bredy et al., 2007). Interference with BDNF activity in these regions is associated with extinction failure and the return of conditioned fear (Heldt et al., 2007; Peters et al., 2010; Rosas-Vidal et al., 2014). Interestingly, ketamine induces a rapid upregulation of

neuronal BDNF that is reminiscent of the natural changes in neurotrophin expression that are induced by fear extinction learning. Subanesthetic ketamine stimulates a significant enhancement of BDNF protein expression in the prefrontal cortex and hippocampus that occurs within only 30 minutes of administration (Autry et al., 2011; Garcia et al., 2008a; Zhang et al., 2015a; Zhang et al., 2015b). Consistent with increased BDNF activity, ketamine also upregulates several synaptic proteins (including synapsin I, PSD-95, and the AMPA subunit GluR1), increases dendritic spine density, and enhances dendritic spine maturity in the mPFC within one hour of treatment (Li et al., 2010; Li et al., 2011; Zhang et al., 2015a). Since the present study introduced an hour delay between ketamine administration and behavioral testing, it is possible that these changes in neurotrophin expression and synaptic plasticity could contribute to the enhanced suppression of conditioned responding we observed during extinction training. It is noteworthy that a similar facilitation of conditioned fear suppression can be produced by directly infusing BDNF into either the IL or hippocampus of rats (Peters et al., 2010). Within an hour of BDNF infusion, these treatments produce a significant reduction in cue-elicited freezing that begins with the first unreinforced stimulus, even in the absence of extinction training (Peters et al., 2010). Peters and colleagues (2010) refer to this phenomenon as “BDNF-extinction,” because the successful and immediate suppression of conditioned responding induced by BDNF infusion was not a facilitation of extinction training (since no extinction training took place), but rather an effective substitution for it. We believe that a similar phenomenon, resulting from a ketamine-induced upregulation of prefrontal and hippocampal BDNF, could underlie the impaired conditioned fear expression observed in the present study.

Since BDNF expression promotes the strength and maturity of synapses in the brain, and increases postsynaptic membrane insertion of AMPA receptor subunits, it is unsurprising that ketamine also induces a long-lasting facilitation of neurotransmission (Autry et al., 2011; Li et al., 2010; Li et al., 2011). This has potentially significant consequences for extinction, since enhancements in prefrontal and hippocampal synaptic efficacy play an important role in the suppression of conditioned fear. Extinction learning is associated with the induction of LTP in the IL, which reverses a conditioning-induced depression of neuron excitability in this region (Cruz et al., 2014; Farinelli et al., 2006; Herry & Garcia, 2002; Hugues et al., 2006). Conversely, depressed IL neuron excitability accompanies the spontaneous weakening of extinction expression over time, illustrating the important role that LTP plays in the maintenance of

extinction memory (Cruz et al., 2014; Farinelli et al., 2006; Herry & Garcia, 2002; Herry & Mons, 2004; Hugues et al., 2006). Furthermore, extinction enhances synaptic efficacy between the hippocampus and mPFC, a process that is critical in the long-term maintenance of fear suppression (Farinelli et al., 2006; Garcia et al., 2008b; Hugues et al., 2006; Hugues & Garcia, 2007). Once again, ketamine appears to induce changes in neurotransmission that parallel those seen in the natural process of extinction. Past research has demonstrated that subanesthetic ketamine potentiates synaptic efficacy in both the hippocampus (Autry et al., 2011; Nosyreva et al., 2013) and PFC (Li et al., 2011), elaborating upon the means by which ketamine could facilitate the suppression of conditioned fear.

By the end of extinction training, animals in both the pre-conditioning ketamine group and control group expressed similar levels of conditioned freezing. Interestingly, despite having significantly reduced freezing at the beginning of extinction training, pre-extinction ketamine treatment had no effect on behavior during extinction recall testing on the following day. While it may appear contradictory that ketamine can suppress conditioned responding during fear recall but not during extinction recall, it is possible that the induction of extinction learning involves processes that retroactively interfere with ketamine's ability to further influence behavior. Since previous research has shown that ketamine retains the ability to reduce conditioned responding for at least 48 hours after administration (when administration and testing are separated by a rest period), it is doubtful that the behavioral effects of ketamine in the present study expired due to the 24 hour passage of time between drug administration and extinction recall testing (Amann et al., 2009; Zhang et al., 2015b). It is also unlikely that the lack of treatment effect during extinction recall is a result of state-dependent memory recall, since animals in the pre-conditioning ketamine group exhibited no more cue-elicited freezing than animals in the control group did. Instead, the process of extinction learning appears to override the behavioral effects of ketamine, leading to a weakened suppression of conditioned fear in subsequent recall tests. Further research is needed in order to evaluate this possibility.

4.4. Clinical Implications

Subanesthetic doses of ketamine have demonstrated impressive efficacy in the treatment of human depression (Aan Het Rot et al., 2012; Berman et al., 2000; Cusin et al., 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price

et al., 2009; Rasmussen et al., 2013; Zarate et al., 2006), but it has been uncertain if ketamine also helps challenge negative cognitive biases in memory that are associated with this disorder. The findings from the present study suggest that ketamine rapidly suppresses the expression of negative emotional memory in rats, even when the initial acquisition of aversive learning is undisturbed. If a similar attenuation of negatively biased memories is induced in humans, this could contribute to the antidepressant qualities of ketamine. Additional research is needed to determine if ketamine produces a similar effect on memory in animal models of depression and in human patients with depression.

The ability for a pharmacological intervention to inhibit fear associations after they have been acquired is also of potential value to the treatment of PTSD. Individuals with PTSD express poor extinction memory, which is accompanied by decreased activation in the hippocampus and mPFC, and enhanced activity in the amygdala (Milad et al., 2009b). The present study found that a single subanesthetic dose of ketamine was capable of attenuating the long-term expression of conditioned fear in rats when administered immediately following an aversive learning task. This observation appears consistent with a study by McGhee and colleagues, which found that military servicemen who received ketamine anesthesia during post-trauma surgery had a lower future prevalence of PTSD than those who were administered other anesthetic compounds (McGhee, Maani, Garza, Gaylord, & Black, 2008). However, attempts to replicate this finding have been mixed. A larger study by the same group found no effect of ketamine on reducing future PTSD prevalence, although these results may have been confounded by the fact that ketamine-treated individuals experienced injuries of greater severity than those who did not receive ketamine (McGhee et al., 2014). Furthermore, a separate investigation found that peritraumatic administration of (s)-ketamine (esketamine) in moderately injured accident victims elevated the future expression of PTSD symptoms, while racemic ketamine (as used in the present study) had no effect (Schönenberg, Reichwald, Domes, Badke, & Hautzinger, 2005). Further research is needed in order to better characterize the ways that the dosage, stereoisomer mixture, and time point of ketamine administration can influence the consolidation of negative emotional memory.

5. Conclusions

The results of this study demonstrate that the effect of a single subanesthetic dose of ketamine on the expression of auditory fear conditioning is dependent upon the time at which ketamine is administered relative to fear learning and recall. When administered during the consolidation phase of fear learning, ketamine impaired the expression of cue-elicited freezing in a recall test 10 days later. However, the same dose of ketamine was ineffective at inducing a long-lasting changes in behavior when injected prior to fear conditioning training. Ketamine treatment an hour before recall assessment attenuated conditioned responding immediately at the start of the trial, promoting an extinction-like suppression of conditioned fear. We believe that the differential effects of pre-conditioning, post-conditioning, and pre-extinction ketamine arise from interference with different cognitive processes of memory. None of the ketamine treatments examined in this study had a significant effect on the final degree of extinction acquisition, nor the recall of extinction behavior the next day. The findings of the present study could have implications for the treatment of disorders that feature a pathological bias towards negative memory, such as PTSD and depression.

CHAPTER 3

Ketamine Prevents Repeated Corticosterone-Induced Reemergence of Extinguished Auditory Fear

Brian D. Kulyk¹ and Lisa E. Kalynchuk²

¹ Department of Psychology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

² Department of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Abstract

The experience of chronic stress plays an important role in the pathogenesis of major depressive disorder. Prolonged stress induces a state of chronically elevated glucocorticoid exposure in the brain, which is neurotoxic and can lead to the dysfunction of glutamatergic signaling. Since memory is highly dependent upon glutamatergic neurotransmission, patients with depression commonly display alterations memory processing that bias the recollection of past events towards negative emotional information. Past research has revealed that prolonged exposure to the glucocorticoid CORT produces a number of depression-like behaviors in rats, and makes extinguished negatively-valenced associations more prone to reinstatement in a contextual fear conditioning and extinction paradigm. The aim of the present study was to investigate the effects of chronic exogenous CORT exposure on auditory fear conditioning and extinction, and to evaluate the efficacy of the NMDA glutamate receptor antagonist ketamine in modulating long-term fear and extinction recall in repeated CORT- and vehicle-treated rats. Rats received 40 mg/kg injections of either CORT or vehicle for 21 consecutive days. Following repeated CORT/vehicle treatment, all animals were fear conditioned, extinction trained (two sessions), and then submitted to a sub-conditioning retraining procedure that is designed to reinstate conditioned fear in previously trained, but not conditioning-naive rats. Regardless of prior CORT exposure, pre-extinction administration of a subanesthetic 15 mg/kg dose of ketamine induced an immediate and substantial attenuation of cue-elicited freezing during conditioned fear recall assessment. Repeated CORT treatment provoked a spontaneous recovery of conditioned freezing between extinction sessions and induced a reinstatement of freezing following sub-conditioning retraining, an effect which was entirely prevented by pre-extinction ketamine administration. The present study therefore demonstrates that chronic exposure to the glucocorticoid CORT induces a failure of long-term extinction retrieval, and establishes ketamine as a powerful modulator of fear memory and emotionally-driven behavior.

1. Introduction

The recent experience of an adverse life event frequently precedes the onset of a major depressive episode (Farmer & McGuffin, 2003; Kessing et al., 2003; Slavich et al., 2010), which has lead researchers to identify chronic stress as an important factor in the pathogenesis of depression (Deutschenbaur et al., 2015; Pittenger & Duman, 2008; Willner et al., 2013). Stress initiates a cascade of endocrine responses within the HPA axis, which culminate in the secretion of glucocorticoids (cortisol in humans; CORT in rodents) into the bloodstream (Herman & Cullinan, 1997). Although acute glucocorticoid release is an adaptive response that serves to promote an organism's survival when faced with adversity, prolonged glucocorticoid exposure is neurotoxic and produces a number of serious maladaptive alterations in morphological neuroplasticity and behavior (de Kloet et al., 1999). In animal studies, chronic exogenous CORT administration has been shown to produce significant dendritic atrophy in the hippocampus (Magariños et al., 1998; Magariños et al., 1999; Morales-Medina et al., 2009; Sousa et al., 2000; Watanabe et al., 1992a; Woolley et al., 1990) and PFC (Liu & Aghajanian, 2008; Wellman, 2001), while stimulating dendritic hypertrophy in the amygdala (Mitra & Sapolsky, 2008). A similar pattern of hippocampal/prefrontal atrophy and amygdalar hypertrophy has been observed in the brains of human patients with depression (Campbell et al., 2004; Cole et al., 2010; Colla et al., 2007; Lange & Irle, 2004; Phillips et al., 2003; Savitz & Drevets, 2009; Sheline et al., 1999; Sheline et al., 2003; van Tol et al., 2010; Videbeck & Ravnkilde, 2004). In addition to morphological deficits, chronic CORT exposure also produces depression-like behaviors in rodents, such as anhedonia (David et al., 2009; Gorzalka et al., 1999; Gorzalka et al., 2001; Gorzalka et al., 2003; Gorzalka & Hanson, 1998; Gourley et al., 2008a; Gourley et al., 2008b; Gupta et al., 2015; Wu et al., 2013; Yau et al., 2014) and learned helplessness (Brummelte et al., 2006; David et al., 2009; Fenton et al., 2015; Gourley et al., 2008a; Gregus et al., 2005; Hill et al., 2003; Johnson et al., 2006; Kalynchuk et al., 2004; Koike et al., 2013; Marks et al., 2009; Murray et al., 2008; Wu et al., 2013; Zhao et al., 2008a; Zhao et al., 2008b).

Given the fundamental role that these limbic structures play in learning and emotional regulation, it is unsurprising that depression-associated changes in neuronal morphology are accompanied by pathological modifications in cognition and behavior. For example, individuals with depression experience maladaptive alterations in memory that skew the recollection of past events towards negative emotional information (Balut et al., 2013; Gerritsen et al., 2012; Gupta

& Kar, 2012; Harmer et al., 2009a; Harmer et al., 2009b; Hsu et al., 2010; Liu et al., 2012; Mogg et al., 2014; Taylor & John, 2004). Negative cognitive biases in attention, processing, interpretation, and memory are believed to support the development and maintenance of depression (Elliott et al., 2011; Harmer et al., 2009a; Nolen-Hoeksema, 2000; Robinson & Sahakian, 2008), emphasizing the need for antidepressant treatments to effectively combat these insidious symptoms (Clark et al., 2009; Elliott et al., 2011; Roiser et al., 2012).

To experimentally investigate stress-induced memory biases in animals, researchers often use classical fear conditioning, a behavioral paradigm that promotes a learned association between an emotionally neutral conditioned stimulus (e.g., tone cue) and an innately aversive and negatively-valenced unconditioned stimulus (e.g., footshock). Following successful fear conditioning, presentation of the conditioned stimulus alone will elicit fear-motivated behavioral responses such as defensive freezing. Fear learning is rapidly acquired and generally very robust, but under proper conditions can later be challenged through a form of novel learning known as extinction. Extinction learning occurs when a conditioned stimulus is repeatedly presented in the absence of unconditioned stimulus reinforcement, and consists of the formation of a new memory that competes with the original fear memory to suppress fear-motivated behavior (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). However, extinction memory is far less robust than conditioned fear memory, and conditioned responding can easily return under a number of circumstances. Previously extinction-trained animals can experience a recovery of conditioned fear spontaneously with the passage of time (Cruz et al., 2014; Pape & Paré, 2010), and are particularly susceptible to trained fear reinstatement with weak aversive stimuli that are incapable of inducing fear conditioning in naive animals (Deschaux et al., 2011a; Deschaux et al., 2011b; Deschaux et al., 2013; Laurent & Westbrook, 2010; Zheng et al., 2013).

Successful retention of fear extinction relies upon the integrity of prefrontal and hippocampal circuits that serve to inhibit activity of the amygdala, which is the primary locus of conditioned stimuli convergence and conditioned response output. Accordingly, high levels of circulating glucocorticoids and stress-induced amygdalar hypertrophy strengthen the ability to form fearful memories, while prefrontal and hippocampal atrophy simultaneously compromise the means by which the brain regulates fear-motivated behavior (Pape & Paré, 2010; Rodrigues et al., 2009). Previous research has shown that chronic stress exposure impairs extinction memory recall in rodents (despite successful short-term extinction acquisition), leading to the

spontaneous recovery of fear-motivated behaviors such as defensive freezing (Baran et al., 2009; Farrell et al., 2010; Gourley et al., 2009; Hoffman et al., 2014; Miracle et al., 2006; Wilber et al., 2011). This apparent stress-induced bias towards negative emotional memory makes fear conditioning and extinction paradigms particularly valuable in the study of negative cognitive biases and the pursuit of pharmacotherapeutic agents capable of correcting them.

SSRIs are often prescribed to treat depression, but several common SSRIs have surprisingly been shown to enhance fear memory (Burghardt et al., 2004; Burhans et al., 2013; Cassaday & Thur, 2015; Montezinho et al., 2010; Ravinder et al., 2013) and impair extinction (Burghardt et al., 2013) in animal models of conditioned fear. This apparent facilitation of negatively-biased memory might be counter-productive in the treatment of depression, so the identification of drugs that instead inhibit fear memory could prove to be of great clinical value. One promising drug is ketamine, a noncompetitive antagonist of the NMDA receptor that blocks receptor activation by binding to the phencyclidine site of the complex's cation channel (Sanacora et al., 2008). Clinical trials have demonstrated that subanesthetic infusions of ketamine produce rapid and long-lasting antidepressant effects in patients with treatment-resistant depression (Aan Het Rot et al., 2012; Berman et al., 2000; Cusin et al., 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price et al., 2009; Rasmussen et al., 2013; Zarate et al., 2006). Ketamine is well-equipped to mediate memory for negatively valenced stimuli, since it upregulates synthesis of the plasticity-enhancing BDNF protein in the hippocampus and frontal cortex (Autry et al., 2011; Zhang et al., 2015a; Zhang et al., 2015b), and reverses stress-induced dendritic atrophy in the PFC (Li et al., 2011). In nonstressed animals, infusion of a subanesthetic dose of ketamine prior to fear training reduces fear-motivated behavior in both auditory (Pietersen et al., 2006) and contextual (Calzavara et al., 2009) fear conditioning, while simultaneously inhibiting neuronal activity in the amygdala (Pietersen et al., 2006). However, the ability for ketamine to prevent stress-induced impairments in long-term extinction memory retention has not yet been examined. Furthermore, past investigations into the effects of ketamine on auditory fear conditioning have only administered ketamine prior to fear conditioning training, which could potentially interfere with the initial consolidation of fear memory. Since patients with depression typically only seek medical treatment following the development of depressive symptoms, administering ketamine

at a time point subsequent to successful and undisturbed fear conditioning training could offer a more valid animal model of treating negative cognitive biases with antidepressants.

The aim of the present study was twofold: first, to characterize the effects of chronic glucocorticoid exposure on auditory fear conditioning, extinction, and reinstatement using a repeated exogenous CORT animal model of depression; and second, to evaluate the efficacy of ketamine in facilitating the expression and retention of extinction behavior in both CORT-exposed and nonstressed animals. To induce a stress-like state, rats were injected with 40 mg/kg CORT for 21 consecutive days prior to fear conditioning. All animals were fear conditioned, extinction trained, and then submitted to a sub-conditioning retraining procedure that is designed to reinstate conditioned fear in previously trained, but not conditioning-naïve rats. Defensive freezing was analyzed throughout the conditioning, extinction, and reinstatement trials to evaluate the effects of each treatment condition on the expression of fear-motivated behavior and the retention of extinction training. We hypothesized that repeated CORT treatment would impair the long-term retention of fear extinction between extinction training sessions and following sub-conditioning retraining. In addition, we hypothesized that a pre-extinction subanesthetic dose of ketamine would reduce freezing during extinction training in both CORT-treated and healthy rats, and prevent any potential CORT-induced deficits in extinction retention.

2. Materials and Methods

2.1. Animals

Thirty-three adult male Long-Evans rats (Charles River Laboratories; Montreal, Quebec, Canada), weighing 200-250 g upon arrival, were used in the study. Rats were housed individually in clear plastic cages with free access to food and water. The thermally-controlled colony room maintained a 12 hr/12 hr light/dark cycle. All procedures were approved by the University of Saskatchewan Animal Research Ethics Board, and conducted in accordance with the Canadian Council on Animal Care.

2.2. Experimental Design and Drug Injection Paradigm

Handling of all rats took place for 7 consecutive days to habituate the animals to the procedure room and researcher before the first injection day. On the final day of handling, rats were assigned to one of four weight-matched experimental conditions: repeated vehicle injection

+ pre-extinction vehicle injection (Veh-21/Veh; n=9; i.e., control), repeated vehicle injection + pre-extinction 15 mg/kg ketamine injection (Veh-21/Ket; n=8), repeated 40 mg/kg CORT injection + pre-extinction vehicle injection (CORT-21/Veh; n=8), and repeated 40 mg/kg CORT injection + pre-extinction 15 mg/kg ketamine injection (CORT-21/Ket; n=8).

2.2.1. CORT

Animals in the CORT-21/Veh and CORT-21/Ket group received subcutaneous injections of CORT for 21 consecutive days at a dose of 40 mg/kg and a volume of 1 ml/kg. CORT (Steraloids Inc.; Newport, Rhode Island, United States) was suspended in saline with 2% Tween-80 (VWR International; West Chester, Pennsylvania, United States). This dose and injection paradigm was selected based on previous findings that 21 days of 40 mg/kg CORT produces reliable increases in depression-like behavior in rodents (Fenton et al., 2015; Gregus et al., 2005; Johnson et al., 2006; Kalynchuk et al., 2004; Lussier et al., 2013; Mao et al., 2012; Marks et al., 2009; O'Donovan et al., 2014; Wu et al., 2013; Yau et al., 2011). Animals in the Veh-21/Veh and Veh-21/Ket group received subcutaneous injections of saline with 2% Tween-80 for 21 days instead.

2.2.2. Ketamine

For animals in the Veh-21/Ket and CORT-21/Ket group, ketamine hydrochloride (Vetalar; Bioniche Animal Health Canada Inc.; Belleville, Ontario, Canada) was suspended in saline and injected intraperitoneally at a dose of 15 mg/kg, and a volume of 1 ml/kg. This dose was selected as it has been shown to be efficacious in disturbing behavioral and neural correlates of fear conditioning when administered before training (Amann et al., 2009; Li et al., 2011; Pietersen et al., 2006), as well as reducing depression-like behaviors in chronically stressed rats (Garcia et al., 2009; Koike et al., 2013; Li et al., 2011). Injections took place 60 minutes prior to extinction training to avoid the potentially confounding transient effects of ketamine on spontaneous locomotor activity. Since general locomotor activity in rats is normalized within 40 minutes of a 16 mg/kg dose of ketamine (Imre et al., 2006), any ketamine-associated changes in freezing beyond this time point testing cannot be attributed to alterations in baseline movement. Administration of all injections took place in a procedure room separate from the animal colony

and fear conditioning apparatus. Animals in the Veh-21/Veh and CORT-21/Veh group received a subcutaneous injection of saline with 2% Tween-80 at this time instead.

2.3. Fear Conditioning and Extinction

Fear conditioning and extinction followed a modified version of a protocol previously used by Deschaux et al. (2013).

2.3.1. Apparatus

All behavioral testing procedures took place in standard operant chambers (VFC-008; Med Associates Inc., St. Albans, Vermont, United States) contained within sound-attenuating cubicles. Each operant chamber was constructed of aluminum walls and a clear plastic door through which a video camera could record the rat's behavior. An overhead fluorescent lamp illuminated the chamber through a clear plastic roof. A high-frequency speaker (ANL-926) mounted high within the chamber wall delivered auditory cues. The floor of the chamber consisted of a grid of stainless steel rods that delivered aversive footshocks through a shock generator (ENV-414S). Video Freeze Software (Med Associates Inc.) controlled the delivery of tone and shock stimuli, and recorded video from the camera at 30 frames per second.

2.3.2. Conditioning

One day after the final CORT or vehicle injection, rats were habituated to the conditioning context of the operant chamber for 10 minutes (day 22). On day 23, each rat was placed inside the operant chamber again and given 60 s to habituate before fear conditioning began. Conditioning consisted of 5 tone cues (2 kHz, 32 s, 90 dB) that co-terminated with an aversive footshock (0.7 mA, 250 ms). Each tone-shock pairing was followed by a 60 s inter-trial interval. Upon completion of fear conditioning, rats were transported back to the colony room, and the operant chamber was cleaned with 0.4% glacial acetic acid before training for the next subject began.

2.3.3. Novel context extinction

Since the rats would form fearful associations with the contextual cues of the training environment in addition to the discrete tone cues, the context during extinction was altered in a

number of ways so that only auditory-cued conditioned fear would be measured and extinguished during this phase. During transit to and from the conditioning room, the rats' cages were covered with dark curtain, and an alternative route was followed. The general environment of the conditioning room was changed with colored curtains draped over the walls, and overhead fluorescent lighting was replaced with a red lamp. Vanilla-scented deodorizer provided novel olfactory cues in the extinction context. Most importantly, the operant chambers were modified in shape and appearance with a colorful curved wall insert, and the metal grid floor was replaced with a smooth white plastic cover. A striped ceiling cover panel altered the lighting pattern within the chamber. Contextual auditory cues were obscured with white noise played through the chamber's speaker (65 dB). After each extinction session, the chamber was cleaned with 75% ethanol so that any residual scent of the cleaner would be unfamiliar.

Rats rested for 2 consecutive days before extinction training began (day 26). Rats were placed into the novel extinction context and given 60 s to habituate. Extinction consisted of 20 tone cues (2 kHz, 32 s, 90 dB) presented in the absence of footshock. Each tone was followed by a 60 s inter-trial interval. On day 27, long-term retention of extinction memory was evaluated by presenting an additional 10 tone cues, using the same extinction protocol as described above. Upon completion of each extinction trial, rats were transported back to the colony room.

2.3.4. Sub-conditioning retraining

After a day of rest, rats underwent a sub-conditioning retraining procedure on day 29. All contextual cues were reverted back to those present during fear conditioning training. Each rat was placed inside the operant chamber and given 60 s to habituate before retraining began. Retraining consisted of 5 tone cues (2 kHz, 32 s, 90 dB) that co-terminated with a low-amperage footshock (0.3 mA, 250 ms), followed by a 60 s inter-trial interval. These mild footshocks are not strong enough to induce significant fear conditioning in naive animals, but can successfully reinstate fear responses in previously trained rats that have undergone extinction (Deschaux et al., 2013). Rats were returned to the colony room upon completion of the retraining trial.

2.3.5. Extinction retention test

Following a day of rest, the rats were tested for retention of extinction behavior (day 31). Contextual cues present during this test were consistent with those used during the extinction

phase. Rats were placed inside the operant chambers, and after 60 s of acclimation received 5 tone cues (2 kHz, 32 s, 90 dB) in the absence of footshock. Once extinction retention testing concluded, animals were transported back to the colony room and sacrificed the following day.

2.3.6. Behavioral measurement

Fear conditioning was measured by freezing, defined as the absence of any movement not required for respiration. Using Video Freeze Software (Med Associates Inc.; St. Albans, Vermont, United States), movement was calculated as a change in pixel composition between adjacent video frames. To account for individual variation in baseline breathing movement, a motion threshold was set for each rat that defined the absolute limit beyond which movement would be registered as non-respiratory in nature. Immobility was required to last for at least 1 s before a freezing episode would be recorded in the data output. The percentage of time spent freezing during habituation and tone cue presentation was then calculated by Video Freeze Software and used as the dependent variable in analysis.

2.3.7. Body weight

Rats were weighed daily during the 21 days of CORT or vehicle injections, and the data was recorded for later analysis of change in body weight.

2.4. Statistical Analysis

Mean freezing data was analyzed using a 2x2 factorial ANOVA, with ketamine treatment (vehicle or 15 mg/kg ketamine) and CORT treatment (vehicle or 40 mg/kg CORT) as between subjects factors. Post-hoc analyses were performed using independent samples *t*-tests, which examined group differences in cue-elicited freezing behavior in order to test our hypotheses. First, mean freezing was compared between the Veh-21/Veh and Veh-21/Ket group to investigate the effect of ketamine on the behavior of nonstressed rats. Second, mean freezing was compared between the Veh-21/Veh and CORT-21/Veh group to assess the effect of CORT on behavior. Third, mean freezing was compared between the CORT-21/Veh and CORT-21/Ket group to evaluate the influence of ketamine on the behavior of CORT-exposed animals. Finally, mean freezing was compared between Veh-21/Veh and CORT-21/Ket groups was compared to determine if ketamine treatment in CORT-exposed animals modified behavior compared to

nonstressed and untreated animals. Within subjects *t*-tests were used to investigate changes in average tone-elicited freezing across fear extinction for each group.

Body weight data was analyzed using a 2x4 mixed design ANOVA, with CORT treatment (vehicle or 40 mg/kg CORT) as a between subjects factor and injection day (1, 7, 14, or 21) as a within subjects factor.

Animals that exhibited levels of freezing exceeding 1.5 standard deviations of the mean during extinction training or recall were considered outliers and excluded from statistical analysis.

3. Results

3.1. Fear Conditioning Training Day

Freezing during the 60 s habituation period prior to fear conditioning training was analyzed to determine if CORT treatment affected baseline behavior in the training context. All treatment groups exhibited near-zero levels of freezing during habituation, and no significant main effects [CORT Treatment: $F(1, 26) = 1.156, p = 0.292$; Ket Treatment: $F(1, 26) = 1.156, p = 0.292$] or interactions [$F(1, 26) = 1.156, p = 0.292$] were found (Fig. 3-1A).

Average freezing during tone cue presentation was also analyzed to assess potential treatment effects on behavior during fear conditioning acquisition. No significant main effects [Ket Treatment: $F(1, 26) = 0.008, p = 0.931$; CORT Treatment: $F(1, 26) = 0.149, p = 0.702$] or interaction effects [$F(1, 26) = 1.661, p = 0.209$] were found (Fig. 3-1B).

3.2. Fear Extinction Training Day

Freezing during the 60 s habituation period prior to fear extinction training was analyzed to determine if CORT or Ket treatment affected baseline behavior in the novel extinction context. All treatment groups exhibited near-zero levels of freezing during novel context habituation, and no significant main effects [CORT Treatment: $F(1, 26) = 0.149, p = 0.702$; Ket Treatment: $F(1, 26) = 0.008, p = 0.931$] or interaction effects [$F(1, 26) = 1.661, p = 0.209$] were found (Fig. 3-2A; Fig. 3-3A; Fig. 3-3C; Fig 3-3E).

The long-term expression of auditory fear conditioning was investigated by analyzing the average level of freezing across the first five tone cues of extinction training (early phase extinction training; T1-T5). No significant main effect of CORT [$F(1, 26) = 0.227, p = 0.638$] or

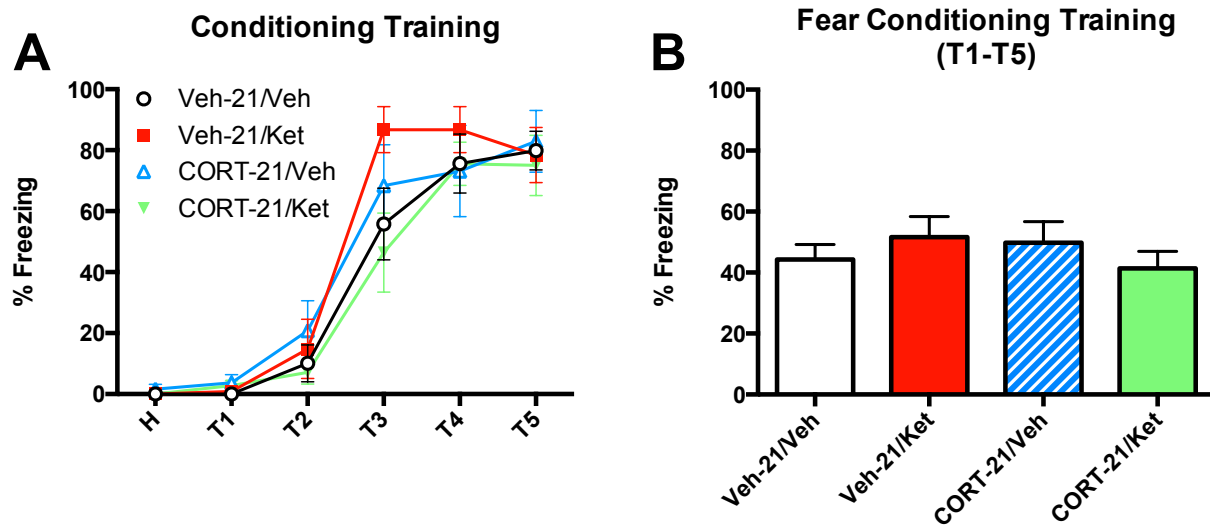


Figure 3-1. (A) Percentage of time spent freezing during fear conditioning training. None of the treatments examined significantly affected freezing during habituation. (B) None of the treatments examined significantly affected the acquisition of fear conditioning.

interaction effect was noted [$F(1, 26) = 0.121, p = 0.731$], although a significant main effect of Ketamine treatment on freezing across T1-T5 was found [$F(1, 26) = 19.391, p < 0.001$] (Fig. 3-2C). Post-hoc analysis confirmed that repeated CORT treatment alone did not affect initial conditioned responding; both the CORT-21/Veh and Veh-21/Veh group exhibited similar levels of average freezing across T1-T5 [$t(13) = 0.080, p = 0.938$]. Post-hoc analysis also revealed that ketamine treatment successfully reduced freezing in both nonstressed and CORT-treated animals. Rats in the Veh-21/Veh control group exhibited high levels of freezing across T1-T5, while rats in the Veh-21/Ket group immediately displayed significantly less freezing behavior during these initial tones [$t(14) = 2.874, p = 0.012$]. Similarly, the CORT-21/Ket treatment group demonstrated less cue-elicited freezing during the first quarter of extinction training than either the Veh/Veh [$t(13) = 3.352, p = 0.005$] or CORT/Veh group [$t(12) = 3.388, p = 0.005$].

Average freezing across the last five tone cues of extinction training was analyzed to assess the relative level of extinction behavior acquisition at the end of the session (late phase extinction training; T16-T20). All treatment groups exhibited low levels of freezing at this time, and no significant main effects [CORT Treatment: $F(1, 26) = 0.606, p = 0.443$; Ket Treatment: $F(1, 26) = 0.180, p = 0.675$] or interaction effects [$F(1, 26) = 0.876, p = 0.358$] were found (Fig. 3-2D).

A within subjects analysis was performed to investigate changes in average tone-elicited freezing between the first quarter (T1-T5) and last quarter (T16-T20) of extinction training within each group. Rats in the Veh-21/Veh group [$t(7) = -1.967, p = 0.090$] and CORT-21/Veh group [$t(6) = 2.754, p = 0.033$] exhibited significantly more freezing behavior across the first quarter of extinction training than across the latter quarter of the session. However, rats in the Veh-21/Ket group [$t(7) = -1.032, p = 0.337$] and CORT-21/Ket group [$t(6) = 0.042, p = 0.968$] exhibited uniformly low levels of freezing across both time periods .

3.3. Fear Extinction Recall Day

All treatment groups exhibited near-zero levels of freezing during the 60 s habituation period preceding fear extinction recall testing, and no significant main effects [CORT Treatment: $F(1, 26) = 0.040, p = 0.844$; Ket Treatment: $F(1, 26) = 0.035, p = 0.853$] or

interaction effects [$F(1, 26) = 1.893, p = 0.181$] were found (Fig. 3-2B; Fig. 3-3B; Fig. 3-3D; Fig. 3-3F).

The initial long-term expression of extinction behavior was investigated by analyzing freezing behavior during the first tone cue of extinction recall testing (early phase extinction recall; T1). No significant main effect of ketamine treatment [$F(1, 26) = 1.379, p = 0.251$] or interaction effect [$F(1, 26) = 2.160, p = 0.154$] was found. Although the main effect of CORT treatment [$F(1, 26) = 3.085, p = 0.091$] failed to reach our pre-established threshold of significance, further post-hoc analysis revealed that CORT treatment alone produced a statistically significant enhancement in freezing behavior, an effect which was prevented by co-treatment with CORT and ketamine (Fig. 3-2E). Rats in the CORT-21/Veh group froze significantly more during T1 than rats in either the Veh-21/Veh [$t(13) = -2.403, p = 0.034$] or CORT-21/Ket group [$t(12) = 2.617, p = 0.022$]. However, ketamine treatment did not affect freezing at T1 when compared to nonstressed animals, as no significant differences were found between the Veh-21/Veh and Veh-21/Ket group [$t(14) = -0.180, p = 0.860$] or Veh-21/Veh and CORT-21/Ket group [$t(13) = -0.433, p = 0.673$].

Average freezing across the second to fifth tones of extinction recall testing was also analyzed to investigate any possible treatment effects on behavior during the remainder of the test session's first quarter (sustained extinction recall; T2-T5). No significant main effects [CORT Treatment: $F(1, 26) = 1.221, p = 0.279$; Ket Treatment: $F(1, 26) = 0.303, p = 0.587, p = 0.853$] or interaction effects [$F(1, 26) = 0.011, p = 0.917$] were found (Fig. 3-2F).

Average freezing across the final five tones of extinction recall testing (late phase extinction recall; T16-T20) was analyzed to assess the relative level of extinction behavior at the end of the session. No significant main effects [CORT Treatment: $F(1, 26) = 0.426, p = 0.520$; Ket Treatment: $F(1, 26) = 1.460, p = 0.238$] or interaction effects [$F(1, 26) = 1.326, p = 0.260$] were found (Fig. 3-2G).

3.4. Sub-Conditioning Retraining Day

Freezing during the 60 s habituation period prior to sub-conditioning retraining was analyzed to determine if CORT or Ket treatment affected baseline behavior when animals were reintroduced to the training context. All groups exhibited minimal freezing, and no significant

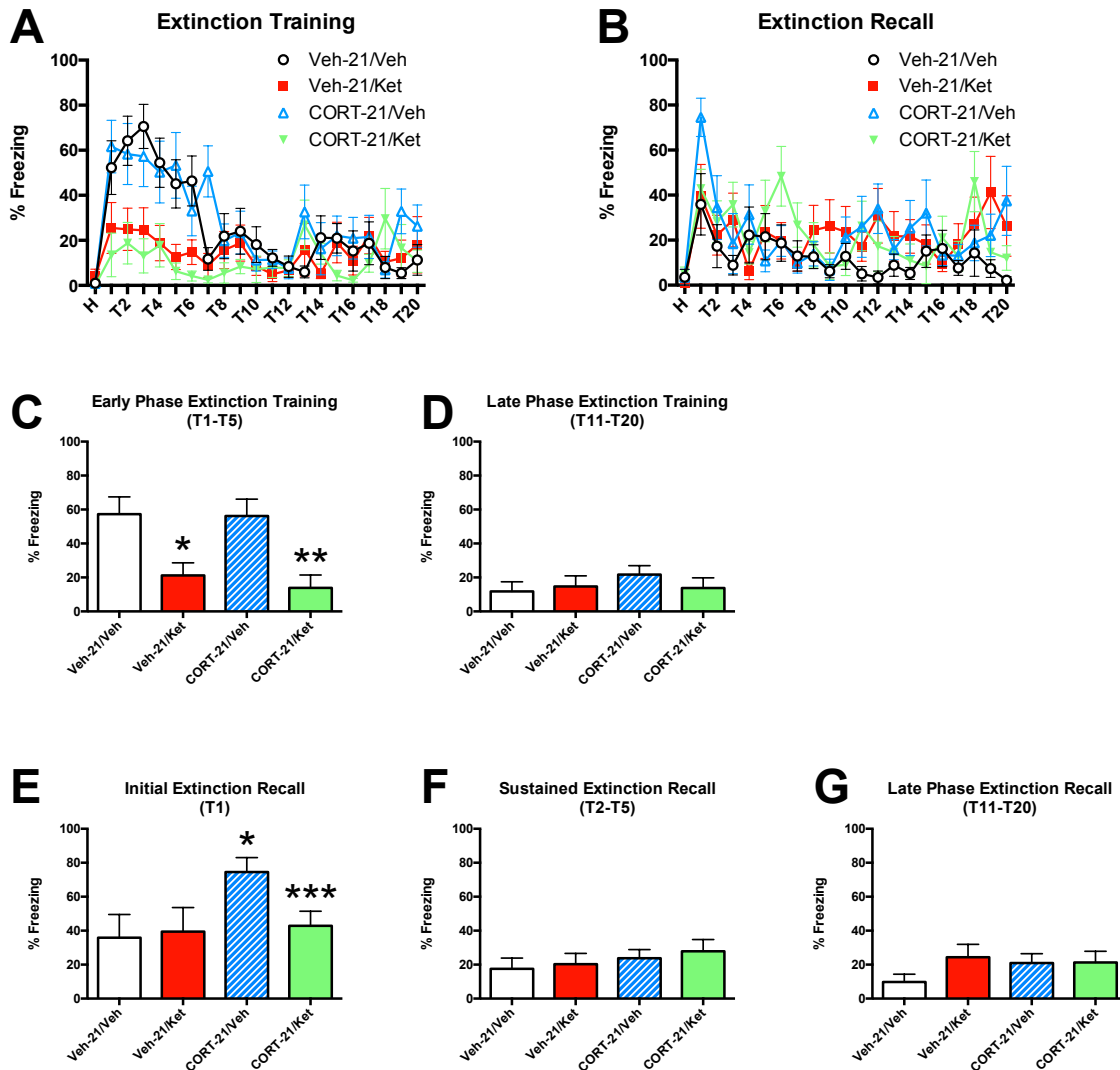


Figure 3-2. (A) Percentage of time spent freezing during extinction training. None of the treatments examined significantly affected freezing during habituation. (B) Percentage of time spent freezing during extinction recall testing. None of the treatments examined significantly affected freezing during habituation. (C) Ketamine significantly reduced freezing in both repeated vehicle- and CORT-treated rats across the first five tones of extinction training. (D) None of the treatments examined significantly impacted freezing across the last five tones of extinction training. (E) CORT significantly increased freezing during the first tone of extinction recall testing, and co-treatment with ketamine prevented this effect. (F) None of the treatments examined significantly impacted freezing across the second through fifth tones of extinction recall testing. (G) None of the treatments examined significantly impacted freezing across the last five tones of extinction recall testing.

* Significantly different from Veh-21/Veh group ($p < 0.05$)

** Significantly different from Veh-21/Veh group and CORT-21/Veh group ($p < 0.05$)

*** Significantly different from CORT-21/Veh group ($p < 0.05$)

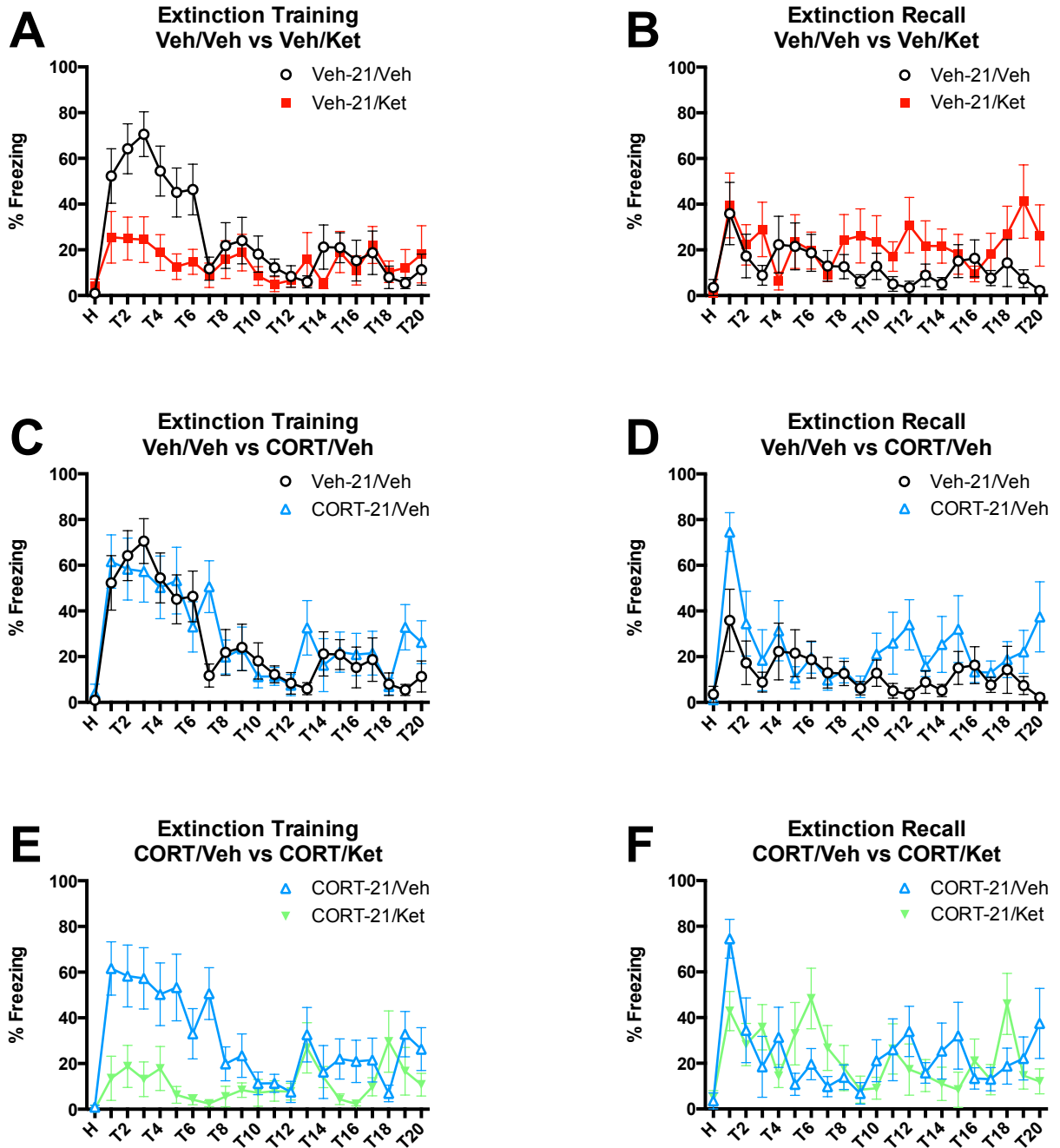


Figure 3-3. (A-F) Group comparisons in percentage of time spent freezing during extinction training and extinction recall testing.

main effects [CORT Treatment: $F(1, 26) = 0.009, p = 0.924$; Ket Treatment: $F(1, 26) = 1.008, p = 0.325$] or interaction effects [$F(1, 26) = 1.715, p = 0.202$] were found (Fig. 3-4A).

Average freezing during tone cue presentation was also analyzed to assess potential treatment effects on behavior during sub-conditioning fear acquisition. No significant main effects [CORT Treatment: $F(1, 26) = 0.107, p = 0.746$; Ket Treatment: $F(1, 26) = 0.618, p = 0.439$;] or interaction effects [$F(1, 26) = 0.012, p = 0.915$] were found (Fig. 3-4B).

3.5. Fear Extinction Retention Test

All treatment groups exhibited near-zero levels of freezing during the 60 s habituation period preceding fear extinction retention testing, and no significant main effects [CORT Treatment: $F(1, 26) = 2.458, p = 0.129$; Ket Treatment: $F(1, 26) = 2.458, p = 0.129$] or interaction effects [$F(1, 26) = 2.458, p = 0.129$] were found (Fig. 3-5A; Fig. 3-5B; Fig. 3-5C; Fig. 3-5D).

The initial retention of extinction memory was investigated by analyzing freezing behavior during the first tone cue of extinction retention testing (T1). A significant main effect of CORT treatment [$F(1, 26) = 4.532, p = 0.043$], a significant main effect of ketamine treatment [$F(1, 26) = 5.526, p = 0.027$], and a significant interaction effect [$F(1, 26) = 11.398, p = 0.002$] was found (Fig. 3-5E). Post-hoc analysis revealed that CORT treatment alone enhanced freezing compared to nonstressed animals, and that this effect that was prevented by co-treatment with CORT and ketamine. Rats in the CORT-21/Veh group froze significantly more at T1 than rats in either the Veh-21/Veh group [$t(13) = -2.839, p = 0.028$] or CORT-21/Ket group [$t(12) = 2.997, p = 0.024$]. Near-zero levels of freezing in both the Veh-21/Veh and CORT-21/Ket group yielded no statistically significant differences [$t(13) = 0.555, p = 0.589$]. Ketamine had no effect on behavior at T1 in nonstressed animals, as rats in both the Veh-21/Veh and Veh-21/Ket group exhibited comparably low levels of freezing [$t(14) = -1.201, p = 0.258$].

Average freezing across the second to fifth tones of extinction retention testing was also analyzed to investigate any possible treatment effects on behavior during the remainder of the test session (sustained extinction retention; T2-5). No significant main effects [CORT Treatment: $F(1, 26) = 0.012, p = 0.914$; Ket Treatment: $F(1, 26) = 0.121, p = 0.731$] were found, although a significant interaction effect was noted [$F(1, 26) = 4.551, p = 0.042$] (Fig. 3-5F). However,

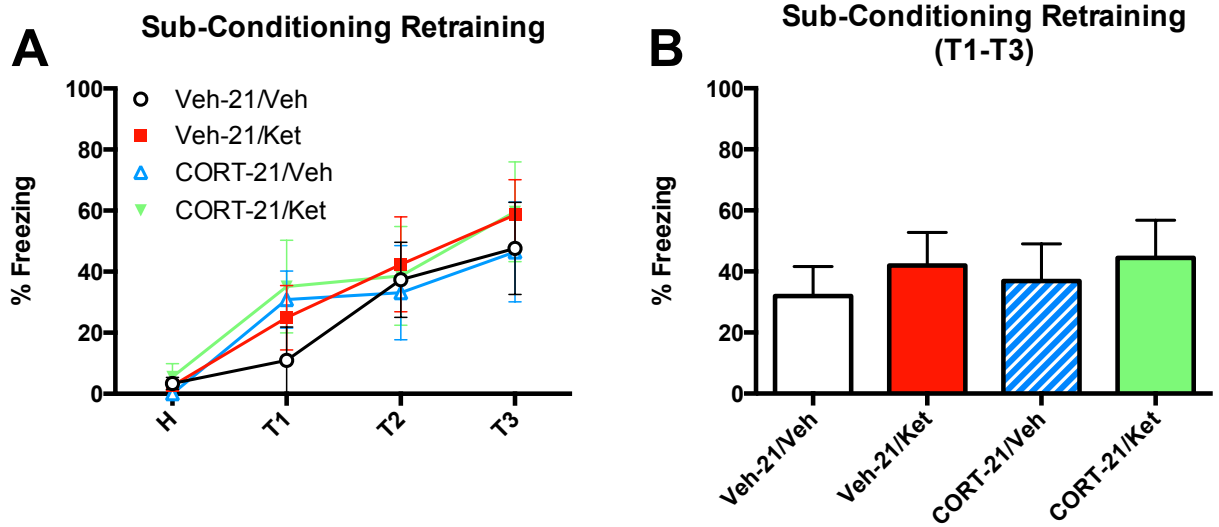


Figure 3-4. (A) Percentage of time spent freezing during sub-conditioning retraining. None of the treatments examined significantly affected freezing during habituation. (B) None of the treatments examined significantly affected the acquisition of sub-conditioning retraining.

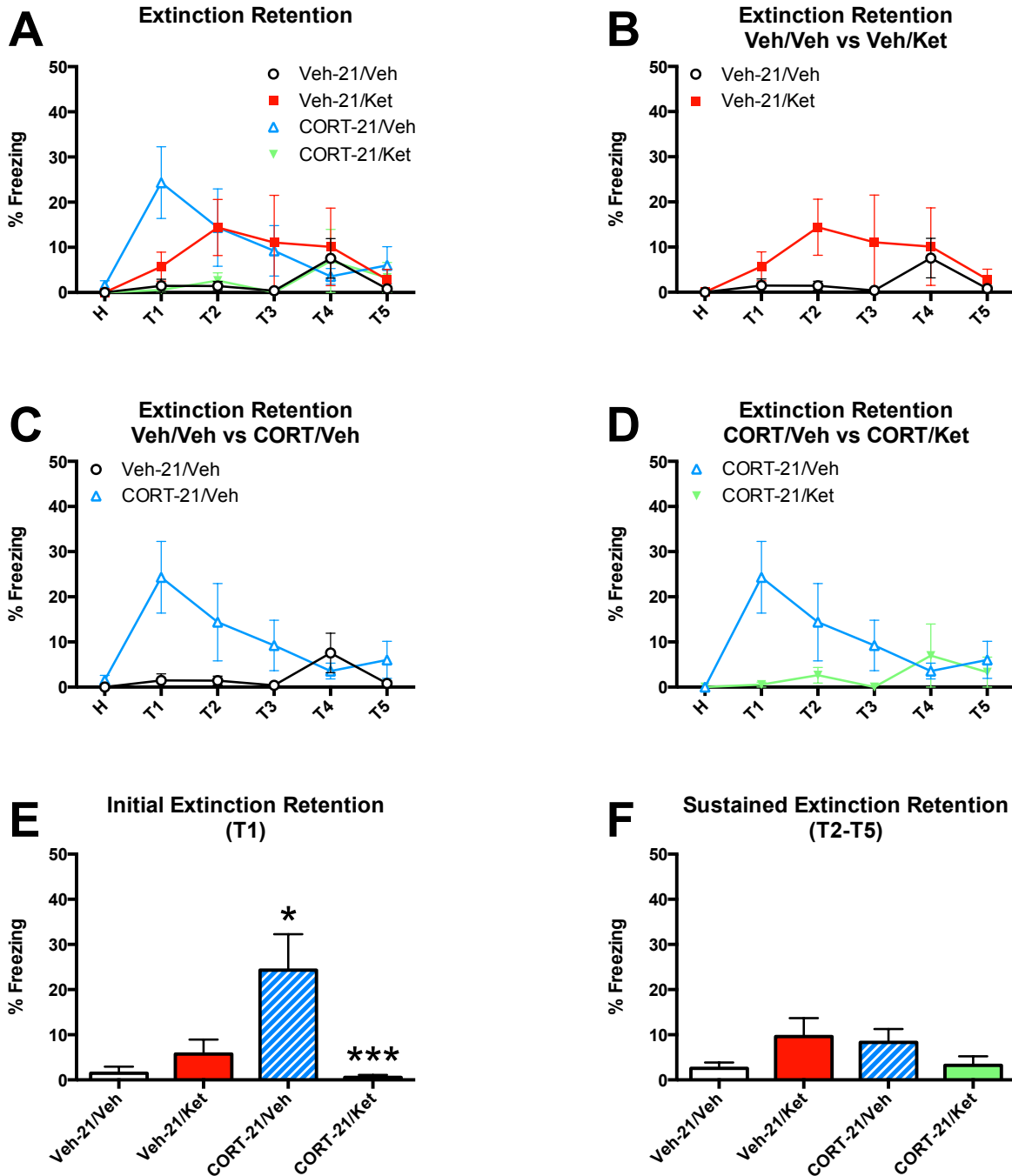


Figure 3-5. (A-D) Percentage of time spent freezing during extinction retention testing. None of the treatments examined significantly affected freezing during habituation. (E) CORT significantly increased freezing during the first tone of extinction retention testing, and co-treatment with ketamine prevented this effect. (F) None of the treatments examined significantly impacted freezing across the last five tones of extinction retention testing.

* Significantly different from Veh-21/Veh group ($p < 0.05$)

*** Significantly different from CORT-21/Veh group ($p < 0.05$)

post-hoc analyses revealed no statistically significant differences between groups (all p values \geq 0.110).

3.6. Body Weight

Daily injections of 40 mg/kg CORT prevented weight gain in rats over time, while animals injected with vehicle instead displayed continuous weight gain throughout the 21 days preceding behavioral testing. A mixed design ANOVA revealed a significant main effect of injection day [$F(3, 48) = 115.205, p < 0.001$], a significant main effect of CORT treatment ($F(1, 28) = 113.934, p < 0.001$), and a significant injection day x CORT treatment interaction effect [$F(3, 48) = 135.058, p < 0.001$]. Post-hoc analyses revealed that no significant differences in weight between CORT and vehicle groups were present on the first injection day [$t(28) = 0.512, p = 0.613$]. However, rats in the CORT-treated group weighed significantly less on average than rats in the vehicle-treated group on day 7 [$t(28) = 10.391, p < 0.001$], day 14 [$t(28) = 11.242, p < 0.001$], and day 21 [$t(28) = 12.765, p < 0.001$] (Fig. 3-6A).

4. Discussion

The aim of this study was to characterize the effects of a single subanesthetic dose of ketamine on the extinction and reinstatement of auditory conditioned fear in animals exposed to repeated CORT or vehicle injections. We observed that regardless of CORT treatment, administration of a 15 mg/kg dose of ketamine significantly impaired the expression of conditioned freezing during early phase extinction training. Chronic CORT exposure had no effect on extinction acquisition, but was found to enhance initial cue-elicited freezing during extinction recall and sub-conditioning retention testing. These CORT-induced enhancements in the spontaneous recovery and trained reinstatement of conditioned fear were prevented by pre-extinction ketamine treatment, which produced a normalization of freezing comparable to that of control animals. Together, our results suggest that a single subanesthetic dose of ketamine can restore the long-term expression of extinction behavior in animals with a history of chronic exogenous CORT exposure, and dramatically suppress the initial recall of conditioned fear irrespective of past CORT treatment. The findings of the present study are discussed in further detail below.

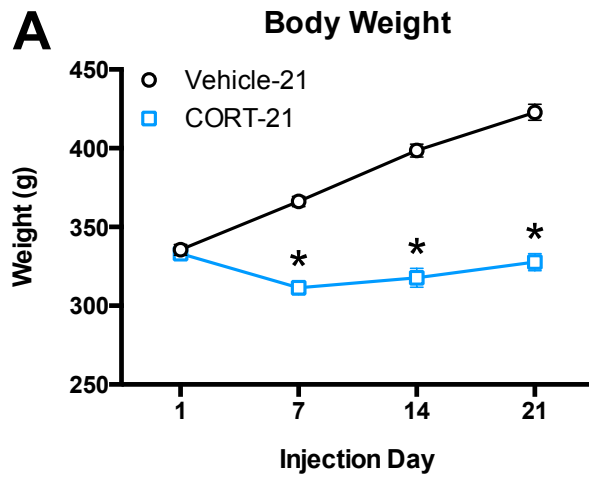


Figure 3-6. (A) Rats treated with CORT weighed significantly less than vehicle-injected rats on injection days 7, 14, and 21.

* Significantly different from CORT group ($p < 0.05$)

4.1. The Effect of Repeated CORT on Auditory Fear Conditioning, Extinction, and Reinstatement in Animals Not Treated With Ketamine

The present study demonstrated that 21 days of CORT treatment did not enhance the acquisition of cue-elicited freezing during fear conditioning training compared to vehicle-treated controls. This finding is corroborated by several past studies, which have reported that chronic CORT treatment either has no effect on fear acquisition, or induces a slight reduction of freezing during associative fear learning (Conrad et al., 2004; Dagnino-Subiabre et al., 2012; Gourley et al., 2009; Marks et al., 2015; Monsey et al., 2014). Similarly, repeated restraint models of chronic stress have shown that both stressed and nonstressed animals exhibit comparable auditory fear acquisition (Baran et al., 2009; Conrad et al., 1999; Miracle et al., 2006; Zhang & Rozencranz, 2013). The absence of a treatment effect on fear acquisition may be the result of a failure for CORT to affect short-term memory. Chronic CORT exposure does not enhance the recall of conditioned fear 1-2 hours after training, despite being capable of increasing cue-elicited freezing 1-14 days later (Gourley et al., 2009). In addition, chronic stress does not alter the level of electrical current needed to induce a pain response in rats (Baran et al., 2009; Conrad et al., 1999; Dagnino-Subiabre et al., 2012; Zhang & Rozencranz, 2013), so the unconditioned stimuli used in the current study should be equally aversive to both CORT- and vehicle-treated groups, and thus produce a similar behavioral response.

Repeated CORT treatment in our study did not significantly affect the long-term expression of auditory conditioned fear during early phase and late phase extinction training. The acquisition of extinction behavior was unimpaired by prolonged exogenous CORT exposure, and both CORT-21/Veh and Veh-21/Veh animals displayed a significant reduction in freezing between the beginning and end of extinction training. Previous research on the effect of chronic stress on conditioned fear recall has yielded inconsistent results. While a couple studies have reported that chronic CORT exposure produces an enhancement of freezing during auditory fear recall testing (Dagnino-Subiabre et al., 2012; Monsey et al., 2014), others have found no effect of chronic CORT on initial long-term auditory (Conrad et al., 2004; Marks et al., 2015) and contextual (Gourley et al., 2009) fear retrieval. Similarly, studies using behavioral models of chronic stress have been unable to reliably produce an enhancement of initial conditioned fear recall (Baran et al., 2009; Bisaz & Sandi, 2010; Conrad et al., 1999; Farrell et al., 2010; Hoffman et al., 2014; Miracle et al., 2006; Wilber et al., 2011; Zhang & Rozenkranz, 2013). It is possible

that conflicting reports on the effect of chronic stress on long-term fear memory could be the result of differences in experimental protocol, especially when auditory and contextual fear are examined within the same study. Our lab has previously demonstrated that repeated CORT treatment increases auditory cue-elicited freezing when tone testing follows contextual testing, but that CORT has no effect on auditory fear memory when tone testing occurs first (Marks et al., 2015). It is unlikely that the effect of CORT on cognition and behavior “wore off” in the 4 days between the final CORT injection and extinction training, as previous studies have reported that much lower doses of CORT than those used in the present study can continue to influence fear-motivated behavior for at least 2 weeks after cessation of administration (Gourley et al., 2009; Monsey et al., 2014).

Despite unimpaired extinction acquisition, animals in the CORT treatment group displayed a 2X increase in freezing compared to control during the first tone of extinction recall testing. This enhancement in cue-elicited freezing is consistent with a spontaneous recovery of conditioned fear, and suggests that either the consolidation or recall of extinction memory was impaired. However, since CORT-treated animals quickly returned to normal freezing levels after the first tone, it seems likely that the recovery of conditioned responding is primarily the result of extinction retrieval failure. Previous studies using restraint and chronic mild stress models have similarly produced enhancements in cue-elicited freezing during early phase, but not late phase extinction recall testing (Baran et al., 2009; Garcia et al., 2008b; Hoffman et al., 2014; Miracle et al., 2006; Wilber et al., 2011). Although one study found that chronic administration of CORT in drinking water did not affect the recall of auditory fear extinction, rats in that experiment were exposed to a significantly smaller dose of CORT compared to the present investigation (Dagnino-Subiabre et al., 2012). Since CORT facilitates conditioned freezing in a dose-dependent manner (Marks et al., 2015), it is possible that the dosage of CORT used in this previous study was not high enough to produce a long-term change in freezing behavior during extinction recall assessment. However, another study found that when CORT was chronically administered through drinking water before contextual fear conditioning and extinction, the between-session recall of extinction behavior was impaired and CORT-treated animals exhibited a spontaneous recovery of contextual fear (Gourley et al., 2009).

The spontaneous recovery of conditioned freezing we observed cannot be ascribed to a generalized inhibition of locomotor activity, as both CORT-21/Veh and Veh-21/Veh rats

exhibited comparable levels of freezing during the 60 s habituation period prior to extinction recall testing. Furthermore, past research has established that chronic behavioral stress has no effect on cue-elicited freezing during fear conditioning, extinction acquisition, and extinction recall when tone and shock cues are unpaired during training, suggesting that prolonged stress does not enhance non-associative fear-motivated behavior (Baran et al., 2009; Conrad et al., 2001; Wilber et al., 2011). CORT treatment in the present study also did not alter freezing behavior during fear conditioning or fear extinction training, so increased freezing during extinction recall testing can neither be accounted for by a facilitation of fear acquisition, nor an impairment of extinction acquisition, respectively.

The present study is the first to our knowledge to examine the effects of chronic exogenous CORT exposure on the sub-conditioning reinstatement of conditioned fear. Similar to initial fear conditioning, repeated CORT treatment did not alter freezing behavior during the sub-conditioning retraining session. However, CORT-treated animals displayed a 16X increase in freezing behavior compared to controls during the first tone of extinction retention testing, which is consistent with conditioned fear reinstatement. This reinstatement of conditioned freezing was limited to the first unreinforced cue presentation, suggesting that extinction retrieval, rather than consolidation, was impaired by CORT. Once again, the CORT-induced enhancement of cue-elicited freezing cannot be ascribed to a generalized inhibition of locomotor activity, as both CORT-21/Veh and Veh-21/Veh rats exhibited comparable levels of freezing during the 60 s habituation period prior to extinction retention testing. These findings suggest that chronic exposure to glucocorticoids can induce a long-lasting impairment of fear extinction that lasts for at least 10 days following the cessation of exogenous CORT administration. Contrary to our expectations, animals in the Veh-21/Veh group did not display a reinstatement of conditioned fear following sub-conditioning retraining. The extensive extinction training protocol used in this study, which consisted of 40 total unreinforced tone cues, apparently created a robust extinction memory that was resistant to change in healthy animals. However, our sub-conditioning retraining protocol appeared to be sensitive to animals with a history of CORT exposure, whose ability to retain long-term extinction expression was visibly impaired in our testing.

Endogenous glucocorticoids play an essential role in normal associative fear learning (Conrad et al., 2001; Cordero et al., 2002; Donley et al., 2005; Jin et al., 2007; Kolber et al., 2008; Pugh et al., 1997a; Pugh et al., 1997b; Rodrigues & Sapolsky, 2009; Roozendaal &

McGaugh, 1997), but excessive glucocorticoid exposure can significantly alter the morphology and function of brain regions responsible for the processing of fear-related stimuli. Neurons in the hippocampus, PFC, and amygdala are abundant in glucocorticoid receptors, making these structures highly vulnerable to the deleterious effects of chronic CORT exposure (Feldman & Weidenfeld, 1999; Herman et al., 2003; Herman et al., 2005; Mitra & Sapolsky, 2008; Morimoto et al., 1996). These same structures are also heavily implicated in the expression and suppression of fear memory, so pathological alterations to their morphology and function could underlie the CORT-induced recovery and reinstatement of conditioned fear that we observed in the current study.

As previously mentioned, the amygdala is responsible for forming a predictive association between conditioned and unconditioned fear stimuli, and acts as a gateway for the expression of conditioned responding (e.g. freezing; Pape & Paré, 2010). However, amygdalar output of conditioned fear can be suppressed through descending projections from the IL mPFC, which stimulate inhibitory GABAergic ITC neurons within the amygdala (Quirk & Mueller, 2008; Pape & Paré, 2010; VanElzakker et al., 2013). The mPFC plays an essential role in the long-term retention of fear extinction, as lesioning or chemical inactivation of this region causes the spontaneous recovery of cue-elicited freezing, despite unimpaired extinction acquisition (Fernandez, 2003; Lebrón et al., 2004; Morgan et al., 1993; Morgan et al., 2003; Quirk et al., 2000; Sierra-Mercado et al., 2006). The hippocampus also mediates activity within fear memory circuitry (Maren & Hobin, 2009; Sotres-Bayon et al., 2012), and communicates with the mPFC to facilitate extinction memory recall and suppress conditioned fear in a contextually appropriate manner (Farinelli et al., 2006; Garcia et al., 2008b; Hugues et al., 2006; Hugues & Garcia, 2007; Peters et al., 2010; Quirk & Mueller, 2008). Impairment of hippocampal neuronal activity during extinction learning results in the recovery of conditioned fear, presumably due to disturbed consolidation of contextual information (Corcoran et al., 2005). Together, the mPFC and hippocampus comprise the fundamental structures underlying the induction and long-term expression of fear extinction, but the integrity of these structures is pathologically altered by exposure to chronic stress. Posing a significant threat to the prefrontal inhibition of conditioned fear, chronic behavioral stress and chronic exogenous CORT exposure have been found to induce dendritic atrophy and an impairment of dendritic spine density in both the PFC and hippocampus of rodents (Cook & Wellman, 2004; Delgado et al., 2011; Dias-Ferreira et al.,

2009; Izquierdo et al., 2006; Li et al., 2011; Liston et al., 2006; Liu & Aghajanian, 2008; Magariños et al., 1996; Magariños et al., 1998; Magariños et al., 1999; Magariños & McEwen, 1995a; Magariños & McEwen, 1995b; Morales-Medina et al., 2009; Radley et al., 2004; Shansky & Morrison, 2009; Sousa et al., 2000; Tata et al., 2006; Watanabe et al., 1992a; Watanabe et al., 1992b; Wellman, 2001; Woolley et al., 1990). Based on the findings that chronic stress impairs prefrontal and hippocampal morphology, and that damage to these structures impairs extinction memory recall, it appears plausible that the spontaneous recovery of conditioned freezing we observed in CORT-exposed rats could in part be the result of stress-induced prefrontal and hippocampal morphological damage.

In addition to morphological deficits, chronic stress induces pathological neurobiological changes in extinction circuits that endanger the ability to successfully suppress conditioned fear. One particularly important component underlying extinction learning is BDNF, a growth factor that mediates synaptic plasticity by promoting dendritic protein synthesis, dendritogenesis, synaptogenesis, and the induction of LTP (Horch et al., 1999; Jourdi et al., 2009; Kang et al., 1997; Korte et al., 1996; Patterson et al., 1996; Takei et al., 2004; Yoshii & Constantine-Paton, 2007; Yoshii & Constantine-Paton, 2010). While fear conditioning impairs hippocampal BDNF transcription (Rasmusson et al., 2002), fear extinction is associated with an upregulation of BDNF in fear suppression pathways (Bredy et al., 2007; Chhatwal et al., 2006; Rosas-Vidal et al., 2014). Following fear extinction, the presence of BDNF protein is increased in the ventral hippocampus (Rosas-Vidal et al., 2014), and BDNF mRNA is upregulated in the PFC (Bredy et al., 2007), suggesting a role in the consolidation of extinction memory. BDNF also appears to mediate extinction memory retrieval, as hippocampal BDNF levels are correlated with the degree of successful long-term fear extinction recall (Peters et al., 2010). Past research has demonstrated that suppression of BDNF activity in the hippocampal-mPFC pathway impairs the long-term expression of fear extinction, resulting in the recovery of conditioned fear (Heldt et al., 2007; Peters et al., 2010; Rosas-Vidal et al., 2014). Chronic stress and CORT exposure oppose the natural changes in BDNF expression that underlie fear extinction by inducing a downregulation of BDNF expression in the hippocampus and PFC (Aboul-Fotouh, 2015; Chen et al., 2008; Dwivedi et al., 2006; Filho et al., 2015; Fu et al., 2014; Gourley et al., 2008; Grønli et al., 2006; Guo et al., 2014; Hamani et al., 2012; Jacobsen & Mørk, 2006; Jin et al., 2015; Li et al., 2012; Liang et al., 2012a; Liang et al., 2012b; Liu et al., 2014; Liu et al., 2015; Luoni et al., 2014;

Monsey et al., 2014; Nibuya et al., 1999; Nitta et al., 1997; Ray et al., 2011; Smith et al., 1995; Yan et al., 2015; Zhang et al., 2015a; Zhu et al., 2016). Since BDNF is an inducer of LTP (Kang et al., 1997; Korte et al., 1996; Patterson et al., 1996), a stress-induced downregulation of this important neurotrophin could produce impairments in neuronal activity as well. Accordingly, chronic stress impairs LTP in both the hippocampus and hippocampal-PFC pathway of rodents (Cerqueira et al., 2007; Foy et al., 1987; Garcia et al., 1997; Kim et al., 1996; Pavildes et al., 2002; Shors et al., 1989; Xu et al., 1997). This carries significant consequences for long-term extinction retention, since the long-term maintenance of extinction behavior depends upon the sustained reversal of a conditioning-induced depression of IL neuron excitability (Cruz et al., 2014; Herry & Mons, 2004), but stress impairs IL neuronal activity during extinction recall (Wilber et al., 2011). Hippocampal and prefrontal LTP also exerts powerful influence over the prevention of sub-conditioning fear reinstatement (Deschaux et al., 2015; Herry & Garcia, 2008b; Zheng et al., 2013), so an impairment of LTP in these regions might underlie the reinstatement of conditioned freezing we observed in rats exposed to chronic CORT.

Although an impairment of hippocampal and prefrontal plasticity could be sufficient to compromise fear extinction alone, a stress-induced facilitation of amygdalar function might also contribute to these behavioral deficits. Acute or chronic exposure to CORT induces dendritic hypertrophy, enhances neuronal excitability, and reduces GABAergic inhibition within the BLA (Duvarci & Paré, 2007; Kavushansky & Richter-Levin, 2006; Mitra & Sapolsky, 2008). Thus, by facilitating neuroplasticity and neuronal activity within the amygdala, exposure to CORT might result in an even greater imbalance between the strength of fear conditioning and extinction memory circuits.

4.2. The Effect of Subanesthetic Ketamine on Auditory Fear Extinction and Reinstatement in Repeated Vehicle- and CORT-Treated Animals

We observed that pre-extinction administration of 15 mg/kg ketamine significantly attenuated the long-term expression of auditory fear in repeated vehicle-injected animals, as evidenced by a 63% reduction in cue-elicited freezing during early phase extinction training compared to control. This same dose of ketamine was also effective at reducing the cue-elicited freezing of CORT-exposed rats by 75% compared to their vehicle-treated CORT-exposed counterparts. Interestingly, rats in the CORT-21/Ket group displayed even less freezing at this

time than rats in the Veh-21/Veh control group, indicating a supranormal suppression of conditioned responding despite their history of chronic CORT exposure. Although this inhibition of freezing is reminiscent of successful extinction learning behavior, the fact that ketamine suppressed conditioned responding immediately at the start of the trial (before extinction learning could begin) suggests that this is not a facilitation of natural extinction learning, but rather an induction of extinction-like behavior. Using nonstressed rats, our lab has previously demonstrated a milder 21% reduction in cue-elicited freezing during early phase extinction as a result of a lower pre-extinction 10 mg/kg dose of ketamine (unpublished data). A similar attenuation of conditioned responding has previously been found when chronic low-dose ketamine treatment precedes the assessment of contextual fear memory recall in nonstressed rodents (Amann et al., 2009; Zhang et al., 2015b), but the present study is the first to our knowledge to investigate the effect of ketamine on animals with a history of CORT exposure. We also found that ketamine significantly altered the acquisition profile of auditory fear extinction. While vehicle-treated rats displayed a reduction of cue-elicited freezing between early phase and late phase extinction training, ketamine-treated rats exhibited an immediate inhibition of cue-elicited freezing that remained consistently low throughout the learning trial. However, the lack of a change in freezing behavior over time should not be interpreted as a failure for these animals to acquire fear extinction, since they demonstrated normal extinction recall in subsequent testing sessions. Instead, the acquisition process is “invisible” in ketamine-treated animals due to the drug’s dramatic and immediate suppression of conditioned freezing.

Since all treatment groups exhibited similar levels of freezing during fear conditioning training, the ketamine-induced reduction in conditioned responding we observed during early phase extinction training cannot be explained by group differences in initial fear acquisition. Our findings can also not be ascribed to a generalized increase in locomotor activity, as ketamine- and vehicle-treated groups displayed equivalent levels of freezing during the 60 s habituation period prior to extinction training. Furthermore, previous research has established that spontaneous locomotor activity in the open field test is normalized within 40 minutes following a 16 mg/kg injection of ketamine in rats (Imre et al., 2006). Since we maintained an hour delay between drug administration and extinction training, ketamine-treated animals in the present study had ample time for locomotor normalization, and exhibited behavior that was indistinguishable from control animals before extinction training began. It is also unlikely that

ketamine induced perceptual distortions that impaired the perception of conditioned stimuli, as previous research has shown that subanesthetic ketamine does not affect auditory evoked potentials in rats (de Bruin et al., 1999).

When administered to rats with a history of repeated vehicle injections, ketamine had no effect on the long-term expression of fear extinction 24 hours after training. Past research in our lab has similarly demonstrated that a lower dose of 10 mg/kg ketamine is also unable to influence long-term fear extinction retrieval in nonstressed rats (unpublished data). Since other researchers have shown that ketamine retains the ability to reduce conditioned responding for at least 48 hours after administration (when administration and testing are separated by a rest period), it is doubtful that the behavioral effects of ketamine in the present study expired due to the 24 hour passage of time between drug administration and extinction recall testing (Amann et al., 2009; Zhang et al., 2015b). It is also unlikely that the lack of treatment effect during extinction recall is a result of state-dependent memory recall, since animals in the pre-extinction ketamine group exhibited no more cue-elicited freezing than animals in the control group did. Rather, it appears that ketamine's ability to facilitate fear suppression is limited to the first extinction session following drug administration in healthy animals. However, the present study revealed that ketamine has an enduring impact on extinction retention in rats with a history of chronic CORT exposure. While CORT-21/Veh rats exhibited a 2X increase in cue-elicited freezing compared to control during initial extinction recall assessment, this abnormal recovery of conditioned fear was abolished in the CORT-21/Ket group. Ketamine treatment induced a complete normalization of long-term extinction expression in these animals, who displayed a low level of freezing in both early and late phase extinction recall testing that was comparable to that of control subjects. Since all groups exhibited similarly low levels of freezing at the end of extinction training, this prevention of conditioned freezing recovery cannot be the result of between-group differences in extinction acquisition. Nor can it be attributed to a ketamine-induced enhancement of general locomotor activity, since ketamine had no effect on freezing during the 60 s habituation period prior to extinction recall testing.

Ketamine did not influence freezing behavior during the sub-conditioning retraining session, and all treatment groups reacted similarly when re-exposed to the training context and tone-shock pairings. When extinction retention was tested 48 hours later, Veh-21/Ket animals exhibited low levels of freezing that did not significantly differ from control animals. This

finding is not surprising, since ketamine also failed to affect freezing behavior during the first extinction recall assessment; however, control group freezing during this trial was also so low that further conditioned fear suppression could not even be possible. Despite a lack of treatment effect in nonstressed animals, ketamine continued to demonstrate an enduring facilitation of extinction retention in animals given chronic CORT. While CORT-21/Veh rats underwent a significant reinstatement of cue-elicited freezing during the first tone of extinction retention testing, this effect was completely abolished in the CORT-21/Ket group. Ketamine successfully prevented the reinstatement of conditioned responding in animals with a history of chronic CORT exposure, producing near-zero levels of freezing that did not differ significantly from the control group. Once again, general locomotor behavior was unchanged in ketamine-treated animals.

Overall, ketamine demonstrated remarkable efficacy in suppressing initial conditioned fear expression, regardless of an animal's history of CORT exposure. However, the benefits of ketamine treatment were limited to the first extinction session in nonstressed rats, which showed no differences in freezing behavior compared to control for the remainder of the study. Conversely, ketamine induced a long-lasting normalization of fear extinction retention in animals with chronic exogenous CORT exposure, preventing both the spontaneous recovery and sub-conditioning reinstatement of conditioned freezing. Furthermore, the present study demonstrated that ketamine's facilitation of healthy fear extinction function lasts for at least 5 days following drug administration, which is consistent with the duration of ketamine's other antidepressant-like behavioral effects in animal models (Autry et al., 2011; Jett et al., 2015; Li et al., 2011; Maeng et al., 2008).

Although the present study's focus was limited to the effects of CORT and ketamine on behavior, past research on the morphological and neurobiological changes induced by these agents can help inform speculation into some of the mechanisms that might underlie our observed results. One potential explanation for the attenuation of cue-elicited freezing we observed during early phase extinction training is that ketamine impaired the cognitive process of memory recall by inactivating NMDA receptors in the amygdala. The lateral amygdala in particular plays a crucial role in integrating fear-relevant stimuli, and mediates the induction and expression of conditioned fear (Pape & Paré, 2010). Infusion of the NMDA receptor antagonist APV into the lateral amygdala prior to fear recall testing has been shown to attenuate

conditioned responding, so it is possible that a systemic injection of ketamine in a high enough dose and close enough temporal proximity to behavioral testing could elicit a similar effect. However, we believe that transient NMDA receptor inactivation itself likely plays a minor and non-essential role in the present study's observed effects of pre-extinction ketamine on the expression of conditioned fear. Past studies that impaired conditioned fear recall through NMDA receptor antagonism administered APV only 5 minutes prior to behavioral testing, ensuring that drug binding to NMDA receptors would be high at the time of conditioned stimulus presentation. Conversely, animals in the present study received ketamine at least one hour before extinction training, which considering ketamine's short 1.3 hour half-life (Veilleux-Lemieux et al., 2013), likely reduced binding to NMDA receptors significantly at the time of behavioral assessment. Furthermore, previous research has demonstrated that ketamine can induce a similar attenuation of conditioned fear recall long after NMDA receptor antagonism has ceased. Chronic administration of 5 mg/kg ketamine, terminated 48 hours before recall testing, was shown to significantly reduce freezing in a contextual fear conditioning paradigm (Amann et al., 2009). We also observed in the present study that ketamine continued to suppress conditioned freezing in CORT-treated animals for 5 days following drug administration, an effect which cannot be explained by short-lived NMDA receptor inactivation. Thus, while transient NMDA receptor antagonism at the time of fear recall may play a role in the ketamine's early phase suppression of conditioned freezing, it is likely an insufficient explanation for the short- and long-term facilitation of extinction-like behavior we observed.

Instead, we propose that ketamine likely facilitates the suppression of conditioned fear by stimulating enhancements in neurotransmission and synaptic plasticity that mirror those underlying the natural process of fear extinction. In addition, we suggest that ketamine prevents the recovery and reinstatement of conditioned fear in CORT-treated rats by reversing neurobiological impairments in fear extinction circuits caused by chronic glucocorticoid exposure.

Past research has illustrated a clear relationship between prefrontal and hippocampal neuronal activity and the successful suppression of conditioned fear. During the consolidation and recall of extinction memory, activity within the IL mPFC is positively correlated with the degree to which extinction behavior is successfully expressed (Burgos-Robles et al., 2007; Milad & Quirk, 2002; Wilber et al., 2001). Extinction recall is also associated with increased

expression of c-Fos, a protein biomarker of recent neuronal activity, within the IL, hippocampus (CA1, CA3, dentate gyrus), and ITC neurons of the amygdala (Knapska & Maren, 2009). Interestingly, experimentally applied electrical stimulation of IL neurons can successfully inhibit fear expression and induce extinction-like behavior in the absence of extinction training (Milad & Quirk, 2002; Milad et al., 2004; Vidal-Gonzalez et al., 2006). Ketamine possesses the potential to similarly modulate conditioned fear suppression because it stimulates presynaptic excitatory neurotransmitter release (i.e. glutamate) in the PFC and hippocampus, namely by reducing the activity of inhibitory GABAergic interneurons in these regions (Chowdhury et al., 2012; Homayoun & Moghaddam, 2007; Moghaddam et al., 1997; Stone et al., 2012). This facilitation of glutamatergic neurotransmission is an essential underlying component of ketamine's antidepressant behavioral effects (Autry et al., 2011; Koike et al., 2011). Accordingly, subanesthetic ketamine has been found to increase c-Fos expression in the mPFC (including the IL) and hippocampus (CA1, CA3, dentate gyrus) at rest (Imre et al., 2006). It is possible that an enhancement of activity in these regions facilitates the downstream inhibition of amygdalar fear output, as ketamine has also demonstrated efficacy in preventing conditioning-induced enhancement of c-Fos expression in the amygdala, while simultaneously inhibiting the expression of conditioned fear (Pietersen et al., 2006). Based on these findings, an immediate enhancement of presynaptic glutamate release in the mPFC and hippocampus might be one of the mechanisms underlying the immediate ketamine-induced suppression of conditioned responding we observed during early phase extinction training in both nonstressed and CORT-exposed rats.

A transient ketamine-induced burst of glutamatergic neurotransmission could also promote long-term changes in synaptic plasticity, since activation of the AMPA glutamate receptor stimulates BDNF release in the brain (Jourdi et al., 2009). The BDNF protein's ability to strengthen synapses, even in the absence of electrical stimulation, makes it a powerful modulator of memory and behavior (Autry et al., 2011; Liu et al., 2012; Messaoudi et al., 2002; Peters et al., 2010). As previously discussed, fear extinction reverses a conditioning-induced inhibition of hippocampal BDNF transcription (Rasmusson et al., 2001; Rosas-Vidal et al., 2014). Hippocampal BDNF is projected to the IL to suppress conditioned fear expression (Peters et al., 2010), and the presence of BDNF within the hippocampus even enhances the excitability of extinction-critical IL neurons (Rosas-Vidal et al., 2014). Furthermore, extinction training

initiates epigenetic regulation of BDNF gene expression in the IL, increasing histone H4 acetylation of BDNF promoter regions to result in an upregulation of prefrontal BDNF mRNA (Bredy et al., 2007). Interestingly, ketamine induces a rapid upregulation of neuronal BDNF that is reminiscent of the natural changes in neurotrophin expression that are induced by fear extinction learning. Subanesthetic ketamine stimulates a significant enhancement of BDNF protein expression in the prefrontal cortex and hippocampus that occurs within only 30 minutes of administration (Autry et al., 2011; Garcia et al., 2008a; Zhang et al., 2015a; Zhang et al., 2015b). Consistent with increased BDNF activity, ketamine also upregulates several synaptic proteins (including synapsin I, PSD-95, and the AMPA subunit Gria1), increases dendritic spine density, and enhances dendritic spine maturity in the mPFC within one hour of treatment (Li et al., 2010; Li et al., 2010; Zhang et al., 2015a). Since the present study introduced an hour delay between ketamine administration and behavioral testing, it is possible that these changes in neurotrophin expression and synaptic plasticity could contribute to the suppression of conditioned responding we observed during early phase extinction training. It is noteworthy that a similar facilitation of conditioned fear suppression can be produced by directly infusing BDNF into either the IL or hippocampus of rats (Peters et al., 2010). Within an hour of BDNF infusion, these treatments produce a significant reduction in cue-elicited freezing that begins with the first unreinforced stimulus, even in the absence of extinction training (Peters et al., 2010). Peters and colleagues (2010) refer to this phenomenon as “BDNF-extinction,” because the successful and immediate suppression of conditioned responding induced by BDNF infusion was not a facilitation of extinction training (since no extinction training took place), but rather an effective substitution for it. We believe that a similar phenomenon, resulting from a ketamine-induced upregulation of prefrontal and hippocampal BDNF, could underlie the impaired conditioned fear expression observed in the present study.

Not only does ketamine upregulate BDNF expression in the brains of nonstressed rats, but it also reverses stress-induced neurobiological impairments in memory circuits that could contribute to long-term extinction failure. In the present study, animals with chronic CORT exposure showed a spontaneous recovery and sub-conditioning reinstatement of conditioned freezing (suggesting that their ability to recall extinction memory was impaired), an effect that was prevented by ketamine treatment. As previously discussed, prefrontal and hippocampal BDNF play a critical role in the long-term recall of fear extinction (Heldt et al., 2007; Peters et

al., 2010; Rosas-Vidal et al., 2014), but BDNF expression is impaired by chronic stress exposure (Aboul-Fotouh, 2015; Chen et al., 2008; Dwivedi et al., 2006; Filho et al., 2015; Fu et al., 2014; Gourley et al., 2008; Grønli et al., 2006; Guo et al., 2014; Hamani et al., 2012; Jacobsen & Mørk, 2006; Jin et al., 2015; Li et al., 2012; Liang et al., 2012a; Liang et al., 2012b; Liu et al., 2014; Liu et al., 2015; Luoni et al., 2014; Monsey et al., 2014; Nibuya et al., 1999; Nitta et al., 1997; Ray et al., 2011; Smith et al., 1995; Yan et al., 2015; Zhang et al., 2015a; Zhu et al., 2016). Conversely, ketamine ameliorates the suppression of prefrontal and hippocampal BDNF seen in stressed rats, supporting healthy neuronal function in these extinction-critical structures (Zhang et al., 2015a; Zhang et al., 2015b). Furthermore, this ketamine-induced enhancement of BDNF expression lasts for at least 8 days (Zhang et al., 2015a), which is consistent with the duration of therapeutic effect we observed in CORT-treated rats in the present study. Since BDNF induction plays a necessary role in the antidepressant behavioral effects of ketamine (Autry et al., 2011; Liu et al., 2012), it is plausible that BDNF modulation could also underlie the long-term extinction facilitation seen in CORT + ketamine-treated rats. Subanesthetic ketamine also reverses stress-induced deficits in synaptic proteins such as synapsin I, PSD-95, and AMPA subunit Gria1 (Li et al., 2010; Li et al., 2011; Zhang et al., 2015a). On a larger morphological scale, ketamine reverses chronic stress-induced impairments in prefrontal dendritic spine density and maturity within 24 hours of administration, offering an additional means by which ketamine might support long-term extinction retention (Li et al., 2010; Li et al., 2011; Monsey et al., 2014).

Since BDNF expression promotes the strength and maturity of synapses in the brain, and increases postsynaptic membrane insertion of AMPA receptor subunits, it is unsurprising that ketamine also induces a long-lasting facilitation of neurotransmission (Autry et al., 2011; Li et al., 2010; Li et al., 2011). This potentially has significant consequences for extinction, since enhancements in prefrontal and hippocampal synaptic efficacy play an important role in the continued suppression of conditioned fear. Extinction learning is associated with the induction of LTP in the IL, which reverses a conditioning-induced depression of neuron excitability in this region (Cruz et al., 2014; Farinelli et al., 2006; Herry & Garcia, 2002; Hugues et al., 2006). Conversely, depressed IL neuron excitability accompanies the spontaneous weakening of extinction expression over time, illustrating the important role that LTP plays in the maintenance of extinction memory (Cruz et al., 2014; Farinelli et al., 2006; Herry & Garcia, 2002; Herry &

Mons, 2004; Hugues et al., 2006). Fear extinction also enhances synaptic efficacy between the hippocampus and mPFC, and experimental disruption of this process results in long-term extinction recall failure (Farinelli et al., 2006; Garcia et al., 2008b; Hugues et al., 2006; Hugues & Garcia, 2007). Posing a significant threat to the long-term retention of fear extinction, chronic stress impairs LTP in both the hippocampus and hippocampal-PFC pathway of rodents (Cerqueira et al., 2007; Foy et al., 1987; Garcia et al., 1997; Kim et al., 1996; Pavlides et al., 2002; Shors et al., 1989; Xu et al., 1997), and suppresses extinction-critical IL neuronal activity during extinction recall testing (Wilber et al., 2011). Once again, ketamine appears to induce a facilitation of neurotransmission which parallels that seen in the natural process of extinction, and ameliorates deficits in synaptic efficacy that are caused by chronic stress. Past research has demonstrated that subanesthetic ketamine potentiates synaptic efficacy in the hippocampus (Autry et al., 2011; Nosyreva et al., 2013), and reverses stress-induced prefrontal synaptic depression (Li et al., 2011). Facilitation of LTP in the mPFC has been shown to prevent the reinstatement of conditioned fear following sub-conditioning retraining (Deschaux et al., 2011a; Zheng et al., 2013), so it is plausible that ketamine-induced synaptic potentiation might similarly strengthen long-term extinction.

If ketamine is facilitating long-term extinction retention by reversing impairments in BDNF expression, neuronal morphology, and synaptic efficacy, then this might explain why an enduring therapeutic response was only found in CORT-treated rats. Since animals that were administered vehicle injections for 21 days should have generally unimpaired neuronal functioning, their ability to suppress conditioned fear during extinction recall testing and following sub-conditioning retraining should remain intact. Indeed, we observed that Veh-21/Veh and Veh-21/Ket animals exhibited comparable levels of freezing during these times to that of control animals. Only CORT-21/Veh rats displayed an abnormal spontaneous recovery and sub-conditioning reinstatement of conditioned fear, suggesting an underlying pathology that impairs long-term extinction memory recall. Addressing this pathology through pharmacological treatment would then be expected to produce a facilitation long-term fear suppression only in CORT-treated animals, which is what we observed in the present study. Furthermore, CORT-treated rats that received ketamine behaved no differently than control animals, exhibiting normal responses to tone cue re-exposure. Thus, ketamine's enduring effects on extinction retention in CORT-treated animals likely reflect a reinstatement of normal neurological function,

as opposed to a supranormal augmentation of freezing suppression like we observed during early phase extinction training.

4.3. Clinical Implications

Subanesthetic doses of ketamine have demonstrated impressive efficacy in the treatment of human depression (Aan Het Rot et al., 2012; Berman et al., 2000; Cusin et al., 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Murrough et al., 2013; Phelps et al., 2009; Price et al., 2009; Rasmussen et al., 2013; Zarate et al., 2006), but it has been uncertain if ketamine also helps challenge negative cognitive biases in memory that are associated with this disorder. The findings from the present study suggest that a single subanesthetic dose of ketamine can rapidly suppress the expression of negative emotional memory in rats with chronic CORT exposure, even when the initial acquisition of aversive learning undisturbed. Furthermore, ketamine treatment can ameliorate CORT-induced failures in long-term extinction retrieval, and restore normal extinction memory function. By shifting recollection away from emotionally aversive events, ketamine could possess utility in overcoming depression-associated negative cognitive biases. Additional research is needed to determine if ketamine produces a similar facilitation of fear extinction in human patients with depression.

5. Conclusions

This study is the first to demonstrate the efficacy of ketamine in treating the negative emotional biases in memory that result from chronic glucocorticoid exposure. We found that a single subanesthetic dose of ketamine can induce a rapid and long-lasting suppression of auditory fear expression, particularly in animals exposed to chronic exogenous CORT. Within an hour of ketamine administration, both repeated vehicle- and CORT-treated animals exhibited a significant reduction of cue-elicited freezing when conditioned fear recall was assessed. Chronic CORT exposure induced a spontaneous recovery of conditioned freezing, and a reinstatement of freezing following sub-conditioning retraining, suggesting a failure of long-term extinction retention in these animals. However, ketamine treatment completely eliminated these CORT-induced extinction retrieval deficits, an effect which lasted for at least 5 days following drug administration. Since negative cognitive biases are believed to support the development and

maintenance of depression in humans, the findings of the present study could have implications for the treatment of pathologically altered memory in this disorder.

CHAPTER 4

General Discussion

1. Overview of Main Findings

The aim of this dissertation was to explore ketamine's ability to modulate the expression of negative emotional memory and prevent stress-induced deficits in the regulation of emotionally-driven behavior. To accomplish this, I conducted two experiments that examined the effect of acute ketamine treatment on fear-motivated behavior in fear conditioning and extinction paradigms.

In **Chapter 2**, I sought to dissociate the effects of ketamine on distinct stages of auditory fear conditioning and extinction in order to gain a better understanding of the underlying cognitive processes that the NMDA receptor antagonist might modulate. I found that ketamine's influence on cue-elicited freezing in rats is dependent upon the time at which ketamine is administered relative to fear learning and recall. Ketamine did not disturb the long-term expression of conditioned fear behavior when administered prior to fear conditioning training, suggesting that the drug does not interfere with the acquisition of fear memories. However, immediate post-conditioning ketamine administration attenuated conditioned freezing in a recall test 10 days later, suggesting that NMDA receptor antagonism impaired fear memory consolidation. Pre-extinction ketamine treatment induced a similar reduction of long-term fear expression, a phenomenon which could either be explained by an impairment of fear memory recall or a facilitation of extinction-like fear suppression. None of the ketamine treatments examined affected the long-term recall of extinction behavior. The results from this study help fill an important gap in the literature by identifying the specific stages of fear learning that are affected by subanesthetic ketamine injection.

Chapter 3 aimed to expand upon these findings by examining the effect of ketamine on the long-term retention of extinction behavior in a repeated CORT animal model of depression. Regardless of prior CORT exposure, pre-extinction administration of a subanesthetic 15 mg/kg dose of ketamine induced an immediate and substantial attenuation of cue-elicited freezing during conditioned fear recall assessment. Chronic CORT exposure provoked a spontaneous recovery of conditioned freezing between extinction sessions and induced a reinstatement of freezing following sub-conditioning retraining, suggesting that the ability to recall extinction memory had been impaired. However, ketamine treatment completely abolished these CORT-

induced extinction retrieval deficits and facilitated healthy fear extinction expression for at least 5 days following drug administration. The findings of this study contribute to our understanding of the influence that prolonged glucocorticoid exposure has on memory for aversive emotional events, and further demonstrate the rapid and long-lasting antidepressant-like effects of ketamine on negatively biased memory.

2. The Effects of Ketamine on Fear Conditioning

The findings of this dissertation make a significant contribution to our understanding of the effects that ketamine has on the induction and expression of conditioned fear. Although previous studies have repeatedly demonstrated ketamine's ability to impair fear conditioning, the experimental designs used in these investigations limited the conclusions that could be drawn regarding the specific stages of fear conditioning that ketamine modulates. For example, most experiments have only administered ketamine prior to fear conditioning training, which makes it difficult to identify the cognitive processes that are affected by the drug, since ketamine has long-lasting effects on neurobiology and behavior (Amann et al., 2009; Calzavara et al., 2009; Pietersen et al., 2006; Pietersen et al., 2007; Zhang et al., 2015). When alternative administration timelines have been investigated using chronic ketamine injections, a lack of comparable comparison groups has confounded the ability to distinguish the unique effects of each treatment (Amann et al., 2009; Zhang et al., 2015).

Thus, characterizing the effects of acute ketamine treatment on auditory fear conditioning requires special consideration in methodological design, and this is the issue I sought to address in Chapter 2. Surprisingly, I found that pre-conditioning ketamine treatment had no effect on the long-term expression of conditioned fear when recall was assessed 10 days after administration. However, when ketamine was administered an hour before recall assessment, an attenuation of conditioned freezing was observed. This suggests that ketamine impairs fear conditioning by interfering with the expression of conditioned responding, rather than the initial acquisition of fear learning. One implication of this finding is that most fear conditioning studies have been administering ketamine at a time point at which the drug has little to no effect on fear-relevant cognition and behavior. Therefore, future research on the modulatory effects of ketamine on fear conditioning should instead administer ketamine before recall assessment, when the drug is most effective. Furthermore, pre-recall ketamine injection paradigms possess greater clinical relevance

in the study of depression-associated negative cognitive biases, since patients typically only seek medical treatment once symptoms of the disorder have already developed (and thus negative associations have been formed).

This was also the first study to my knowledge to experimentally investigate the effect of ketamine on the consolidation of conditioned fear memory. Immediate post-conditioning ketamine administration induced a significant attenuation of cue-elicited freezing during recall assessment 10 days later. Since pre-conditioning ketamine failed to impair conditioned responding after this same time delay, post-conditioning ketamine likely reduced conditioned responding by impairing the consolidation, rather than the recall, of associative fear memory. Past research has failed to influence long-term fear expression with post-conditioning APV infusion, which has lead some researchers to hypothesize that acute NMDA receptor antagonism is unable to interfere with the process of fear memory consolidation. (Kim et al., 1991; Maren et al., 1996b). However, the present findings suggest that this might not be the case, and past studies may have produced different results due to the competitive binding characteristics of APV, compared to the non-competitive binding of ketamine. Furthermore, a study of wounded military servicemen found that post-trauma ketamine anesthesia reduced the likelihood of developing PTSD in the future, supporting the possibility that ketamine interferes with the consolidation of aversive memories (McGhee et al., 2008). These preliminary findings warrant further experimental investigation into the matter, as the ability to modulate the consolidation of negative emotional memory following a trauma could be of great clinical value in the prevention of PTSD.

3. The Delicate Balance of Fear Extinction

Fear extinction is a unique cognitive and behavioral phenomenon, the expression of which is guided by a delicate balance between the competing influence of opposing associative memories. Since fear extinction involves the formation of a new benign associative memory, as opposed to the erasure of the original fear memory, an extinguished animal that is presented with a CS can interpret and respond to the cue in two different ways (Pape & Paré, 2010; Quirk & Mueller, 2008; VanElzakker et al., 2013). Utilization of the predictive fear memory will provoke a freezing response, while utilization of the extinction memory will elicit a suppression of freezing behavior. The level of freezing that is expressed in response to a CS is dependent upon

the relative strength of fear and extinction memories and the neuronal circuits that underlie them (Cruz et al., 2014; Deschaux et al., 2011; Herry & Mons, 2004; Milad & Quirk, 2002; Wilber et al., 2011; Zheng et al., 2013).

Extinction memory is strong shortly after acquisition (Deschaux et al., 2011a; Deschaux et al., 2011b; Deschaux et al., 2013; Zheng et al., 2013), but research has shown that a number of internal and external factors can erode the strength of this fragile predictive association, causing a reemergence of cue-elicited fear-motivated behavior. The mere passage of time is sufficient to induce progressive extinction retrieval failure (Cruz et al., 2014; Pape & Paré, 2010), a phenomenon that is associated with the decay of extinction-induced IL potentiation (Cruz et al., 2014; Herry & Mons, 2004). Extinction is also poorly expressed in novel contexts, as the requisite environmental cues of the extinction training context are no longer present to trigger extinction retrieval (Bouton, 2004; Bouton & Bolles, 1979a; Bouton & Bolles, 1979b; Bouton et al., 2006; Bouton & King, 1983). Re-exposure to the US alone or a weak CS-US pairing provokes a reinstatement of conditioned responding in previously extinguished animals (Deschaux et al., 2011a; Deschaux et al., 2011b; Deschaux et al., 2013; McAllister & McAllister, 2006; Laurent & Westbrook, 2010; Rescorla & Heth, 1975; Shen et al., 2013; Zheng et al., 2013). Fear reinstatement is the result of a relearning process that strengthens the original CS-US fear association that was acquired during fear conditioning, causing it to overwhelm the ability for extinction memory circuits to suppress amygdalar fear output (Deschaux et al., 2011a; Deschaux et al., 2015; Shen et al., 2013; Zheng et al., 2013). Interestingly, acute post-extinction stress exposure elicits a similar reemergence of extinguished fear (Deschaux et al., 2013; Izquierdo et al., 2006; Zheng et al., 2013). Acute stress likely impairs extinction expression through the depotentiation and dendritic atrophy of prefrontal extinction circuits (Maroun & Richter-Levin, 2003; Rocher, Spedding, Monoz, & Jay, 2004; Zheng et al., 2013) in addition to a stress hormone-induced facilitation of fear memory recall (Izquierdo et al., 2002).

Past research has found that chronic pre-conditioning behavioral stress and CORT exposure also impairs the long-term expression of fear extinction (Baran et al., 2009; Farrell et al., 2010; Gourley et al., 2009; Hoffman et al., 2014; Miracle et al., 2006; Wilber et al., 2011). The results from Chapter 3 corroborate this inverse relationship between chronic stress and long-term extinction retrieval, as repeated CORT injections induced a spontaneous recovery of conditioned fear between extinction sessions. In addition, this study further demonstrated the

vulnerability of extinction memory to stress through the sub-conditioning reinstatement of conditioned freezing that occurred exclusively in CORT-exposed animals. Although vehicle-treated animals did not exhibit fear reinstatement following sub-conditioning retraining (perhaps due to the extensive extinction training protocol used), it appears that chronic CORT exposure lowered the threshold required for weak tone-shock pairings to restrengthen the CS-US fear association.

I propose that chronic stress and prolonged glucocorticoid exposure compromises the long-term retention of extinction behavior by impairing the ability for prefrontal and hippocampal extinction circuits to regulate the fear output of the amygdala. Both chronic behavioral stress and repeated CORT treatment damage the dendritic morphology of the PFC and hippocampus in rodents (Cook & Wellman, 2004; Delgado et al., 2011; Dias-Ferreira et al., 2009; Izquierdo et al., 2006; Li et al., 2011; Liston et al., 2006; Liu & Aghajanian, 2008; Magariños et al., 1996; Magariños et al., 1998; Magariños et al., 1999; Magariños & McEwen, 1995a; Magariños & McEwen, 1995b; Morales-Medina et al., 2009; Radley et al., 2004; Shansky & Morrison, 2009; Sousa et al., 2000; Tata et al., 2006; Watanabe et al., 1992a; Watanabe et al., 1992b; Wellman, 2001; Woolley et al., 1990), and suppress BDNF expression in these same regions (Aboul-Fotouh, 2015; Chen et al., 2008; Dwivedi et al., 2006; Filho et al., 2015; Fu et al., 2014; Gourley et al., 2008; Grønli et al., 2006; Guo et al., 2014; Hamani et al., 2012; Jacobsen & Mørk, 2006; Jin et al., 2015; Li et al., 2012; Liang et al., 2012a; Liang et al., 2012b; Liu et al., 2014; Liu et al., 2015; Luoni et al., 2014; Monsey et al., 2014; Nibuya et al., 1999; Nitta et al., 1997; Ray et al., 2011; Smith et al., 1995; Yan et al., 2015; Zhang et al., 2015a; Zhu et al., 2016). Chronically stressed animals also exhibit impairments in LTP within the hippocampal-PFC pathway that is essential to long-term fear extinction retention, a phenomenon which could explain the recovery and spontaneous reinstatement of conditioned fear observed in CORT-treated animal in Chapter 3 (Cerqueira et al., 2007; Deschaux et al., 2015; Foy et al., 1987; Garcia et al., 1997; Herry & Garcia, 2008b; Kim et al., 1996; Pavildes et al., 2002; Wilber et al., 2011; Shors et al., 1989; Xu et al., 1997; Zheng et al., 2013). Furthermore, chronic stress and glucocorticoid exposure induces dendritic hypertrophy, enhances neuronal excitability, and reduces GABAergic inhibition within the BLA, the primary gateway for conditioned fear responding (Duvarci & Paré, 2007; Kavushansky & Richter-Levin, 2006; Mitra & Sapolsky, 2008). Collectively, these studies suggest that chronic stress impairs the function of neural

circuits that serve to inhibit amygdalar activity during extinction testing, while simultaneously strengthening the function of circuitry responsible for conditioned fear recall.

Although the balance between extinction expression and fear recall can easily be biased in the favor fear reemergence through a number of means, relapses of conditioned responding are preventable through interventions that enhance the efficacy of extinction circuits. Application of high-frequency stimulation, which is known to induce LTP in its targets, to the mPFC or hippocampus successfully prevents the sub-conditioning reinstatement of conditioned fear (Deschaux et al., 2011a; Zheng et al., 2013). Theoretically, if a drug were to similarly induce LTP in fear extinction pathways, it would stand to reason that this drug might also promote the long-term retention of fear extinction. In Chapter 3, I demonstrated that a single subanesthetic dose of ketamine prevents both the spontaneous recovery and sub-conditioning reinstatement of auditory fear in rats with chronic CORT exposure. Not only does ketamine potentiate the synaptic efficacy of the PFC and hippocampus (Autry et al., 2011; Li et al., 2011; Nosyreva et al., 2013), but it also stimulates excitatory neurotransmitter release (Chowdhury et al., 2012; Homayoun & Moghaddam, 2007; Moghaddam et al., 1997; Stone et al., 2012), increases neuronal activity (Imre et al., 2006), and reverses stress-induced deficits in BDNF (Zhang et al., 2015a; Zhang et al., 2015b) and synaptic protein expression (Li et al., 2010; Li et al., 2011; Zhang et al., 2015a) within these same regions. It is evident that ketamine facilitates the inhibition of amygdalar fear output, as amygdalar expression of the neuronal activity marker c-Fos is reduced in conjunction with the ketamine-induced impairment of conditioned fear recall (Pietersen et al., 2006). The findings of these past studies and those of Chapter 3 illustrate the ability for ketamine to overcome the stress-induced bias towards conditioned fear retrieval, instead promoting a robust maintenance of extinction expression. Figure 4-1 schematically summarizes the means by which chronic CORT exposure and ketamine might mediate the balance between conditioned fear and extinction expression.

4. Depression-Like Behavior Induced by Chronic CORT Exposure

Chronic exogenous CORT administration has become an increasingly popular preclinical model of depression over the past 15 years. One of the CORT model's virtues lies in its precise control over sustained glucocorticoid exposure, which limits individual variability in stressor

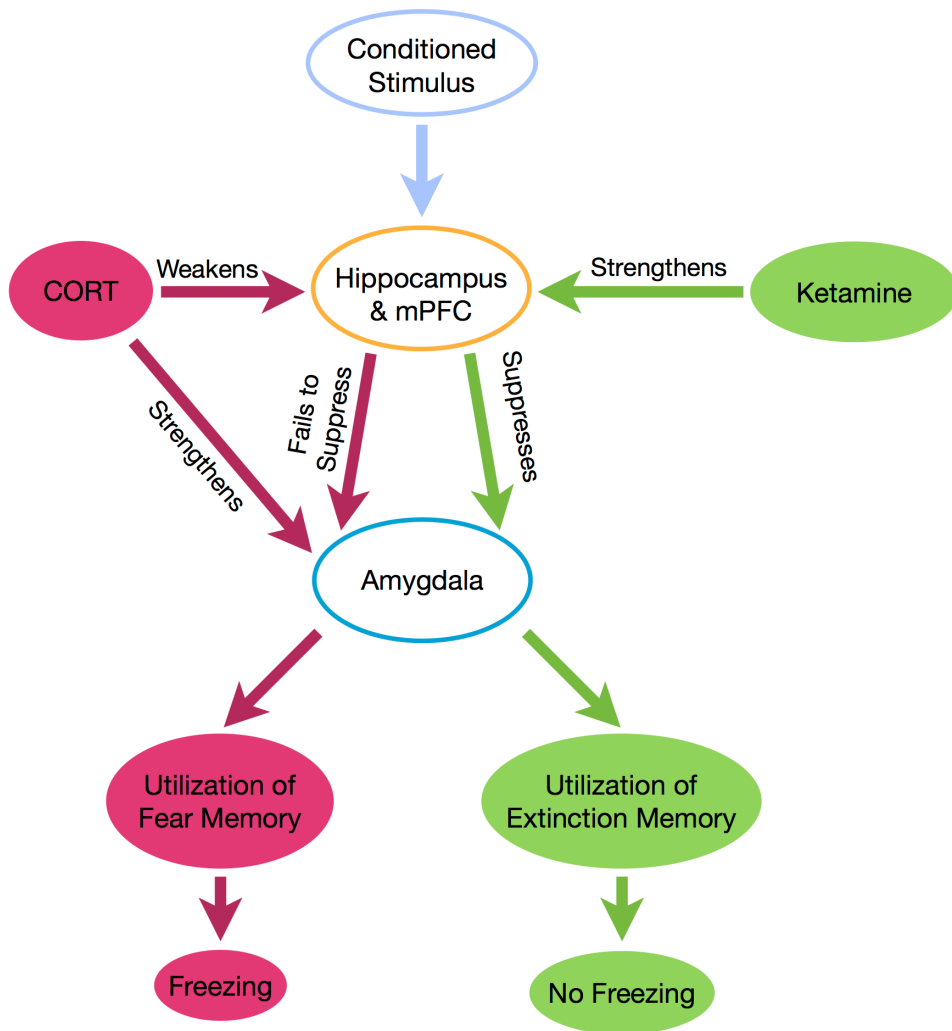


Figure 4-1. Schematic representation of a possible model by which CORT and ketamine modulate conditioned fear behavior. Chronic exposure to CORT impairs the integrity and function of the hippocampus and mPFC, which in turn compromises the ability for these structures to suppress amygdalar fear output. As a result, CORT-treated animals express recall of the associative fear memory through defensive freezing. Conversely, treatment with ketamine strengthens hippocampal and prefrontal function, facilitating successful inhibition of the amygdala and prevention of cue-elicited freezing.

response. Prolonged CORT exposure also induces pathological alterations in neuroplasticity that provide mechanistic similarity to the pathogenesis of depression in humans (Bremner et al., 2000; Campbell et al., 2004; Cole et al., 2010; Colla et al., 2007; Frodl et al., 2002; Lange & Irle, 2004; Liu & Aghajanian, 2008; Magariños et al., 1998; Magariños et al., 1999; Mitra & Sapolsky, 2008; Morales-Medina et al., 2009; Seib & Wellman, 2003; Sheline et al., 1999; Sheline et al., 2003; Sousa et al., 2000; Tata et al., 2006; Videbech & Ravnkilde, 2004; Watanabe et al., 1992a; Wellman, 2001; Woolley et al., 1990). Imperative to the face validity of the model, chronic CORT administration produces several depression-like behaviors in rodents, including various forms of anhedonia (David et al., 2009; Gorzalka et al., 1999; Gorzalka et al., 2001; Gorzalka et al., 2003; Gorzalka & Hanson, 1998; Gourley et al., 2008a; Gourley et al., 2008b; Gupta et al., 2015; Wu et al., 2013; Yau et al., 2014) in addition to learned helplessness (Brummelte et al., 2006; David et al., 2009; Fenton et al., 2015; Gourley et al., 2008a; Gregus et al., 2005; Hill et al., 2003; Johnson et al., 2006; Kalynchuk et al., 2004; Koike et al., 2013; Marks et al., 2009; Murray et al., 2008; Wu et al., 2013; Zhao et al., 2008a; Zhao et al., 2008b). However, it is also important for animal models to recapitulate the negative cognitive biases that are associated with depression, as these pathological alterations in cognition are believed to contribute to the development and maintenance of the disorder (Elliott et al., 2011; Harmer et al., 2009a; Robinson & Sahakian, 2008). For example, individuals with depression exhibit a bias towards the recall of negative autobiographical events and negatively-valenced information (Balut et al., 2013; Gerritsen et al., 2012; Gupta & Kar, 2012; Harmer et al., 2009a; Hsu et al., 2010; Liu et al., 2012; Mogg et al., 2014; Taylor & John, 2004). Thus far, attempts to model depression-associated negative cognitive biases in CORT-treated animals have mostly remained limited to measuring changes in contextual and auditory fear conditioning recall (Conrad et al., 2004; Marks et al., 2015; Monsey et al., 2014; Skórzewska et al., 2006; Thompson et al., 2004). By demonstrating a persistent failure of extinction recall in CORT-treated animals, the results of Chapter 3 help further establish the ability for the chronic CORT animal model of depression to produce depression-like biases in memory, bolstering the model's face validity. Furthermore, this study showed that the effects of CORT exposure on extinction recall last for at least 5 days following the cessation of CORT injections. Negative cognitive memory biases are also persistent in humans with depression, and can even endure beyond remission off the disorder (Gupta & Kar, 2012).

The usefulness of an animal model also depends upon its predictive validity. Drugs that are effective at treating depression in humans should likewise be effective in reversing the symptoms of pathology produced in the animal model, and vice versa (Belzung & Lemoine, 2011). Although the chronic CORT model of depression has demonstrated excellent predictive validity in measures of learned helplessness (Ago et al., 2013; Chen et al., 2014; Crupi et al., 2010; Fenton et al., 2015; Gourley et al., 2008; Huang et al., 2011; Koike et al., 2013; O'Donovan et al., 2014; Mao et al., 2012; Mao et al., 2014; Ulloa et al., 2010; Wu et al., 2013), investigation into the effect of antidepressants on negative cognitive biases in CORT-exposed animals has previously been limited to fluoxetine (Anderson et al., 2013). The predictive validity of the CORT model is supported by the observation in Chapter 3 that acute treatment with ketamine, a powerful antidepressant in humans (Aan Het Rot et al., 2012; Berman et al., 2000; Cusin et al., 2012; Diazgranados et al., 2010; Ibrahim et al., 2011; Kranaster et al., 2011; Murrough et al., 2013; Okamoto et al., 2010; Phelps et al., 2009; Price et al., 2009; Rasmussen et al., 2013; Wang et al., 2012; Zarate et al., 2006), is also capable of preventing the development of depression-like behavior in an auditory fear extinction paradigm. Furthermore, ketamine in this study demonstrated a similar time course of therapeutic effect to that observed in humans (Diazgranados et al., 2010; Murrough et al., 2013; Zarate et al., 2006), producing rapid antidepressant-like effects within an hour following administration, which then lasted for several days following acute treatment. These findings suggest that repeated CORT injection is a viable model for studying the antidepressant-like effects of ketamine on glucocorticoid-induced negative cognitive biases in memory.

The results of Chapter 3 also help illustrate the importance of examining extinction retention and inducing chronic stress states when investigating antidepressant efficacy in attenuating pathological memory biases. Previous studies corroborate my observation that chronic CORT exposure does not always facilitate auditory fear recall (Conrad et al., 2004; Marks et al., 2015), so analyzing long-term extinction recall in addition to fear conditioning can reveal CORT-induced changes in memory that would otherwise go unnoticed. Extinction retention following sub-conditioning retraining is a particularly valuable measure in the evaluation of antidepressant-like effects on memory, as previous research has shown that fluoxetine's facilitation of auditory fear extinction is not apparent until extinction is challenged through mild tone-shock pairings (Deschaux et al., 2013). Thus, by not examining long-term

extinction retention, a study might erroneously conclude that a drug of interest is ineffective in alleviating negative emotional memory bias. Studies on the effect of antidepressants on fear conditioning and extinction should also strive to utilize animal models of depression, as this can yield additional information compared to what could be achieved by examining healthy animals alone. In Chapter 3, although both repeated vehicle- and CORT-injected animals demonstrated a significant reduction of initial conditioned fear expression as a result of ketamine treatment, only CORT animals experienced a ketamine-induced facilitation of long-term extinction recall. Had this study been conducted using only nonstressed animals, the results would not have revealed ketamine's enduring antidepressant-like facilitation of extinction retention. For these reasons, future preclinical studies on depression-like memory biases should include extinction retention measures and use chronically stressed animals in addition to nonstressed animals.

5. Antidepressant-Like Effects of Ketamine

Subanesthetic ketamine has demonstrated efficacy in ameliorating several depression-like behavioral deficits induced by chronic stress in rodents, including immobility in the forced swim test (Autry et al., 2011; Gigliucci et al., 2013; Jett et al., 2015; Koike et al., 2013; Perrine et al., 2014), anhedonia in the sucrose preference test (Autry et al., 2011; Garcia et al., 2009; Li et al., 2011), impaired cognitive flexibility in the attentional set-shifting task (Jett et al., 2015), and passivity in the shock-probe defensive burying task (Jett et al., 2015). Although several previous studies have revealed antidepressant-like effects of ketamine on fear conditioning (Amann et al., 2009; Calzavara et al., 2009; Pietersen et al., 2006; Pietersen et al., 2007; Zhang et al., 2008), none of these investigations evaluated the ability for ketamine to prevent depression-like alterations in memory during extinction, nor memory deficits caused by chronic stress. By addressing these gaps in the existing literature, the studies presented in Chapter 2 and Chapter 3 advance our current understanding of ketamine's antidepressant-like influence on negative emotional memory. These experiments not only confirmed that a single pre-conditioning subanesthetic dose of ketamine can impair next-day fear recall, but also demonstrated that ketamine can prevent long-term extinction retrieval failure (both in spontaneous fear recovery and sub-conditioning fear reinstatement) provoked by chronic CORT exposure. These antidepressant-like behavioral effects were observed within only one hour of ketamine administration and were maintained for at least 5 days, mirroring the rapid and long-lasting

therapeutic response that ketamine has previously demonstrated in several other models of depression-like behavior (Autry et al., 2011; Carrier & Kabbaj, 2013; Gigliucci et al., 2013; Maeng et al., 2008; Nosyreva et al., 2013).

Compared to typical antidepressants, ketamine in the present studies also demonstrated some clear advantages in its effects on negative emotional memory. For example, acute treatment with SSRIs such as paroxetine and tricyclic antidepressants such as tianeptine can fail to reduce conditioned fear recall in rodents, requiring weeks of continuous treatment in order to have a therapeutic effect (Burghardt et al., 2004; Takahashi et al., 2006). Furthermore, chronic treatment with the antidepressants citalopram or tianeptine can surprisingly impair the acquisition of fear extinction (Burghardt et al., 2013), while acute treatment with fluoxetine or tianeptine impairs both extinction acquisition and recall in rats (Godsil et al., 2015; Lebrón-Milad et al., 2013). These findings raise concerns that treatment with these drugs might be counter-productive to challenging negative emotional memory biases, particularly during the early phases of antidepressant treatment. Conversely, ketamine induced a rapid and long-lasting facilitation of extinction behavior in the present investigations, demonstrating none of the limitations observed with typical antidepressant drugs.

Together, the findings from Chapter 2 and Chapter 3 help establish ketamine's antidepressant-like modulation of fear conditioning and ability to prevent stress-induced biases towards negatively-valenced memory. If these therapeutic cognitive effects are preserved across species, ketamine could have additional value in the treatment of depression in humans, as the memory of depressed individuals is biased towards the recollection of and fixation upon negative events (Gupta & Kar, 2012; Nolen-Hoeksema, 2000). By shifting recollection away from emotionally aversive events, ketamine could possess utility in overcoming depression-associated memory biases. This would be an important therapeutic asset, as the degree to which memory is biased towards negative information is correlated with depression symptom severity (Liu et al., 2012), and negative cognitive biases contribute to the maintenance of depression (Elliott et al., 2011; Harmer et al., 2009a; Robinson & Sahakian, 2008). Furthermore, various forms of negative cognitive biases do not operate in isolation, but rather co-exist and influence the expression of one another (Balut et al., 2013; Gupta & Kar, 2012; Mogg et al., 2006). It is possible that a pathological fixation on negative memories (as seen in depression-associated rumination; Nolen-Hoeksema, 2000) could also contribute to the unrealistically pessimistic

outlook depressed individuals have of their future (Strunk & Adler, 2009; Strunk et al., 2006). If memory is constantly biased towards focus on negative emotional experiences, then one might have little reason to expect positive outcomes in the future, and instead develop a sense of hopelessness. Suicidality is a serious concern in depression, so it is important to address the contributing symptoms of depression as quickly as possible through treatment. Clinical trials have shown that ketamine rapidly reduces suicidal ideation in patients in addition to all other depressive symptoms (Berman et al., 2000; Diazgranados et al., 2010; Price et al., 2009; Zarate et al., 2006). Thus, the remarkable rapidity of ketamine's antidepressant effects would make it particularly useful during the early stages of therapeutic intervention, when typical antidepressants would be ineffective at reducing symptoms (Judd et al., 2002; Thase et al., 2005; Trivedi et al., 2006).

6. Limitations

6.1. Pathogenic Validity of Repeated Exogenous CORT Administration in Modeling Depression

Any attempt to model a human psychiatric disorder in animals will be met with an inherent compromise between validity, consistency, and practicality. As previously discussed, there are a number of unique experimental paradigms that researchers use to induce depression-like behavior in rodents, and each model possesses its own set of strengths and weaknesses. Although repeated restraint stress is procedurally simple, progressive habituation to this single stressor can cause excessive variability in results or fail to produce depression-like behaviors altogether (Dunn & Swiergiel, 2008; Gregus et al., 2005; Lussier et al., 2009; Perrot-Sinal et al., 2004; Platt & Stone, 1982). By exposing animals to a variety of mild stressors over a prolonged period of time, the chronic unpredictable stress model offers excellent pathogenic validity, in that the processes leading to disease in animals are semantically similar to those believed to lead to depression in humans. However, the chronic unpredictable stress model is difficult to implement, and results are still liable to be affected by individual variation in stress susceptibility and HPA axis response. Repeated exogenous CORT injection avoids the problems of stressor habituation and stress response variability by administering glucocorticoids directly and circumventing behavioral stress induction entirely. Unfortunately, these advantages come at the cost of reduced pathogenic validity, since chronic high levels of circulating corticosterone in this model are artificially induced. In addition, a 40 mg/kg dose of CORT exceeds the level of glucocorticoid

release that is triggered by natural stressors. Although a lower 5 mg/kg dose would more accurately reproduce physiological CORT concentrations (Sandi, Venero, & Guaza, 1996), past research has found that this dose is insufficient to reliably produce depression-like behavior in the forced swim test (Johnson et al., 2006), defeating one of the primary advantages of this model. Despite these limitations, exogenous CORT exposure produces similar depression-like deficits in neurobiology and neuronal morphology those observed in humans (Bremner et al., 2000; Campbell et al., 2004; Cole et al., 2010; Colla et al., 2007; Dwivedi et al., 2006; Föcking et al., 2003; Frodl et al., 2002; Gourley et al., 2008a; Jacobsen & Mørk, 2006; Lange & Irle, 2004; Liu & Aghajanian, 2008; Magariños et al., 1998; Magariños et al., 1999; Mitra & Sapolsky, 2008; Monsey et al., 2014; Morales-Medina et al., 2009; Nitta et al., 1997; Seib & Wellman, 2003; Sheline et al., 1999; Sheline et al., 2003; Smith et al., 1995; Sousa et al., 2000; Stein-Behrens et al., 1992; Stein-Behrens et al., 1994; Tata et al., 2006; Venero & Borrell, 1999; Videbech & Ravnkilde, 2004; Virgin et al., 1991; Watanabe et al., 1992a; Wellman, 2001; Woolley et al., 1990), lending the model sound mechanistic validity. Furthermore, prolonged CORT administration produces several depression-like behaviors similar to those induced by behavioral stress models (Brummelte et al., 2006; David et al., 2009; Fenton et al., 2015; Gorzalka et al., 1999; Gorzalka et al., 2001; Gorzalka et al., 2003; Gorzalka & Hanson, 1998; Gourley et al., 2008a; Gourley et al., 2008b; Gregus et al., 2005; Gupta et al., 2015; Hill et al., 2003; Johnson et al., 2006; Kalynchuk et al., 2004; Koike et al., 2013; Marks et al., 2009; Murray et al., 2008; Willner, 1997; Wu et al., 2013; Yau et al., 2014; Zhao et al., 2008a; Zhao et al., 2008b). Thus, while repeated CORT administration models might not be suitable for studying the naturalistic development of depression-like symptoms, there is ample evidence to support the utility of this model in preclinical studies so long as its limitations are kept in mind.

6.2. Sex Differences in Depression-Like Behavior and Response to Ketamine

The animals used within the present studies were exclusively male, which has important implications for the generalizability of the experiments' results. First, female rats exhibit higher basal and stress-induced circulating glucocorticoid levels compared to male rats, and glucocorticoid concentrations and stress responses are dependent upon a female's present stage within the estrous cycle (Atkinson & Waddell, 1997; Carey, Deterd, de Koning, Helmerhorst, & de Kloet, 1995; Critchlow, Liebelt, Bar-Sela, Mountcastle, & Lipscomb, 1963; Dalla et al., 2005;

Figueiredo, Dolgas, & Herman, 2002; Handa, Burgess, Kerr, & O'Keefe, 1994; Kitay, 1961; Pollard, White, Bassett, & Cairncross, 1975; Seale et al., 2004a; Seale, Wood, Atkinson, Harbuz, & Lightman, 2004b; Viau & Meaney, 1991; Weinstock, Razin, Schorer-Apelbaum, Men, & McCarty, 1998). There are also sex differences in behavioral responses to exogenous CORT exposure, as female rats exhibit less depression-like behavior in the forced swim test than males (Hill et al., 2003; Kalynchuk et al., 2004).

Particularly relevant to fear extinction, IL dendritic morphology is enhanced in stressed female rats, while male rats experience dendritic retraction (Garrett & Wellman, 2009; Shansky et al., 2010). Furthermore, some of these IL neurons that undergo dendritic proliferation in response to stress project to the BLA, which could have significant implications for the suppression of conditioned fear (Shansky et al., 2010). Accordingly, one study has found that chronically stressed female rats exhibit enhanced extinction acquisition and recall compared to unstressed females, while chronic stress impairs extinction recall in male rats (Baran et al., 2009). Interestingly, this same study revealed that nonstressed females display deficits in extinction acquisition and recall compared to nonstressed male rats (Baran et al., 2009). However, the behavior of female rats during fear extinction is greatly affected by relatively small changes in experimental protocol (Baran, Armstrong, Niren, & Conrad, 2010), and changes drastically with estrous cycle phase (Milad, Igoe, Lebron-Milad, & Novales, 2009a), causing conflicting results between studies.

Finally, female rodents appear to be more sensitive to the antidepressant-like effects of ketamine than male rats (Carrier & Kabbaj, 2013; Franceschelli, Sens, Herchick, Thelen, & Pitychoutis, 2015; Zanos et al., 2016). While male rats require at least a 5 mg/kg dose of ketamine to reduce swimming immobility in the forced swim test, this same effect can be achieved in females with only a 2.5 mg/kg dose (Carrier & Kabbaj, 2013). This enhanced sensitivity to ketamine is mediated by female sex hormones, as ovariectomy abolishes sex differences in forced swim test behavior (Carrier & Kabbaj, 2013). Interestingly, although chronically stressed female mice are more sensitive to the antidepressant-like effects of ketamine within the first 24 hours after administration, ketamine has longer-lasting effects in stressed male mice (Franceschelli et al., 2015). These sex-dependent behavioral effects might be explained by sex differences in (2*S*,6*S*;2*R*,6*R*)-hydroxynorketamine (HNK) metabolite levels (Franceschelli et

al., 2015). It is uncertain if ketamine also differentially affects fear conditioning in female rats, so future investigation into possible sex differences is warranted.

7. Future Directions

7.1. Retroactive Influence of Chronic CORT on Fear and Extinction Memory

Thus far, the vast majority of research on the effects of stress on negative emotional memory has been conducted by inducing chronic stress states prior to the acquisition and recall of conditioned fear and extinction (Baran et al., 2009; Bisaz & Sandi, 2010; Conrad et al., 1999; Conrad et al., 2004; Cordero et al., 2003a; Farrell et al., 2010; Gourley et al., 2009; Hoffman et al., 2014; Marks et al., 2015; Miracle et al., 2006; Monsey et al., 2014; Sandi et al., 2001; Skórzewska et al., 2006; Thompson et al., 2004; Wilber et al., 2011; Zhang & Rozencranz, 2013). However, it is plausible that chronic stress might also be able influence the expression of fear-relevant memories that have previously been successfully consolidated in nonstressed states. For example, stress-induced amygdalar hypertrophy could potentially facilitate conditioned fear recall, while hippocampal and prefrontal atrophy could impair extinction recall (Rodrigues et al., 2009). This matter is of potential clinical relevance, as it means that individuals with depression might be susceptible to pathological fixation upon all past negative experiences, not just ones acquired during a depressive episode. If this is true, it would likely contribute significantly to the burden of negative cognitive biases on the maintenance of depression. By training rats in a contextual fear conditioning paradigm, and then administering 40 mg/kg CORT daily over 21 days, fear recall could be assessed on the final day of CORT injections to determine the effect that prolonged glucocorticoid exposure has on the recall of negative emotional memory. In an additional experiment, pre-CORT fear extinction could be added to this same protocol in order to elucidate the influence chronic CORT exposure on the suppression of previously extinguished fear memories.

7.2. Antidepressant-Like Effects of Ketamine Metabolites

Although ketamine's rapid and long-lasting antidepressant characteristics are without a doubt impressive, concerns have been raised regarding the drug's dissociative effects and potential for addiction and abuse (Morgan & Curran, 2012). These limitations present a significant barrier to the widespread clinical use of ketamine as an antidepressant, but derivative

pharmacotherapies might be able to offer more practical alternatives. For example, one group has recently found that the metabolism of ketamine to (2*R*,6*R*)-HNK plays an important role in the induction of antidepressant-like effects in the forced swim test and learned helplessness test (Zanos et al., 2016). Importantly, direct administration of (2*R*,6*R*)-HNK to mice produced rapid and enduring antidepressant-like effects in these same measures, and reversed CORT-induced anhedonia in the sucrose preference test (Zanos et al., 2016). Similar to ketamine, (2*R*,6*R*)-HNK appears to exert its antidepressant effects by enhancing AMPA-mediated neurotransmission, and it also induces an upregulation of hippocampal BDNF (Zanos et al., 2016). The advantage of using (2*R*,6*R*)-HNK over ketamine is that by circumventing NMDA receptor antagonism, this metabolite has fewer side effects and appears to possess no abuse potential. While mice will engage in progressive self-administration of ketamine, this effect does not occur with (2*R*,6*R*)-HNK (Zanos et al., 2016). However, the effect that (2*R*,6*R*)-HNK has on fear conditioning, extinction, and stress-induced negative memory biases is uncertain. It is possible that ketamine's antidepressant-like effects in fear conditioning and extinction measures are the result of downstream signaling cascades initiated by NMDA receptor antagonism, which might not be replicated with (2*R*,6*R*)-HNK administration. This uncertainty warrants further investigation into the antidepressant characteristics of this intriguing metabolite.

8. Conclusions

Individuals with depression exhibit a pathological bias towards focus on negatively-valenced memories, which contributes significantly to the development, severity, and chronicity of the disorder (Nolen-Hoeksema, 2000). As a result, antidepressants that can relieve patients of these negative cognitive biases are of great potential clinical value. The aim of this dissertation was to explore ketamine's ability to modulate the expression of negative emotional memory and prevent stress-induced deficits in the regulation of emotionally-driven behavior in rats. The first experiment sought to dissociate the effects of acute subanesthetic ketamine on distinct stages of auditory fear conditioning, and provided evidence that ketamine impairs the consolidation and recall, but not acquisition, of conditioned fear. Ketamine had no effect on the extinction of nonstressed rats in this study, so a second experiment was conducted to examine the effect of pre-extinction ketamine on conditioned fear and extinction behavior in a repeated CORT animal model of depression. The results of this study revealed that chronic CORT exposure impairs the

long-term recall and retention of extinction memory, and that ketamine rapidly abolishes these CORT-induced cognitive and behavioral deficits. Collectively, the findings of this dissertation help establish ketamine as a powerful modulator of negatively-valenced memory and emotionally-driven behavior, and contribute to our understanding of its antidepressant-like characteristics. Having demonstrated ketamine's ability to ameliorate negative memory biases in rodents, future research should determine if these therapeutic effects are preserved in human patients with depression.

REFERENCES

- Aan Het Rot, M., Zarate, C. A., Charney, D. S., & Matthew, S. J. (2012). Ketamine for depression: Where do we go from here? *Biological Psychiatry*, *72*(7), 537-547.
- Abel, T. & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Current Opinion in Neurobiology*, *11*, 180-187.
- Abiri, D., Douglas, C. E., Calkos, K. C., Barbayannis, G., Roberts, A., & Bauer, E. P. (2014). Fear extinction learning can be impaired or enhanced by modulation of the CRF system in the basolateral nucleus of the amygdala. *Behavioural Brain Research*, *271*, 234-239.
- Aboul-Fotouh, S. (2015). Behavioral effects of nicotinic antagonist mecamylamine in a rat model of depression: prefrontal cortex level of BDNF protein and monoaminergic neurotransmitters. *Psychopharmacology (Berlin)*, *232*(6), 1095-1105.
- Adachi, M., Barrot, M., Autry, A. E., Theobald, D., & Monteggia, L. M. (2008). Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. *Biological Psychiatry*, *63*(7), 642-649.
- Ago, Y., Yano, K., Araki, R., Hiramatsu, N., Kita, Y., Kawasaki, T., Onoe, H., Chaki, S., Nakazato, A., Hashimoto, H., Baba, A., Takuma, K., & Matsuda, T. (2013). Metabotropic glutamate 2/3 receptor antagonists improve behavioral and prefrontal dopaminergic alterations in the chronic corticosterone-induced depression model in mice. *Neuropharmacology*, *65*, 29-38.
- Almatura, C., Maes, M., Dai, J., & Meltzer, H. Y. (1995). Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. *European Neuropsychopharmacology*, *5*, 71-75.

- Almeida, R. F., Thomazi, A. P., Godinho, G. F., Saute, J. A., Wofchuk, S. T., Souza, D. O., & Ganzella, M. (2010). Effects of depressive-like behavior of rats on brain glutamate uptake. *Neurochemistry Research*, 35(8), 1164-1171.
- Altamura, C. A., Mauri, M. C., Ferrara, A., Moro, A. R., D'Andrea, G., & Zamberian, F. (1993). Plasma and platelet excitatory amino acids in psychiatric disorders. *The American Journal of Psychiatry*, 150(11), 1731-1733.
- Altamura, C., Maes, M., Dai, J., & Meltzer, H. Y. (1995). Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. *European Neuropsychopharmacology*, 5, 71-75.
- Amann, L. C., Halene, T. B., Ehrlichman, R. S., Luminais, S. N., Ma, N., Abel, T., & Siegel, S. J. (2009). Chronic ketamine impairs fear conditioning and produces long-lasting reductions in auditory evoked potentials. *Neurobiology of Disease*, 35, 311-317.
- American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC.
- Amsterdam, J. D., Maislin, G., Abelman, E., Berwisch, N., & Winokur, A. (1986). Adrenocortical responsiveness to ACTH stimulation test in depressed patients and healthy volunteers. *Journal of Affective Disorders*, 11(3), 265-274.
- Amsterdam, J. D., Maislin, G., Droba, M., & Winokur, A. (1987). The ACTH stimulation test before and after clinical recovery from depression. *Psychiatry Research*, 20(4), 325-336.
- An, Y., Inoue, T., Kitaichi, Y., Izumi, T., Nakagawa, S., Song, N., Chen, C., Li, X., Koyama, T., & Kusumi, I. (2013). Anxiolytic-like effect of mirtazapine mediates its effect in the median raphe nucleus. *European Journal of Pharmacology*, 720(1-3), 192-197.

- An, Y., Inoue, T., Kitaichi, Y., Nakagawa, S., Wang, C., Chen, C., Song, N., & Kusumi, I. (2015). Subchronic lithium treatment increases the anxiolytic-like effect of mirtazapine on the expression of contextual conditioned fear. *European Journal of Pharmacology*, 747, 13-17.
- Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *The Journal of Neuroscience*, 19(13), 1106-1114.
- Anderson, M. H., Munafò, M. R., & Robinson, E. S. (2013). Investigating the psychopharmacology of cognitive affective bias in rats using an affective tone discrimination task. *Psychopharmacology (Berlin)*, 226(3), 601-613.
- Ankarcrona, M., Dypbukt, J. M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. A., & Nicotera, P. (1995). Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. *Neuron*, 15, 961-973.
- Atkinson, H. C., & Waddell, B. J. (1997). Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. *Endocrinology*, 138(9), 3842-3848.
- Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P., Kavalali, E. T., & Monteggia, L. M. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, 475, 91-97.
- Aydemir, O., Deveci, A., & Taneli, F. (2005). The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminary study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 29(2), 261-265.

- Ayensu, W. K., Pucilowski, O., Mason, G. A., Overstreet, D. H., Rezvani, A. H., & Janowsky, D. S. (1995). Effects of chronic mild stress on serum complement activity, saccharin preference, and corticosterone levels in Flinders lines of rats. *Physiology & Behavior*, *57*(1), 165-169.
- Bachis, A., Cruz, M. I., Nosheny, R. L., & Mocchetti, I. (2008). Chronic unpredictable stress promotes neuronal apoptosis in the cerebral cortex. *Neuroscience Letters*, *442*(2), 104-108.
- Bagley, J., & Moghaddam, B. (1997). Temporal dynamics of glutamate efflux in the prefrontal cortex and in the hippocampus following repeated stress: Effects of pretreatment with saline or diazepam. *Neuroscience*, *77*(1), 65-73.
- Baker-Andresen, D., Flavell, C. R., Li, X., & Bredy, T. W. (2013). Activation of BDNF signaling prevents the return of fear in female mice. *Learning & Memory*, *20*(5), 237-240.
- Balut, A., Paulewicz, B., Szastok, M., Prochwicz, K., & Koster, E. (2013). Are attentional bias and memory bias for negative words causally related? *Journal of Behavioral Therapy & Experimental Psychiatry*, *44*(3), 293-299.
- Banasr, M., & Duman, R. S. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biological Psychiatry*, *64*(10), 863-870.
- Banasr, M., Chowdhury, G. M., Terwilliger, R., Newton, S. S., Duman, R. S., Behar, K. L., & Sanacora, G. (2010). Glial pathology in an animal model of depression: Reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Molecular Psychiatry*, *15*(5), 501-511.

- Banasr, M., Valentine, G. W., Li, X. Y., Gourley, S. L., Taylor, J. R., & Duman, R. S. (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biological Psychiatry*, *62*(5), 496-504.
- Baran, S. E., Armstrong, C. E., Niren, D. C., & Conrad, C. D. (2010). Prefrontal cortex lesions and sex differences in fear extinction and perseveration. *Learning & Memory*, *17*(5), 267-278.
- Baran, S. E., Armstrong, C. E., Niren, D. C., Hanna, J. J., & Conrad, C. D. (2009). Chronic stress and sex differences on the recall of fear conditioning and extinction. *Neurobiology of Learning and Memory*, *91*, 323-332.
- Bauer, E. P., Schafe, G. E., & LeDoux, J. E. (2002). NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *The Journal of Neuroscience*, *22*(12), 5239-5249.
- Bear, M. F., & Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Current Opinions in Neurobiology*, *4*(3), 389-399.
- Beck, K. D., & Luine, V. N. (2002). Sex differences in behavioral and neurochemical profiles after chronic stress: role of housing conditions. *Physiology & Behavior*, *5*(5), 661-673.
- Belzung, C., & Lemoine, M. (2011). Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biology of Mood & Anxiety Disorders*, *1*(9), 1-14.
- Benoit, E., Carratù, M. R., Dubois, J. M., & Mitolo-Chieppa, D. (1986). Mechanism of action of ketamine in the current and voltage clamped myelinated nerve fiber of the frog. *British Journal of Pharmacology*, *87*(2), 291-297.

- Beretta, S., Pantazopoulos, H., Caldera, M., Pantazopoulos, P., & Paré, D. (2005). Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience*, *132*(4), 943-953.
- Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Henniger, G. R., Charney, D. S., & Krystal, J. H. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, *47*(4), 351-354.
- Bielajew, C., Konkle, A. T., & Merali, Z. (2002). The effects of chronic mild stress on male Sprague-Dawley and Long Evans rats: I. Biochemical and physiological analyses. *Behavioural Brain Research*, *136*(2), 583-592.
- Bisaz, R., & Sandi, C. (2010). The role of NCAM in auditory fear conditioning and its modulation by stress: a focus on the amygdala. *Genes, Brain, and Behavior*, *9*(4), 353-364.
- Black, I. B. (1999). Trophic regulation of synaptic plasticity. *Journal of Neurobiology*, *41*(1), 108-118.
- Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology*, *81*(2), 281-290.
- Blanchard, R. J., & Blanchard, D. C. (1969). Crouching as an index of fear. *Journal of Comparative and Physiological Psychology*, *67*(3).
- Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*(6407), 31-39.

- Bonanno, G., Giambelli, R., Raiteri, L., Tiraboschi, E., Zappettini, S., Musazzi, L., Raiteri, M., Racagni, G., & Popoli, M. (2005). Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. *The Journal of Neuroscience*, *25*(13), 3270-3279.
- Borsini, F., & Meli, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berlin)*, *94*(2), 147-160.
- Bouhuys, A. L., Geerts, E., & Gordijn, M. C. (1999). Depressed patients' perceptions of facial emotions in depressed and remitted states are associated with relapse: a longitudinal study. *The Journal of Nervous and Mental Disease*, *187*(10), 595-602.
- Bourdeau, I., Bard, C., Noël, B., Leclerc, I., Cordeau, M. P., Béllair, M., Lesage, J., Lafontaine, L., & Lacroix, A. (2002). Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. *The Journal of Clinical Endocrinology & Metabolism*, *87*(5), 1949-1954.
- Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning and Memory*, *11*(5), 485-494.
- Bouton, M. E., & Bolles, R. C. (1979a). Contextual control of the extinction of conditioned fear. *Learning and Motivation*, *10*, 455-466.
- Bouton, M. E., & Bolles, R. C. (1979b). Role of conditioned contextual stimuli in reinstatement of extinguished fear. *Journal of Experimental Psychology: Animal Behavior Processes*, *5*(4), 368-378.
- Bouton, M. E., & King, D. A. (1983). Contextual control of the extinction of conditioned fear: Tests for the associative value of the context. *Journal of Experimental Psychology: Animal Behavior Processes*, *9*(3), 248-265.

- Bouton, M. E., Westbrook, R. F., Corcoran, K. A., & Maren, S. (2006). Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biological Psychiatry*, *60*(4), 352-360.
- Bredy, T. W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y. E., & Barad, M. (2007). Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. *Learning & Memory*, *14*, 268-276.
- Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000). Hippocampal volume reduction in major depression. *American Journal of Psychiatry*, *157*(1), 115-117.
- Broomfield, N. M., Davies, R., MacMahon, K., Ali, F., & Cross, S. M. (2007). Further evidence of attention bias for negative information in late life depression. *International Journal of Geriatric Psychiatry*, *22*(3), 175-180.
- Browning, M., Reid, C., Cowen, P. J., Goodwin, G. M., & Harmer, C. J. (2007). A single dose of citalopram increases fear recognition in healthy subjects. *Journal of Psychopharmacology*, *21*(7), 684-690.
- Brummelte, S., Pawluski, J. L., & Galea, L. A. (2006). High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: A model of post-partum stress and possible depression. *Hormones and Behavior*, *50*(3), 370-382.
- Brunoni, A. R., Lopes, M., & Fregni, F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *The International Journal of Neuropsychopharmacology*, *11*(8), 1169-1180.

- Bui, E., Orr, S. P., Jacoby, R. J., Keshaviah, A., LeBlanc, N. J., Milad, M. R., Pollack, M. H., & Simon, N. M. (2013). Two weeks of pretreatment with escitalopram facilitates extinction learning in healthy individuals. *Human Psychopharmacology Clinical and Experimental*, 28, 447-456.
- Burghardt, N. S., Sigurdsson, T., Gorman, J. M., McEwen, B. S., & LeDoux, J. E. (2013). Chronic antidepressant treatment impairs the acquisition of fear extinction. *Biological Psychiatry*, 73, 1078-1086.
- Burghardt, N. S., Sullivan, G. M., McEwen, B. S., Gorman, J. M., & LeDoux, J. E. (2004). The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: a comparison with tianeptine. *Biological Psychiatry*, 55(12), 1171-1178.
- Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E., & Quirk, G. J. (2007). Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron*, 53(6), 871-880.
- Burhans, L. B., Smith-Bell, C. A., & Schreurs, B. G. (2013). Subacute fluoxetine enhances conditioned responding and conditioning-specific reflex modification of the rabbit nictitating membrane response: implications for drug treatment with selective serotonin reuptake inhibitors. *Behavioural Pharmacology*, 24(1), 55-64.
- Calzavara, M. B., Medrano, W. A., Levin, R., Kameda, S. R., Andersen, M. L., Tufik, S., Silvia, R. H., Frussa-Fihlo, R., & Abilio, V. C. (2009). Neuroleptic drugs revert the contextual fear conditioning deficit presented by spontaneously hypertensive rats: a potential animal model of emotional context processing in schizophrenia? *Schizophrenia Bulletin*, 35(4), 748-759.

- Cammarota, M., Barros, D. M., Vianna, M. R. M., Bevilaqua, L. R. M., Coithinho, A., Szapiro, G., Izquierdo, L. A., Medina, J. H., & Izquierdo, I. (2004). The transition from memory retrieval to extinction. *Anais da Academia Brasileira de Ciências*, 76(3).
- Camp, M. C., Macpherson, K. P., Lederle, L., Graybeal, C., Gaburro, S., Debrouse, L. M., Ihne, J. L., Bravo, J. A., O'Connor, R. M., Ciocchi, S., Wellman, C. L., Lüthi, A., Cryan, J. F., Singewald, N., & Holmes, A. (2012). Genetic strain differences in learned fear inhibition associated with variation in neuroendocrine, autonomic, and amygdala dendritic phenotypes. *Neuropsychopharmacology*, 37(6), 1534-1547.
- Campbell, S., Marriott, M., Nahmias, C., & Macqueen, G. M. (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. *American Journal of Psychiatry*, 161(4), 598-607.
- Campeau, S., & Davis, M. (1995). Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *The Journal of Neuroscience*, 15, 2312-2327.
- Campeau, S., Miserendino, M. J., & Davis, M. (1992). Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behavioral Neuroscience*, 106(3), 569-574.
- Carey, M. P., Deterd, C. H., de Koning, J., Helmerhorst, F., & de Kloet, E. R. (1995). The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *The Journal of Endocrinology*, 144(2), 311-321.
- Carrier, N., & Kabbaj, M. (2013). Sex differences in the antidepressant-like effects of ketamine. *Neuropharmacology*, 70, 27-34.

- Carroll, B. J., Curtis, G. C., & Mendels, J. (1976). Neuroendocrine regulation in depression. II. Discrimination of depressed from nondepressed patients. *Archives of General Psychiatry*, 33(9), 1051-1058.
- Caseras, X., Garner, M., Bradley, B. P., & Mogg, K. (2007). Biases in visual orienting to negative and positive scenes in dysphoria: An eye movement study. *Journal of Abnormal Psychology*, 116(3), 491-497.
- Cassaday, H. J., & Thur, K. E. (2015). Intraperitoneal sertraline and fluvoxamine increase contextual fear conditioning but are without effect on overshadowing between cues. *Pharmacology, Biochemistry, and Behavior*, 129, 111-115.
- Cerqueira, J. J., Mailliet, F., Almeida, O. F., Jay, T. M., & Sousa, N. (2007). The prefrontal cortex as a key target of the maladaptive responses to stress. *The Journal of Neuroscience*, 27(11), 2781-2787.
- Chaturvedi, H. K., Chandra, D., & Bapna, J. S. (1999). Interaction between N-methyl-D-aspartate receptor antagonists and imipramine in shock-induced depression. *Indian Journal of Experimental Biology*, 37(10), 952-958.
- Chen, A. C., Shirayama, Y., Shin, K. H., Neve, R. L., & Duman, R. S. (2001a). Expression of the cAMP response element binding protein (CREB) in hippocampus produces an antidepressant effect. *Biological Psychiatry*, 49(9), 753-762.
- Chen, B., Dowlathahi, D., MacQueen, G. M., Wang, J. F., & Young, L. T. (2001b). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biological Psychiatry*, 50(4), 260-265.

- Chen, J., Li, W., Zhao, X., & Yang, J. X. (2008). Effects of the Chinese traditional prescription Xiaoyaosan decoction on chronic immobilization stress-induced changes in behavior and brain BDNF, TrkB, and NT-3 in rats. *Cellular and Molecular Neurobiology*, 28(5), 745-755.
- Chen, J., Ma, W., Zhang, Y., Wu, X., Wei, D., Liu, G., Deng, Z., Yang, L., & Zhang, Z. (2014). Distinct facial processing related negative cognitive bias in first-episode and recurrent major depression: Evidence from the N170 ERP component. *PLoS ONE*, 9(10), e109176.
- Chen, L., Dai, J., Wang, Z., Zhang, H., Huang, Y., & Zhao, Y. (2014). Ginseng total saponins reverse corticosterone-induced changes in depression-like behavior and hippocampal plasticity-related proteins by interfering with GSK-3 β -CREB signaling pathway. *Evidence-Based Complementary and Alternative Medicine*, EPUB.
- Chhatwal, J. P., Stanek-Rattiner, L., Davis, M., & Ressler, K. J. (2006). Amygdala BDNF signaling is required for consolidation but not encoding of extinction. *Nature Neuroscience*, 9(7), 870-872.
- Choudary, P. V., Molnar, M., Evans, S. J., Tomita, H., Li, J. Z., Vawter, M. P., Myers, R. M., Bunney, W. E., Akil, H., Watson, S. J., & Jones, E. G. (2005). Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proceedings of the National Academy of Sciences of the United States of America*, 102(43), 15653-15658.
- Chowdhury, G. M., Behar, K. L., Cho, W., Thomas, M. A., Rothman, D. L., & Sanacora, G. (2012). ^1H - ^{13}C -nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism. *Biological Psychiatry*, 71(11), 1022-1025.
- Clark, L., Chamberlain, S. R., & Sahakian, B. J. (2009). Neurocognitive mechanisms in depression: implications for treatment. *Annual Review of Neuroscience*, 32, 57-74.

- Clem, R. L., & Huganir, R. L. (2010). Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science*, *330*(6007), 1108-1112.
- Clements, J. A., Nimmo, W. S., & Grant, I. S. (1982). Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. *Journal of Pharmaceutical Sciences*, *71*(5), 539-542.
- Cole, J., Toga, A. W., Hojatkashani, C., Thompson, P., Costafreda, S. G., Cleare, A. J., Williams, S. C., Bullmore, E. T., Scott, J. L., Mitterschiffthaler, M. T., Walsh, N. D., Donaldson, C., Mirza, M., Marquand, A., Nosarti, C., McGuffin, P., & Fu, C. H. (2010). Subregional hippocampal deformations in major depressive disorder. *Journal of Affective Disorders*, *126*(1-2), 272-277.
- Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen, T., Bohrer, M., & Heuser, I. (2007). Hippocampal volume reduction and HPA-system activity in major depression. *Journal of Psychiatric Research*, *41*(7), 553-560.
- Conrad, C. D., MacMillan, D. D., Tsekhanov, S., Wright, R. L., Baran, S. E., & Fuchs, R. A. (2004). Influence of chronic corticosterone and glucocorticoid receptor antagonism in the amygdala on fear conditioning. *Neurobiology of Learning and Memory*, *81*, 185-199.
- Conrad, C. D., Magariños, A. M., LeDoux, J. E., & McEwen, B. S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioral Neuroscience*, *113*(5), 902-913.
- Conrad, C. D., Mauldin-Jourdain, M. L., & Hobbs, R. J. (2001). Metyrapone reveals that previous chronic stress differentially impairs hippocampal-dependent memory. *Stress*, *4*(4), 305-318.

- Conti, A. C., Cryan, J. F., Dalvi, A., Lucki, I., & Blendy, J. A. (2002). cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. *The Journal of Neuroscience*, *22*(8), 3262-3268.
- Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *Journal of Neurobiology*, *60*(2), 236-248.
- Coppell, A. L., Pei, Q., & Zetterström, T. S. (2003). Bi-phasic change in BDNF gene expression following antidepressant drug treatment. *Neuropsychopharmacology*, *44*(7), 903-910.
- Corcoran, K. A., & Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *The Journal of Neuroscience*, *21*(5), 1720-1726.
- Corcoran, K. A., & Maren, S. (2004). Factors regulating the effects of hippocampal inactivation on renewal of conditional fear after extinction. *Learning & Learning*, *11*(5), 598-603.
- Corcoran, K. A., Desmond, T. J., Frey, K. A., & Maren, S. (2005). Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. *The Journal of Neuroscience*, *25*(39), 8978-8987.
- Cordero, M. I., Kruyt, N. D., & Sandi, C. (2003a). Modulation of contextual fear conditioning by chronic stress in rats is related to individual differences in behavioral reactivity to novelty. *Brain Research*, *970*(1-2), 242-245.
- Cordero, M. I., Kruyt, N. D., Merino, J. J., & Sandi, C. (2002). Glucocorticoid involvement in memory formation in a rat model for traumatic memory. *Stress*, *5*(1), 73-79.
- Cordero, M. I., Merino, J. J., & Sandi, C. (1998). Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. *Behavioral Neuroscience*, *112*(4), 885-891.

- Cordero, M. I., Venero, C., Kruyt, N. D., & Sandi, C. (2003b). Prior exposure to a single stress session facilitates subsequent contextual fear conditioning in rats. Evidence for a role of corticosterone. *Hormones and Behavior*, *44*(4), 338-345.
- Corodimas, K. P., LeDoux, J. E., Gold, P. W., & Schulkin, J. (1994). Corticosterone potentiation of conditioned fear in rats. *Annals of the New York Academy of Sciences*, *746*, 392-393.
- Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience*, *112*(5), 1092-1103.
- Coussens, C. M., Kerr, D. S., & Abraham, W. C. (1997). Glucocorticoid receptor activation lowers the threshold for NMDA-receptor-dependent homosynaptic long-term depression in the hippocampus through activation of voltage-dependent calcium channels. *Journal of Neurophysiology*, *78*(1), 1-9.
- Crane, M. K., Bogner, H. R., Brown, G. K., & Gallo, J. J. (2007). The link between depressive symptoms, negative cognitive bias and memory complaints in older adults. *Aging & Mental Health*, *11*(6), 708-715.
- Critchlow, V., Liebelt, R. A., Bar-Sela, M., Mountcastle, W., & Lipscomb, H. S. (1963). Sex difference in resting pituitary-adrenal function in the rat. *The American Journal of Physiology*, *205*(5), 807-815.
- Crupi, R., Mazzon, E., Marino, A., La Spada, G., Bramanti, P., Cuzzocrea, S., & Spina, E. (2010). Melatonin treatment mimics the antidepressant action in chronic corticosterone-treated mice. *Journal of Pineal Research*, *49*(2), 123-129.
- Cruz, E., López, A. V., & Porter, J. T. (2014). Spontaneous recovery of fear reverses extinction-induced excitability of infralimbic neurons. *PLoS ONE*, *9*(8).

- Cryan, J. F., & Holmes, A. (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nature Reviews Drug Discovery*, 4(9), 775-790.
- Cusin, C., Hilton, G. Q., Nierenberg, A. A., & Fava, M. (2012). Long-term maintenance with intramuscular ketamine for treatment-resistant bipolar II depression. *The American Journal of Psychiatry*, 169(8), 868-869.
- Czéh, B., & Lucassen, P. J. (2007). What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *European Archives of Psychiatry and Clinical Neuroscience*, 257(5), 250-260.
- Dagnino-Subiabre, A., Ángel Pérez, M., Terreros, G., Cheng, M. Y., House, P., & Sapolsky, R. (2012). Corticosterone treatment impairs auditory fear learning and the dendritic morphology of the rat inferior colliculus. *Hearing Research*, 294, 104-113.
- Dai, Q., & Feng, Z. (2012). More excited for negative facial expressions in depression: evidence from an event-related potential study. *Clinical Neurophysiology*, 123(11), 2172-2179.
- Dalla, C., Antoniou, K., Drossopoulou, G., Xagoraris, M., Kokras, N., Sfikakis, A., & Papadopoulou-Daifoti, Z. (2005). Chronic mild stress impact: Are females more vulnerable? *Neuroscience*, 135(3), 703-714.
- Dalton, G. L., Wang, Y. T., Floresco, S. B., & Phillips, A. G. (2008). Disruption of AMPA receptor endocytosis impairs the extinction, but not acquisition of learned fear. *Neuropsychopharmacology*, 33(10), 2416-2426.
- David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., Drew, M., Craig, D. A., Guiard, B. P., Guilloux, J. P., Artymyshyn, R. P., Gardier, A. M., Gerald, C., Antonijevic, I. A., Leonardo, E. D., & Hen, R. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*, 62(4), 479-493.

- Davidson, R. J., Irwin, W., Anderle, M. J., & Kalin, N. H. (2003). The neural substrates of affective processing in depressed patients treated with venlafaxine. *The American Journal of Psychiatry*, *160*(1), 64-75.
- de Bruin, N. M., Ellenbroek, B. A., Cools, A. R., Coenen, A. M., & van Luijtelaar, E. L. (1999). Differential effects of ketamine on gating of auditory evoked potentials and prepulse inhibition in rats. *Psychopharmacology (Berlin)*, *142*(1), 9-17.
- de Kloet, E. R., Oitzl, M. S., & Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends in Neurosciences*, *22*(10), 422-426.
- de Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*, *19*(3), 269-301.
- de Vasconcellos-Bittencourt, A. P., Vendite, D. A., Nassif, M., Crema, L. M., Frozza, R., Thomazi, A. P., Nieto, F. B., Wofchuk, S., Salbego, C., da Rocha, E. R., & Dalmaz, C. (2011). Chronic stress and lithium treatments alter hippocampal glutamate uptake and release in the rat and potentiate necrotic cellular death after oxygen and glucose deprivation. *Neurochemical Research*, *36*(5), 793-800.
- Dearing, K. F., & Gotlib, I. H. (2009). Interpretation of ambiguous information in girls at risk for depression. *Journal of Abnormal Child Psychology*, *37*(1), 79-91.
- Delgado, P. R., Campo, A., Henningsen, k., Verhoye, M., Poot, D., Dijkstra, J., Van Audekerke, J., Benveniste, H., Sijbers, J., Wiborg, O., & Van der Linden, A. (2011). Magnetic resonance imaging and spectroscopy reveal differential hippocampal changes in anhedonic and resilient subtypes of the chronic mild stress rat model. *Biological Psychiatry*, *70*(5), 449-457.

- Den, M. L., Graham, B. M., Newall, C., & Richardson, R. (2015). Teens that fear screams: A comparison of fear conditioning, extinction, and reinstatement in adolescents and adults. *Developmental Psychobiology*, [EPUB].
- Deschaux, O., Koumar, O. C., Canini, F., Moreau, J. L., & Garcia, R. (2015). High-frequency stimulation of the hippocampus blocks fear learning sensitization and return of extinguished fear. *Neuroscience*, 286, 423-429.
- Deschaux, O., Motanis, H., Spennato, G., Moreau, J., & Garcia, R. (2011a). Re-emergence of extinguished auditory-cued conditioned fear following a sub-conditioning procedure: Effects of hippocampal and prefrontal tetanic stimulations. *Neurobiology of Learning and Memory*, 95, 510-518.
- Deschaux, O., Spennato, G., Moreau, J., & Garcia, R. (2011b). Chronic treatment with fluoxetine prevents the return of extinguished auditory-cued conditioned fear. *Psychopharmacology*, 215, 231-237.
- Deschaux, O., Zheng, X., Lavigne, J., Nachon, O., Cleren, C., Moreau, J., & Garcia, R. (2013). Post-Extinction fluoxetine treatment prevents stress-induced reemergence of extinguished fear. *Psychopharmacology*, 225, 209-216.
- Deuschle, M., Gilles, M., Scharnholtz, B., Lederbogen, F., Lang, U. E., & Hellweg, R. (2013). Changes of serum concentrations of brain-derived neurotrophic factor (BDNF) during treatment with venlafaxine and mirtazapine: role of medication and response to treatment. *Pharmacopsychiatry*, 46(2), 54-58.
- Deutschenbaur, L., Beck, J., Kiyhankhadiv, A., Mühlhauser, M., Borgwardt, S., Walter, M., Hasler, G., Sollberger, D., & Lang, U. E. (2015). Role of calcium, glutamate and NMDA in major depression and therapeutic application. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 64, 325-333.

- Diamond, D. M., Bennett, M. C., Fleshner, M., & Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus*, 2(4), 421-430.
- Dias-Ferreira, E., Sousa, J. C., Melo, I., Morgado, P., Mesquita, A. R., Cerqueira, J. J., Costa, R. M., & Sousa, N. (2009). Chronic stress causes frontostriatal reorganization and affects decision-making. *Science*, 325(5940), 621-625.
- Diazgranados, N., Ibrahim, L., Brutsche, N. E., Newberg, A., Kronstein, P., Khalife, S., Kammerer, W. A., Quezado, Z., Luckenbaugh, D. A., Salvadore, G., Machado-Vieira, R., Manji, H. K., & Zarate, C. A. (2010). A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Archives of General Psychiatry*, 67(8), 793-802.
- Dienes, K. A., Hammen, C., Henry, R. M., Cohen, A. N., & Daley, S. E. (2006). The Stress sensitization hypothesis: Understanding the course of bipolar disorder. *Journal of Affective Disorders*, 95(1-3), 43-49.
- Dityatev, A. E., & Bolshakov, V. Y. (2005). Amygdala, long-term potentiation, and fear conditioning. *The Neuroscientist*, 11(1), 75-88.
- Donley, M. P., Schulkin, J., & Rosen, J. B. (2005). Glucocorticoid receptor antagonism in the basolateral amygdala and ventral hippocampus interferes with long-term memory of contextual fear. *Behavioural Brain Research*, 164(2), 197-205.
- Dowlatsahi, D., MacQueen, G. M., Wang, J. F., & Young, L. T. (1998). Increased temporal cortex CREB concentrations and antidepressant treatment in major depression. *Lancet (London, England)*, 352(9142), 1754-1755.
- Drevets, W. C. (2003). Neuroimaging abnormalities in the amygdala in mood disorders. *Annals of the New York Academy of Sciences*, 985, 420-444.

- Drevets, W. C., Videen, T. O., Price, J. L., Preskorn, S. H., Carmichael, S. T., & Raichle, M. E. (1992). A functional anatomical study of unipolar depression. *The Journal of Neuroscience*, *12*(9), 3628-3641.
- Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annual Review of Psychology*, *55*, 51-86.
- Duman, R. S., Li, N., Liu, R., Duric, V., & Aghajanian, G. (2012). Signaling pathways underlying the rapid antidepressant actions of ketamine. *Neuropharmacology*, *62*, 35-41.
- Duncan, C. P. (1949). The retroactive effect of electroshock on learning. *Journal of Comparative and Physiological Psychology*, *42*(1), 32-44.
- Dunn, A. J., & Swiergiel, A. H. (2008). Effects of acute and chronic stressors and CRF in rat and mouse tests for depression. *Annals of the New York Academy of Sciences*, *1148*, 118-126.
- Duvarci, S., & Paré, D. (2007). Glucocorticoids enhance the excitability of principal basolateral amygdala neurons. *The Journal of Neuroscience*, *27*(16), 4482-4491.
- Dwivedi, Y., Rizavi, H. S., & Pandey, G. N. (2006). Antidepressants reverse corticosterone-mediated decrease in brain-derived neurotrophic factor expression: differential regulation of specific exons by antidepressants and corticosterone. *Neuroscience*, *139*(3), 1017-1029.
- Dwivedi, Y., Rizavi, H. S., Conley, R. R., Roberts, R. C., Tamminga, C. A., & Pandey, G. N. (2003). Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Archives of General Psychiatry*, *60*(8), 804-815.
- Elliott, R., Zahn, R., Deakin, J. F., & Anderson, I. M. (2011). Affective cognition and its disruption in mood disorders. *Neuropsychopharmacology*, *36*(1), 153-182.

Enkel, T., Gholizadeh, D., von Bohlen Und Halbach, O., Sanchis-Segura, C., Hurlemann, R., Spanagel, R., Gass, P., & Vollmayr, B. (2010). Ambiguous-cue interpretation is biased under stress- and depression-like states in rats. *Neuropsychopharmacology*, *35*(4), 1008-1015.

Entsuah, A. R., Huang, H., & Thase, M. E. (2001). Response and remission rates in different subpopulations with major depressive disorder administered venlafaxine, selective serotonin reuptake inhibitors, or placebo. *The Journal of Clinical Psychiatry*, *62*(11), 869-877.

Essex, M. J., Schirtcliff, E. A., Burk, L. R., Ruttle, P. L., Klein, M. H., Slattery, M. J., Kalin, N. H., & Armstrong, J. M. (2011). Influence of early life stress on later hypothalamic-pituitary-adrenal axis functioning and its covariation with mental health symptoms: a study of the allostatic processes from childhood into adolescence. *Development and Psychopathology*, *23*(4), 1039-1058.

Falls, W. A., Miserendino, M. J., & Davis, M. (1992). Extinction of fear-potentiated startle: Blockade by infusion of an NMDA antagonist into the amygdala. *The Journal of Neuroscience*, *12*(3), 854-863.

Fanselow, M. S., & Kim, J. J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behavioral Neuroscience*, *108*(1), 210-212.

Farinelli, M., Deschaux, O., Hugues, S., Thevenet, A., & Garcia, R. (2006). Hippocampal train stimulation modulates recall of fear extinction independently of prefrontal cortex synaptic plasticity and lesions. *Learning & Memory*, *13*(3), 329-334.

- Farmer, A. E., & McGuffin, P. (2003). Humiliation, loss and other types of life events and difficulties: a comparison of depressed subjects, healthy controls and their siblings. *Psychological Medicine*, *33*(7), 1169-1175.
- Farrell, M. R., Sayed, J. A., Underwood, A. R., & Wellman, C. L. (2010). Lesion of infralimbic cortex occludes stress effects on retrieval of extinction but not fear conditioning. *Neurobiology of Learning and Memory*, *94*(2), 240-246.
- Fatemi, S. H., Laurence, J. A., Araghi-Niknam, M., Strydom, J. M., Schulz, S. C., Lee, S., & Gottesman, I. I. (2004). Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. *Schizophrenia Research*, *69*(1-2), 317-323.
- Feder, A., Parides, M. K., Murrugh, J. W., Perez, A. M., Morgan, J. E., Saxena, S., Kirkwood, K., van der Rot, M., Lapidus, K. A. B., Wan, L., Iosifescu, D., & Charney, D. S. (2014). Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: A randomized clinical trial. *JAMA Psychiatry*, *71*(6), 681-688.
- Feldman, S., & Weidenfeld, J. (1999). Glucocorticoid receptor antagonists in the hippocampus modify the negative feedback following neural stimuli. *Brain Research*, *821*(1), 33-37.
- Fendt, M. (2001). Injections of the NMDA receptor antagonist aminophosphopentanoic acid into the lateral nucleus of the amygdala block the expression of fear-potentiated startle and freezing. *The Journal of Neuroscience*, *21*(11), 4111-4115.
- Fenton, E. Y., Fournier, N. M., Lussier, A. L., Romay-Tallon, R., Caruncho, H. J., & Kalynchuk, L. E. (2015). Imipramine protects against the deleterious effects of chronic corticosterone on depression-like behavior, hippocampal reelin expression, and neuronal maturation. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *60*, 52-59.

- Fernandez, E. E. (2003). Prefrontocortical dopamine loss in rats delays long-term extinction of contextual conditioned fear, and reduces social interaction without affecting short-term social interaction memory. *Neuropsychopharmacology*, 28(3), 490-498.
- Figueiredo, H. F., Dolgas, C. M., & Herman, J. P. (2002). Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. *Endocrinology*, 143(7), 2534-2540.
- Filho, C. B., Jesse, C. R., Donato, F., Giacomeli, R., Del Fabbro, L., da Silva Antunes, M., de Gomes M. G., Goes, A. T., Boeira, S. P., Prigol, M., & Souza, L. C. (2015). Chronic unpredictable mild stress decreases BDNF and NGF levels and Na(+),K(+)-ATPase activity in the hippocampus and prefrontal cortex of mice: antidepressant effect of chrysin. *Neuroscience*, 289, 367-380.
- Föcking, M., Hölker, I., & Trapp, T. (2003). Chronic glucocorticoid receptor activation impairs CREB transcriptional activity in clonal neurons. *Biochemical and Biophysiological Research Communications*, 304(4), 720-723.
- Fontella, F. U., Vendite, D. A., Tabajara, A. S., Porciúncula, L. O., da Silva Torres, I. L., Jardim, F. M., Martini, L., Souza, D. O., Netto, C. A., & Dalmaz, C. (2004). Repeated restraint stress alters hippocampal glutamate uptake and release in rats. *Neurochemical Research*, 29(9), 1703-1709.
- Foy, M. R., Stanton, M. E., Levine, S., & Thompson, R. F. (1987). Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behavioral and Neural Biology*, 48(1), 138-149.
- Franceschelli, A., Sens, J., Herchick, S., Thelen, C., & Pitychoutis, P. M. (2015). Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naïve and "depressed" mice exposed to chronic mild stress. *Neuroscience*, 290, 49-60.

- Francis, D. D., & Meaney, M. J. (1999). Maternal care and the development of stress responses. *Current Opinions in Neurobiology*, 9(1), 128-134.
- Frechilla, D., Otano, A., & Del Rio, J. (1998). Effect of chronic antidepressant treatment on transcription factor binding activity in rat hippocampus and frontal cortex. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 22(5), 787-802.
- Frodl, T., Jäger, M., Smajstrlova, I., Born, C., Bottlender, R., Palladino, T., Reiser, M., Möller, H. J., & Meisenzahl, E. M. (2008). Effect of hippocampal and amygdala volumes on clinical outcomes in major depression: a 3-year prospective magnetic resonance imaging study. *Journal of Psychiatry Neuroscience*, 33(5), 423-430.
- Frodl, T., Meisenzahl, E., Zetzsche, T., Bottlender, R., Born, C., Groll, C., Jäger, M., Leinsinger, G., Hahn, K., & Möller, H. J. (2002). Enlargement of the amygdala in patients with a first episode of major depression. *Biological Psychiatry*, 51(9), 708-714.
- Fu, C. H., Williams, S. C., Brammer, M. J., Suckling, J., Kim J., Cleare, A. J., Walsh, N. D., Mitterschiffthaler, M. T., Andrew, C. M., Pich, E. M., & Bullmore, E. T. (2007). Neural responses to happy facial expressions in major depression following antidepressant treatment. *The American Journal of Psychiatry*, 164(4), 599-607.
- Fu, C. H., Williams, S. C., Cleare, A. J., Brammer, M. J., Walsh, N. D., Kim, J. Andrew, C. M., Pich, E. M., Williams, P. M., Reed, L. J., Mitterschiffthaler, M. T., Suckling, J., & Bullmore, E. T. (2004). Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study. *Archives of General Psychiatry*, 61(9), 877-889.
- Fu, C. H., Williams, S. C., Cleare, A. J., Scott, J., Mitterschiffthaler, M. T., Walsh, N. D., Donaldson, C., Suckling, J., Andrew, C., Steiner, H., & Murray, R. M. (2008). Neural responses to sad facial expressions in major depression following cognitive behavioral therapy. *Biological Psychiatry*, 64(6), 505-512.

- Fu, J., Zhang, Y., Wu, R., Zheng, Y., Zhang, X., Yang, M., Zhao, J., & Liu, Y. (2014). Shuganjiayu capsule increases neurotrophic factor expression in a rat model of depression. *Neural Regeneration Research*, 9(5), 489-497.
- Galea, L. A., McEwen, B. S., Tanapat, P., Deak, T., Spencer, R. L., & Dhabhar, F. S. (1997). Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience*, 81(3), 689-697.
- Garcia, L. S. B., Comim, C. M., Valvassori, S. S., Réus, G. Z., Sterz, L., Kapczinski, F., Gavioli, E. C., & Quevedo, J. (2009). Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 33, 450-455.
- Garcia, L. S., Comim, C. M., Valvassori, S. S., Réus, G. Z., Barbosa, L. M., Andreazza, A. C., Sterz, L., Fries, G. R., Gavioli, E. C., Kapczinski, F., & Quevedo, J. (2008a). Acute administration of ketamine induces antidepressant-like effects in the forced swim test and increases BDNF levels in the rat hippocampus. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 32(1), 140-144.
- Garcia, R., Paquereau, J., Vouimba, R. M., & Jaffard, R. (1998). Footshock stress but not contextual fear conditioning induces long-term enhancement of auditory-evoked potentials in the basolateral amygdala of the freely behaving rat. *The European Journal of Neuroscience*, 10(2), 457-463.
- Garcia, R., Spennato, G., Nilsson-Todd, L., Moreau, J., & Deschaux, O. (2008b). Hippocampal low-frequency stimulation and chronic mild stress similarly disrupt fear extinction memory in rats. *Neurobiology of Learning and Memory*, 89, 560-566.
- Garcia, R., Vouimba, R. M., & Jaffard, R. (1997). Contextual conditioned fear blocks the induction but not the maintenance of lateral septal LTP in behaving mice. *Journal of Neurophysiology*, 78(1), 76-81.

- Garrett, J. E., & Wellman, C. L. (2009). Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence. *Neuroscience*, *162*(1), 195-207.
- Gaynes, B. N., Warden, D., Trivedi, M. H., Wisniewski, S. R., Fava, M., & Rush, A. J. (2009). What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatric Services*, *60*(11), 1439-1445.
- GBD 2013 Mortality and Causes of Death Collaborators. (2015). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *385*(9963), 117-171.
- Gerritsen, L., Rijpkema, M., van Oostrom, I., Buitelaar, J., Franke, B., Fernández, G., & Tendolkar, I. (2012). Amygdala to hippocampal volume ratio is associated with negative memory bias in healthy subjects. *Psychological Medicine*, *42*(2), 335-343.
- Gervasoni, N., Aubry, J. M., Bondolfi, G., Osiek, C., Schwald, M., Bertschy, G., & Karege, F. (2005). Partial normalization of serum brain-derived neurotrophic factor in remitted patients after a major depressive episode. *Neuropsychobiology*, *51*(4), 234-238.
- Gewirtz, J. C., & Davis, M. (1997). Second-order fear conditioning prevented by blocking NMDA receptors in amygdala. *Nature*, *388*(6641), 471-474.
- Gigliucci, V., O'Dowd, G., Casey, S., Egan, D., Gibey, S., & Harkin, A. (2013). Ketamine elicits sustained antidepressant-like activity via a serotonin-dependent mechanism. *Psychopharmacology*, *228*(1), 157-166.

- Godsil, B. P., Bontempi, B., Mailliet, F., Delagrangé, P., Spedding, M., & Jay, T. M. (2015). Acute tianeptine treatment selectively modulates neuronal activation in the central nucleus of the amygdala and attenuates fear extinction. *Molecular Psychiatry*, *20*(11), 1420-1427.
- Gorzalka, B. B., & Hanson, L. A. (1998). Sexual behavior and wet dog shakes in the male rat: regulation by corticosterone. *Behavioural Brain Research*, *97*(1-2), 143-151.
- Gorzalka, B. B., Brotto, L. A., & Hong, J. J. (1999). Corticosterone regulation of 5-HT_{2A} receptor-mediated behaviors: attenuation by melatonin. *Physiological Behavior*, *67*(3), 439-442.
- Gorzalka, B. B., Hanson, L. A., & Hong, J. J. (2001). Ketanserin attenuates the behavioural effects of corticosterone: implications for 5-HT_{2A} receptor regulation. *European Journal of Pharmacology*, *428*(2), 235-240.
- Gorzalka, B. B., Hanson, L., Harrington, J., Killam, S., & Campbell-Meiklejohn, D. (2003). Conditioned taste aversion: modulation by 5-HT receptor activity and corticosterone. *European Journal of Pharmacology*, *471*(2), 129-134.
- Gotlib, I. H., Joormann, J., Minor, K. L., & Hallmayer, J. (2008). HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biological Psychiatry*, *63*(9), 847-851.
- Gotlib, I. H., Krasnoperova, E., Yue, D. N., & Joormann, J. (2004). Attentional biases for negative interpersonal stimuli in clinical depression. *Journal of Abnormal Psychology*, *113*(1), 121-135.
- Gourley, S. L., Espitia, J. W., Sanacora, G., & Taylor, J. R. (2012). Antidepressant-like properties of oral riluzole and utility of incentive disengagement models of depression in mice. *Psychopharmacology (Berlin)*, *219*(3), 805-814.

- Gourley, S. L., Kedves, A. T., Olausson, P., & Taylor, J. R. (2009). A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. *Neuropsychopharmacology*, *34*(3), 707-716.
- Gourley, S. L., Kiraly, D. D., Howell, J. L., Olausson, P., & Taylor, J. R. (2008a). Acute hippocampal brain-derived neurotrophic factor restores motivational and forced swim performance after corticosterone. *Biological Psychiatry*, *64*(10), 884-890.
- Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Kedves, A. T., Duman, R. S., & Taylor, J. R. (2008b). Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. *Biological Psychiatry*, *63*(4), 353-359.
- Gregus, A., Wintink, A. J., Davis, A. C., & Kalynchuk, L. E. (2005). Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behavioural Brain Research*, *156*, 105-114.
- Grissom, N., Iyer, V., Vining, C., & Bhatnagar, S. (2007). The physical context of previous stress exposure modifies hypothalamic-pituitary-adrenal responses to a subsequent homotypic stress. *Hormones and Behavior*, *51*(1), 95-103.
- Grønli, J., Bramham, C., Murison, R., Kanhema, T., Fiske, E., Bjorvatn, B., Ursin, R., & Portas, C. M. (2006). Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. *Pharmacology, Biochemistry, and Behavior*, *85*(4), 842-849.
- Guo, F., Zhang, Q., Zhang, B., Fu, Z., Wu, B., Huang, C., & Li, Y. (2014). Burst-firing patterns in the prefrontal cortex underlying the neuronal mechanisms of depression probed by antidepressants. *The European Journal of Neuroscience*, *40*(10), 3538-3547.
- Gupta, A., Devi, L. A., & Gomes, I. (2011). Potentiation of μ -opioid receptor-mediated signaling by ketamine. *Journal of Neurochemistry*, *119*(2), 294-302.

- Gupta, D., Radhakrishnan, M., & Kurhe, Y. (2015). Effect of a novel 5-HT₃ receptor antagonist 4i, in corticosterone-induced depression-like behavior and oxidative stress in mice. *Steroids*, *96*, 95-102.
- Gupta, R., & Kar, B. R. (2012). Attention and memory biases as stable abnormalities among currently depressed and currently remitted individuals with unipolar depression. *Frontiers in Psychiatry*, *3*(99), 1-7.
- Hamacher-Dang, T. C., Merz, C. J., & Wolf, O. T. (2015). Stress following extinction learning leads to a context-dependent return of fear. *Psychophysiology*, *52*(4), 489-498.
- Hamani, C., Machado, D. C., Hipólido, D. C., Dubiela, F. P., Suchecki, D., Macedo, C. E., Tescarollo, F., Martins, U., Covolan, L., & Nobrega, J. N. (2012). Deep brain stimulation reverses anhedonic-like behavior in a chronic model of depression: role of serotonin and brain derived neurotrophic factor. *Biological Psychiatry*, *71*(1), 30-35.
- Handa, R. J., Burgess, L. H., Kerr, J. E., & O'Keefe, J. A. (1994). Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Hormones and Behavior*, *28*(4), 464-476.
- Harding, E. J., Paul, E. S., & Mendl, M. (2004). Animal behaviour: cognitive bias and affective state. *Nature*, *427*(6972), 312.
- Hardingham, G. E., Fukunaga, Y., & Bading, H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neuroscience*, *5*(5), 405-414.
- Harmer, C. J. (2013). Emotional processing and antidepressant action. *Current Topics in Behavioral Neuroscience*, *14*, 209-222.

- Harmer, C. J., & Cowen, P. J. (2013). 'It's the way that you look at it'--a cognitive neuropsychological account of SSRI action in depression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 368(1615), 20120407.
- Harmer, C. J., Goodwin, G. M., & Cowen, P. J. (2009a). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. *The British Journal of Psychiatry*, 195(2), 102-108.
- Harmer, C. J., O'Sullivan, U., Favaron, E., Massey-Chase, R., Ayres, R., Reinecke, A., Goodwin, G. M., & Cowen, P. J. (2009b). Effect of acute antidepressant administration on negative affective bias in depressed patients. *American Journal of Psychiatry*, 166(10), 1178-1184.
- Hashimoto, K., Sawa, A., & Iyo, M. (2007). Increased levels of glutamate in brains from patients with mood disorders. *Biological Psychiatry*, 62(11), 1310-1316.
- Hashimoto, S., Inoue, T., Muraki, I., & Koyama, T. (2009). Effects of acute citalopram on the expression of conditioned freezing in naive versus chronic citalopram-treated rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 33(1), 113-117.
- Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biological Psychiatry*, 49(12), 1023-1039.
- Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, 33(6), 693-710.
- Heinz, E. R., Martinez, J., & Haenggeli, A. (1977). Reversibility of cerebral atrophy in anorexia nervosa and Cushing's syndrome. *Journal of Computer Assisted Tomography*, 1, 415-418.

- Heldt, S. A., Stanek, L., Chhatwal, J. P., & Ressler, K. J. (2007). Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Molecular Psychiatry*, *12*, 656-670.
- Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience*, *108*(5), 1005-1009.
- Heninger, G. R., Delgado, P. L., & Charney, D. S. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry*, *29*(1), 2-11.
- Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neuroscience*, *20*(2), 78-84.
- Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology*, *24*(3), 151-180.
- Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *29*(8), 1201-1213.
- Herman, J. P., Prewitt, C. M., & Cullinan, W. E. (1996). Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. *Critical Reviews in Neurobiology*, *10*(3-4), 371-394.
- Herry, C., & Garcia, R. (2002). Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. *The Journal of Neuroscience*, *22*(2), 577-583.

- Herry, C., & Mons, N. (2004). Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. *The European Journal of Neuroscience*, *20*(3), 781-790.
- Heuser, I. J. E., Schweiger, U., Gotthardt, U., Schmider, J., Lammers, C. H., Dettling, M., Yassouridis, A., & Holsboer, F. (1996). Pituitary-adrenal system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and in normal comparison subjects. *The American Journal of Psychiatry*, *153*, 93-99.
- Hill, M. N., Brotto, L. A., Lee, T. T., & Gorzalka, B. B. (2003). Corticosterone attenuates the antidepressant-like effects elicited by melatonin in the forced swim test in both male and female rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *27*(6), 905-911.
- Hirota, K., Okawa, H., Appadu, B. L., Grandy, D. K., Devi, L. A., & Lambert, D. G. (1999). Stereoselective interaction of ketamine with recombinant mu, kappa, and delta opioid receptors expressed in Chinese hamster ovary cells. *Anesthesiology*, *90*(1), 174-182.
- Hitchcock, J. M., Sananes, C. B., & Davis, M. (1989). Sensitization of the startle reflex by footshock: blockade by lesions of the central nucleus of the amygdala or its efferent pathway to the brainstem. *Behavioral Neuroscience*, *103*(3), 509-518.
- Hitchcock, J., & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behavioral Neuroscience*, *100*(1), 11-22.
- Hobin, J. A., Ji, J., & Maren, S. (2006). Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. *Hippocampus*, *16*(2), 174-182.

- Hoffman, A. N., Lorson, N. G., Sanabria, F., Olive, M., F., & Conrad, C. D. (2014). Chronic stress disrupts fear extinction and enhances amygdala and hippocampal Fos expression in an animal model of post-traumatic stress disorder. *Neurobiology of Learning and Memory*, *112*, 139-147.
- Holsboer-Trachsler, E., Hemmeter, U., Hatzinger, M., Seifritz, E., Gerhard, U., & Hobbi, V. (1994). Sleep deprivation and bright light as potential augments of antidepressant drug treatment — neurobiological and psychometric assessment of course. *Journal of Psychiatric Research*, *28*, 381-399.
- Holsboer, F. (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *Journal of Affective Disorders*, *62*, 77-91.
- Holsboer, F., Lauer, C. J., Schreiber, W., & Krieg, J. C. (1995). Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorders. *Neuroendocrinology*, *62*(4), 340-347.
- Homayoun, H., & Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *The Journal of Neuroscience*, *27*(43), 11496-11500.
- Hong, I., Kim, J., Lee, J., Park, S., Song, B., Kim, J., An, B., Park, K., Lee, H. W., Lee, S., Kim, H., Park, S., Eom, K. D., Lee, S., & Choi, S. (2011). Reversible plasticity of fear memory-encoding amygdala synaptic circuits even after fear memory consolidation. *PLoS ONE*, *6*(9), e24260.
- Hong, I., Song, B., Lee, S., Kim, J., Kim, J., & Choi, S. (2009). Extinction of cued fear memory involves a distinct form of depotentiation at cortical input synapses onto the lateral amygdala. *European Journal of Neuroscience*, *30*, 2089-2099.

- Horch, H. W., Krüttgen, A., Portbury, S. D., & Katz, L. C. (1999). Destabilization of cortical dendrites and spines by BDNF. *Neuron*, 23(2), 353-564.
- Hoshaw, B. A., Malberg, J. E., & Lucki, I. (2005). Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Research*, 1037, 204-208.
- Hsu, D. T., Langenecker, S. A., Kennedy, S. E., Zubieta, J. K., & Heitzeg, M. M. (2010). fMRI BOLD responses to negative stimuli in the prefrontal cortex are dependent on levels of recent negative life stress in major depressive disorder. *Psychiatry Research*, 183(3), 202-208.
- Huang, Z., Zhon, X. M., Li, Z. Y., Feng, C. R., Pan, A. J., & Mao, Q. Q. (2011). Curcumin reverses corticosterone-induced depressive-like behavior and decrease in brain BDNF levels in rats. *Neuroscience Letters*, 493(3), 145-148.
- Hugues, S., & Garcia, R. (2007). Reorganization of learning-associated prefrontal synaptic plasticity between the recall of recent and remote fear extinction memory. *Learning & Memory*, 14(8), 520-524.
- Hugues, S., Chessel, A., Lena, I., Marsault, R., & Garcia, R. (2006). Prefrontal infusion of PD098059 immediately after fear extinction training blocks extinction-associated prefrontal synaptic plasticity and decreases prefrontal ERK2 phosphorylation. *Synapse*, 60(4), 280-287.
- Hui, G. K., Figueroa, I. R., Poytress, B. S., Roozendaal, B., McGaugh, J. L., & Weinberger, N. M. (2004). Memory enhancement of classical fear conditioning by post-training injections of corticosterone in rats. *Neurobiology of Learning and Memory*, 81(1), 67-74.
- Hui, I. R., Hui, G. K., Roozendaal, B., McGaugh, J. L., & Weinberger, N. M. (2006). Posttraining handling facilitates memory for auditory-cue fear conditioning in rats. *Neurobiology of Learning and Memory*, 86(2), 160-163.

- Hustveit, O., Maurset, A., & Oye, I. (1995). Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. *Pharmacology & Toxicology*, 77(6), 355-359.
- Ibarguen-Vargas, Y., Surget, A., Vourc'h, P., Leman, S., Andres, C. R., Gardier, A. M., & Belzung, C. (2009). Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress. *Behavioral Brain Research*, 202(2), 245-251.
- Ibrahim, L., Diazgranados, N., Luckenbaugh, D. A., Machado-Vieira, R., Baumann, J., Mallinger, A. G., & Zarate, C. A. (2011). Rapid decrease in depressive symptoms with an N-methyl-d-aspartate antagonist in ECT-resistant major depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 35(4), 1155-1159.
- Imre, G., Fokkema, D. S., Boer, J. A., & Ter Horst, G. J. (2006). Dose-response characteristics of ketamine effect on locomotion, cognitive function and central neuronal activity. *Brain Research Bulletin*, 69, 338-345.
- Inoue, T., Li, X. B., Abekawa, T., Kitaichi, Y., Izumi, T., Nakagawa, S., & Koyama, T. (2004). Selective serotonin reuptake inhibitor reduces conditioned fear through its effect in the amygdala. *European Journal of Pharmacology*, 497(3), 311-316.
- Isaac, L., Vrijnsen, J. N., Rinck, M., Speckens, A., & Becker, E. S. (2014). Shorter gaze duration for happy faces in current but not remitted depression: Evidence from eye movements. *Psychiatry Research*, 218, 79-86.
- Ivanov, A., Pellegrino, C., Rama, S., Dumalska, I., Salyha, Y., Ben-Ari, Y., & Medina, I. (2006). Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. *The Journal of Physiology*, 572(3), 789-798.

- Iwata, J., Chida, K., & LeDoux, J. E. (1987). Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. *Brain Research*, *418*(1), 183-188.
- Izquierdo, A., Wellman, C. L., & Holmes, A. (2006). Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. *The Journal of Neuroscience*, *26*(21), 5733-5738.
- Izquierdo, L. A., Barros, D. M., Medina, J. H., & Izquierdo, I. (2002). Stress hormones enhance retrieval of fear conditioning acquired either one day or many months before. *Behavioural Pharmacology*, *13*(3), 203-213.
- Jacobsen, J. P., & Mørk, A. (2006). Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. *Brain Research*, *1110*(1), 221-225.
- Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine Reviews*, *12*(2), 118-134.
- Jankord, R., & Herman, J. P. (2008). Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Annals of the New York Academy of Sciences*, *1148*, 64-73.
- Jett, J. D., Boley, A. M., Girotti, M., Shah, A., Lodge, D. J., & Morilak, D. A. (2015). Antidepressant-like cognitive and behavioral effects of acute ketamine administration associated with plasticity in the ventral hippocampus to medial prefrontal cortex activity. *Psychopharmacology (Berlin)*, EPUB.
- Ji, Y., & Maren, S. (2007). Hippocampal involvement in contextual modulation of fear extinction. *Hippocampus*, *17*(9), 749-758.

- Jin, P., Yu, H. L., Tian-Lan, Zhang, F., & Quan, Z. S. (2015). Antidepressant-like effects of oleylethanolamide in a mouse model of chronic unpredictable mild stress. *Pharmacology, Biochemistry, and Behavior*, *133*, 146-154.
- Jin, X. C., Lu, Y. F., Yang, X. F., Ma, L., & Li, B. M. (2007). Glucocorticoid receptors in the basolateral nucleus of amygdala are required for postreactivation reconsolidation of auditory fear memory. *The European Journal of Neuroscience*, *25*(12), 3702-3712.
- Johansen, J. P., Cain, C. K., Ostroff, L. E., & LeDoux, J. E. (2011). Molecular mechanisms of fear learning and memory. *Cell*, *147*, 509-524.
- Johansen, J. P., Hamanaka, H., Monfils, M. H., Behnia, R., Deisseroth, K., Blair, H. T., & LeDoux, J. E. (2010). Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(28), 12692-12697.
- John, C. S., Smith, K. L., Van't Veer, A., Gompf, H. S., Carlezon, W. A., Cohen, B. M., Öngür, D., & Bechtholt-Gompf, A. J. (2012). Blockade of astrocytic glutamate uptake in the prefrontal cortex induces anhedonia. *Neuropsychopharmacology*, *37*(11), 2467-2475.
- Johnson, S. A., Fournier, N. M., & Kalynchuk, L. E. (2006). Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. *Behavioural Brain Research*, *168*, 280-288.
- Joormann, J., Talbot, L., & Gotlib, I. H. (2007). Biased processing of emotional information in girls at risk for depression. *Journal of Abnormal Psychology*, *116*(1), 135-143.
- Jourdi, H., Hsu, Y., Zhou, M., Qin, Q., Bi, X., & Baudry, M. (2009). Positive AMPA receptor modulation rapidly stimulates BDNF release and increases dendritic mRNA translation. *The Journal of Neuroscience*, *29*(27), 8688-8697.

- Judd, L. L., Akiskal, H. S., Schettler, P. J., Endicott, J., Maser, J., Solomon, D. A., Leon, A. C., Rice, J. A., & Keller, M. B. (2002). The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Archives of General Psychiatry*, *59*(6), 530-537.
- Jun, C., Choi, Y., Lim, S. M., Bae, S., Hong, Y. S., Kim, J. E., & Lyoo, I. K. (2014). Disturbance of the glutamatergic system in mood disorders. *Experimental Neurobiology*, *23*(1), 28-35.
- Jüngling, K., Seidenbecher, T., Sosulina, L., Lesting, J., Sangha, S., Clark, S. D., Okamura, N., Duangdao, D. M., Xu, Y. L., Reinscheid, R. K., & Pape, H. C. (2008). Neuropeptide S-mediated control of fear expression and extinction: Role of intercalated GABAergic neurons in the amygdala. *Neuron*, *59*(2), 298-310.
- Kalynchuk, L. E., Gregus, A., Boudreau, D., & Perrot-Sinal, T. S. (2004). Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. *Behavioral Neuroscience*, *118*(6), 1365-1377.
- Kang, H., Welcher, A. A., Shelton, D., & Schuman, E. M. (1997). Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. *Neuron*, *19*(3), 653-664.
- Kapur, S., & Seeman, P. (2002). NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D(2) and serotonin 5-HT(2) receptors - Implications for models of schizophrenia. *Molecular Psychiatry*, *7*(8), 837-844.
- Kapp, B. S., Gallagher, M., Underwood, M. D., McNall, C. L., & Whitehorn, D. (1982). Cardiovascular responses elicited by electrical stimulation of the amygdala central nucleus in the rabbit. *Brain Research*, *234*(2), 251-262.

- Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J. M., & Bertschy, G. (2005). Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biological Psychiatry*, *57*(9), 1068-1072.
- Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G., & Aubry, J. M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Research*, *109*(2), 143-148.
- Karpova, N. N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kuleskaya, N., Ágústsdóttir, A., Antila, H., Popova, D., Akamine, Y., Sullivan, R., Hen, R., Drew, L. J., & Castrén, E. (2011). Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science*, *334*, 1731-1734.
- Kavushansky, A., & Richter-Levin, G. (2006). Effects of stress and corticosterone on activity and plasticity in the amygdala. *Journal of Neuroscience Research*, *84*(7), 1580-1587.
- Kavushansky, A., Vouimba, R. M., Cohen, H., & Richter-Levin, G. (2006). Activity and plasticity in the CA1, the dentate gyrus, and the amygdala following controllable vs. uncontrollable water stress. *Hippocampus*, *16*(1), 35-42.
- Kendler, K. S., Gardner, C. O., & Prescott, C. A. (2002). Toward a comprehensive developmental model for major depression in women. *The American Journal of Psychiatry*, *159*, 1133-1145.
- Kendler, K. S., Gardner, C. O., & Prescott, C. A. (2006). Toward a comprehensive developmental model for major depression in men. *The American Journal of Psychiatry*, *163*, 115-124.

- Kendler, K. S., Thornton, L. M., & Gardner, C. O. (2001). Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *The American Journal of Psychiatry*, *158*(4), 582-586.
- Kessing, L. V., Agerbo, E., & Mortensen, P. B. (2003). Does the impact of major stressful life events on the risk of developing depression change throughout life? *Physiological Medicine*, *33*, 1177-1184.
- Kim J., Lee, S., Park, K., Hong, I., Song, B., Son, G., Park, H., Kim, W. R., Park, E., Choe, H. K., Kim, H., Lee, C., Sun, W., Kim, K., Shin, K. S. S., & Choi, S. (2007a). Amygdala depotentiation and fear extinction. *PNAS*, *104*(52), 20955-20960.
- Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science*, *256*(5057), 675-677.
- Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. *Neuroscience and Biobehavioral Reviews*, *30*, 188-202.
- Kim, J. J., DeCola, J. P., Landeira-Fernandez, J., & Fanselow, M. S. (1991). N-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. *Behavioral Neuroscience*, *105*(1), 126-133.
- Kim, J. J., Foy, M. R., & Thompson, R. F. (1996). Behavioral stress modifies hippocampal plasticity through N-methyl-D-aspartate receptor activation. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(10), 4750-4753.
- Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience*, *107*(6), 1093-1098.

- Kim, J., Schmid-Burgk, W., Claus, D., & Kornhuber, H. H. (1970). Increased serum glutamate in depressed patients. *Archiv für Psychiatrie und Nervenkrankheiten*, 232(4), 299-304.
- Kim, M., & Davis, M. (1993). Lack of a temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience*, 107(6), 1088-1092.
- Kim, M., Campeau, S., Falls, W. A., & Davis, M. (1993). Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. *Behavioral and Neural Biology*, 59(1), 5-8.
- Kim, Y. K., Lee, H. P., Won, S. D., Park, E. Y., Lee, H. Y., Lee, B. H., Lee, S. W., Yoon, D., Han, C., Kim, D. J., & Choi, S. H. (2007b). Low plasma BDNF is associated with suicidal behavior in major depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 31(1), 78-85.
- Kitaichi, Y., Inoue, T., Nakagawa, S., Omiya, Y., Song, N., An, Y., Chen, C., Kusumi, I., & Koyama, T. (2014). Local infusion of citalopram into the basolateral amygdala decreased conditioned fear of rats through increasing extracellular serotonin levels. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 54, 216-222.
- Kitay, J. I. (1961). Sex differences in adrenal cortical secretion in the rat. *Endocrinology*, 68, 818-824.
- Kitayama, I., Cintra, A., Janson, A. M., Fuxe, K., Agnati, L. F., Eneroth, P., Aronsson, M., Härfstrand, A., Steinbush, H. W., Visser, T. J., Goldstein, M., Vale, W., & Gustafsson, J. A. (1989). Chronic immobilization stress: evidence for decreases of 5-hydroxytryptamine immunoreactivity and for increases of glucocorticoid receptor immunoreactivity in various brain regions of the male rat. *Journal of Neural Transmission*, 77(2-3), 93-130.

- Knapska, E., & Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learning and Memory*, *16*, 486-493.
- Kohda, K., Harada, K., Kato, K., Hoshino, A., Motohashi, J., Yamaji, T., Morinobu, S., Matsuoka, N., & Kato, N. (2007). Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative post-traumatic stress disorder model. *Neuroscience*, *148*(1), 22-33.
- Koike, H., Iijima, M., & Chaki, S. (2011). Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. *Behavioural Brain Research*, *224*(1), 107-111.
- Koike, H., Iijima, M., & Chaki, S. (2013). Effects of ketamine and LY341495 on the depressive-like behavior of repeated corticosterone-injected rats. *Pharmacology, Biochemistry and Behavior*, *107*, 20-23.
- Kolber, B. J., Roberts, M. S., Howell, M. P., Wozniak, D. F., Sands, M. S., & Muglia, L. J. (2008). Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(33), 12004-12009.
- Konkle, A. T., Baker, S. L., Kentner, A. C., Barbagallo, L. S., Merali, Z., & Bielajew, C. (2003). Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: sex and strain compared. *Brain Research*, *992*(2), 227-238.
- Korte, M., Staiger, V., Griesbeck, O., Thoenen, H., & Bonhoeffer, T. (1996). The involvement of brain-derived neurotrophic factor in hippocampal long-term potentiation revealed by gene targeting experiments. *Journal of Physiology, Paris*, *90*(3-4), 157-164.

- Kranaster, L., Krammerer-Ciernioch, J., Hoyer, C., & Sartorius, A. (2011). Clinically favourable effects of ketamine as an anaesthetic for electroconvulsive therapy: a retrospective study. *European Archives of Psychiatry and Clinical Neuroscience*, 261(8), 575-582.
- Krystal, J. H., Sanacora, G., & Duman, R. S. (2013). Rapid-acting glutamatergic antidepressants: the path to ketamine and beyond. *Biological Psychiatry*, 73(12), 1133-1141.
- Küçükbrahimoğlu, E., Saygin, M. Z., Çalışkan, M., Kaplan, O. K., Unsal, C., & Gören, M. Z. (2009). The change in plasma GABA, glutamine and glutamate levels in fluoxetine- or S-citalopram-treated female patients with major depression. *European Journal of Clinical Pharmacology*, 65(6), 571-577.
- Lan, M. J., McLoughlin, G. A., Griffin, J. L., Tsang, T. M., Huang, J. T., Yuan, P., Manji, H., Holmes, E., & Bahn, S. (2009). Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. *Molecular Psychiatry*, 14(3), 269-279.
- Lange, C., & Irle, E. (2004). Enlarged amygdala volume and reduced hippocampal volume in young women with major depression. *Psychological Medicine*, 34(6), 1059-1064.
- Laurent, V., & Westbrook, R. F. (2008). Distinct contributions of the basolateral amygdala and the medial prefrontal cortex to learning and relearning extinction of context conditioned fear. *Learning & Memory*, 15(9), 657-666.
- Laurent, V., & Westbrook, R. F. (2010). Role of the basolateral amygdala in the reinstatement and extinction of fear responses to a previously extinguished conditioned stimulus. *Learning & Memory*, 17(2), 86-96.

- Laurent, V., Marchand, A. R., & Westbrook, R. F. (2008). The basolateral amygdala is necessary for learning but not relearning extinction of context conditioned fear. *Learning & Memory, 15*(5), 304-314.
- Lebrón-Milad, K., Tsareva, A., Ahmed, N., & Milad, M. R. (2013). Sex differences and estrous cycle in female rats interact with the effects of fluoxetine treatment on fear extinction. *Behavioural Brain Research, 253*, 217-222.
- Lebrón, K., Milad, M. R., & Quirk, G. J. (2004). Delayed recall of fear extinction in rats with lesions of ventral medial prefrontal cortex. *Learning & Memory, 11*(5), 544-548.
- LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184.
- LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *The Journal of Neuroscience, 10*(4), 1062-1069.
- LeDoux, J. E., Iwata, J., Cicchetti, P., & Reis, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *The Journal of Neuroscience, 8*(7), 2517-2529.
- Lee, B. H., Kim, H., Park, S. H., & Kim, Y. K. (2007). Decreased BDNF level in depressive patients. *Journal of Affective Disorders, 101*(1-3), 239-244.
- Lee, H. J., Choi, J. S., Brown, T. H., & Kim, J. J. (2001). Amygdalar nmda receptors are critical for the expression of multiple conditioned fear responses. *The Journal of Neuroscience, 21*(11), 4116-4124.
- Lee, H., & Kim, J. J. (1998). Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. *The Journal of Neuroscience, 18*(20), 8444-8454.

Lee, Y., Walker, D., & Davis, M. (1996). Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience*, *110*(4), 836-839.

Lesage, A. D., Boyer, R., Grunberg, F., Vanier, C., Morissette, R., Ménard-Buteau, C., & Loyer, M. (1994). Suicide and mental disorders: A case-control study of young men. *The American Journal of Psychiatry*, *151*(7), 1063-1068.

Leventopoulos, M., Rüedi-Bettschen, D., Knuesel, I., Feldon, J., Pryce, C. R., & Opacka-Juffry, J. (2007). Long-term effects of early life deprivation on brain glia in Fischer rats. *Brain Research*, *1142*, 1119-1126.

Li, L. F., Lu, J., Li, X. M., Xu, C. L., Deng, J. M., Qu, R., & Ma, S. P. (2012). Antidepressant-like effect of magnolol on BDNF up-regulation and serotonergic system activity in unpredictable chronic mild stress treated rats. *Phytotherapy Research*, *26*(8), 1189-1194.

Li, N., Lee, B., Liu, R., Banasr, M., Dwyer, J. M., Iwata, M., Li, X., Aghajanian, G., & Duman, R. S. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*, *329*, 959-964.

Li, N., Liu, R., Dwyer, J. M., Banasr, M., Lee, B., Son, H., Li, X., Aghajanian, G., & Duman, R. S. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological Psychiatry*, *69*(8), 754-761.

Li, X., Inoue, T., Hashimoto, S., & Koyama, T. (2001). Effect of chronic administration of flesinoxan and fluvoxamine on freezing behavior induced by conditioned fear. *European Journal of Pharmacology*, *425*(1), 43-50.

- Liang, J., Lu, J., Cui, S. F., Wang, J. R., & Tu, Y. (2012a). Effect of acupuncture on expression of brain-derived neurotrophic factor gene and protein in frontal cortex and hippocampus of depression rats. *Acupuncture Research*, 37(1), 20-24.
- Liang, S., Huang, R., Lin, X., Huang, J., Huang, Z., & Liu, H. (2012b). Effects of Yulangsan polysaccharide on monoamine neurotransmitters, adenylylase activity and brain-derived neurotrophic factor expression in a mouse model of depression induced by unpredictable chronic mild stress. *Neural Regeneration Research*, 7(3), 191-196.
- Likhtik, E., Popa, D., Apergis-Schoute, J., Fidacaro, G. A., & Paré, D. (2008). Amygdala intercalated neurons are required for expression of fear extinction. *Nature*, 454(7204), 642-645.
- Liston, C., Miller, M., Goldwater, D. S., Radley, J. J., Rocher, A. B., Hof, P. R., Morrison, J. H., & McEwen, B. S. (2006). Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *The Journal of Neuroscience*, 26(30), 7870-7874.
- Liu, D., Zhang, Q., Gu, J., Wang, X., Xie, K., Xian, X., Wang, J., Jiang, H., & Wang, Z. (2014). Resveratrol prevents impaired cognition induced by chronic unpredictable mild stress in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 49, 21-29.
- Liu, J. L., Li, M., Dang, X. R., Wang, Z. H., Rao, Z. R., Wu, S. X., Li, Y. Q., & Wang, W. (2009). A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory fear responses in a pavlovian model. *PLoS One*, 4(10), e7548.
- Liu, Q., Li, B., Zhu, H. Y., Wang, Y. Q., Yu, J., & Wu, G. C. (2011). Glia atrophy in the hippocampus of chronic unpredictable stress-induced depression model rats is reversed by electroacupuncture treatment. *Journal of Affective Disorders*, 128(3), 309-313.

- Liu, R., & Aghajanian, G. K. (2008). Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: Role of corticosterone-mediated apical dendritic atrophy. *PNAS*, *105*(1), 359-364.
- Liu, R., Lee, F. S., Li, X., Bambico, F., Duman, R. S., & Aghajanian, G. K. (2012). Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biological Psychiatry*, *71*, 996-1005.
- Liu, W. H., Wang, L. Z., Zhao, S. H., Ning, Y. P., & Chan, R. C. (2012). Anhedonia and emotional word memory in patients with depression. *Psychiatry Research*, *200*(2-3), 361-367.
- Liu, Y., Lan, N., Ren, J., Wu, Y., Wang, S. T., Huang, X. F., & Yu, Y. (2015). Orientin improves depression-like behavior and BDNF in chronic stressed mice. *Molecular Nutrition & Food Research*, *59*(6), 1130-1142.
- Lowy, M. T., Gault, L., & Yamamoto, B. K. (1993). Adrenalectomy attenuates stress-induced elevations in extracellular glutamate concentrations in the hippocampus. *Journal of Neurochemistry*, *61*(5), 1957-1960.
- Luoni, A., Macchi, F., Papp, M., Molteni, R., & Riva, M. A. (2014). Lurasidone exerts antidepressant properties in the chronic mild stress model through the regulation of synaptic and neuroplastic mechanisms in the rat prefrontal cortex. *The International Journal of Neuropsychopharmacology*, *18*(4).
- Lussier, A. L., Caruncho, H. J., & Kalynchuk, L. E. (2009). Repeated exposure to corticosterone, but not restraint, decreases the number of reelin-positive cells in the adult rat hippocampus. *Neuroscience Letters*, *460*(2), 170-174.

- Lussier, A. L., Lebedeva, K., Fenton, E. Y., Guskjolen, A., Caruncho, H. J., & Kalynchuk, L. E. (2013). The progressive development of depression-like behavior in corticosterone-treated rats is paralleled by slowed granule cell maturation and decreased reelin expression in the adult dentate gyrus. *Neuropharmacology*, *71*, 174-183.
- Machado-Vieira, R., Salvadore, G., DiazGranados, N., & Zarate, C. A. (2009). Ketamine and the next generation of antidepressants with a rapid onset of action. *Pharmacology & Therapeutics*, *123*, 143-150.
- Maeng, S., & Zarate, C. A. (2007). The role of glutamate in mood disorders: Results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. *Current Psychiatry Reports*, *9*(6), 467-474.
- Maeng, S., Zarate, C. A., Du, J., Schloesser, R. J., McCammon, J., Chen, G., & Manji, H. K. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of α -Amino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid receptors. *Biological Psychiatry*, *63*, 349-352.
- Magariños, A. M., & McEwen, B. S. (1995a). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: a comparison of stressors. *Neuroscience*, *69*(1), 83-88.
- Magariños, A. M., & McEwen, B. S. (1995b). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience*, *69*(1), 89-98.
- Magariños, A. M., McEwen, B. S., Flügge, G., & Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *The Journal of Neuroscience*, *69*(1), 83-88.

- Magariños, A. M., Orchinik, M., & McEwen, B. S. (1998). Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: a paradox. *Brain Research*, *809*, 314-318.
- Magariños, A. M., Somoza, G., & de Nicola, A. F. (1987). Glucocorticoid negative feedback and glucocorticoid receptors after hippocampectomy in rats. *Hormone and Metabolic Research*, *19*(3), 105-109.
- Mahanty, N. K., & Sah, P. (1999). Excitatory synaptic inputs to pyramidal neurons of the lateral amygdala. *The European Journal of Neuroscience*, *11*(4), 1217-1222.
- Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An embarrassment of riches. *Neuron*, *44*(1), 5-21.
- Malinow, R., & Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience*, *25*, 103-126.
- Manev, H., Favaron, M., Guidotti, A., & Costa, E. (1989). Delayed increase of CA²⁺ influx elicited by glutamate: Role in neuronal death. *Molecular Pharmacology*, *36*, 106-112.
- Mao, Q. Q., Huang, Z., Ip, S. P., Xian, Y. F., & Che, C. T. (2012). Peony glycosides reverse the effects of corticosterone on behavior and brain BDNF in rats. *Behavioural Brain Research*, *227*, 305-309.
- Mao, Q. Q., Huang, Z., Zhong, X. M., Xian, Y. F., & Ip, S. P. (2014). Piperine reverses the effects of corticosterone on behavior and hippocampal BDNF expression in mice. *Neurochemistry International*, *74*, 36-41.
- Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *The Journal of Neuroscience*, *18*(8), 3088-3097.

- Maren, S. (1999a). Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends in Neuroscience*, 22(12), 561-567.
- Maren, S. (1999b). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditioned fear in rats. *The Journal of Neuroscience*, 19(19), 8696-8703.
- Maren, S., & Hobin, J. A. (2007). Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. *Learning & Memory*, 14(4), 318-324.
- Maren, S., Aharonov, G., & Fanselow, M. S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. *Behavioral Neuroscience*, 110(4), 718-726.
- Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural Brain Research*, 88(2), 261-274.
- Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996b). N-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience*, 110(6), 1365-1374.
- Marks, W. (2014). Fear learning as a component of a depressive phenotype in rodents. (Doctoral dissertation). University of Saskatchewan, Saskatoon.
- Marks, W. N., Fenton, E. Y., Guskjolen, A. J., & Kalynchuk, L. E. (2015). The effect of chronic corticosterone on fear learning and memory depends on dose and the testing protocol. *Neuroscience*, 289, 324-333.

- Marks, W., Fournier, N. M., & Kalynchuk, L. E. (2009). Repeated exposure to corticosterone increases depression-like behavior in two different versions of the forced swim test without altering nonspecific locomotor activity or muscle strength. *Physiology and Behavior*, *98*, 67-72.
- Maroun, M., & Richter-Levin, G. (2003). Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. *The Journal of Neuroscience*, *23*(11), 4406-4409.
- Matthews, S. C., Strigo, I. A., Simmons, A. N., Yang, T. T., & Paulus, M. P. (2008). Decreased functional coupling of the amygdala and supragenual cingulate is related to increased depression in unmedicated individuals with current major depressive disorder. *Journal of Affective Disorders*, *111*(1), 13-20.
- Mauri, M. C., Ferrara, A., Boscati, L., Bravin, S., Zamberlan, F., Alecci, M., & Invernizzi, G. (1998). Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology*, *37*(3), 124-129.
- McAllister, W. R., & McAllister, D. E. (2006). Recovery of conditioned fear by a single postextinction shock: Effect of similarity of shock contexts and of time following extinction. *Learning & Behavior*, *34*(1), 44-49.
- McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science*, *153*(3742), 1351-1358.
- McGhee, L. L., Maani, C. V., Garza, T. H., Gaylord, K. M., & Black, I. H. (2008). The correlation between ketamine and posttraumatic stress disorder in burned service members. *Journal of Trauma and Acute Care Surgery*, *64*(2), 195-199.

- McGhee, L. L., Maani, C. V., Garza, T. H., Slater, T. M., Petz, L. N., & Fowler, M. (2014). The intraoperative administration of ketamine to burned U.S. service members does not increase the incidence of post-traumatic stress disorder. *Military Medicine*, *179*(8), 41-46.
- McKernan, M. G., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature*, *390*(6660), 607-611.
- Messaoudi, E., Ying, S. W., Kanhema, T., Croll, S. D., & Bramham, C. R. (2002). Brain-derived neurotrophic factor triggers transcription-dependent, late phase long-term potentiation in vivo. *The Journal of Neuroscience*, *22*(17), 7453-7461.
- Michael-Titus, A. T., Bains, S., Jeetle, J., & Whelpton, R. (2000). Imipramine and phenelzine decrease glutamate overflow in the prefrontal cortex--a possible mechanism of neuroprotection in major depression? *Neuroscience*, *100*(4), 681-684.
- Miguel-Hidalgo, J. J., Waltzer, R., Whittom, A. A., Austin, M. C., Rajkowska, G., & Stockmeier, C. A. (2010). Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *Journal of Affective Disorders*, *127*(1-3), 230-240.
- Miguel-Hidalgo, J. J., Wei, J., Andrew, M., Overholser, J. C., Jurjus, G., Stockmeier, C. A., & Rajkowska, G. (2002). Glia pathology in the prefrontal cortex in alcohol dependence and without depressive symptoms. *Biological Psychiatry*, *52*(12), 1121-1133.
- Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature*, *420*(6911), 70-74.
- Milad, M. R., Igoe, S. A., Lebron-Milad, K., & Novales, J. E. (2009a). Estrous cycle phase and gonadal hormones influence conditioned fear extinction. *Neuroscience*, *164*(3), 887-895.

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., Zeidan, M. A., Handwerker, K., Orr, S. P., & Rauch, S. L. (2009b). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biological Psychiatry*, *66*, 1075-1082.

Milad, M. R., Vidal-Gonzalez, I., & Quirk, G. J. (2004). Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. *Behavioral Neuroscience*, *188*(2), 389-394.

Mineur, Y. S., Picciotto, M. R., & Sanacora, G. (2007). Antidepressant-like effects of ceftriaxone in C57BL/6J mice. *Biological Psychiatry*, *61*(2), 250-252.

Miracle, A. D., Brace, M. F., Huyck, K. D., Singler, S. A., & Wellman, C. L. (2006). Chronic stress impairs recall of extinction of conditioned fear. *Neurobiology of Learning and Memory*, *85*(3), 213-218.

Miserendino, M. J., Sananes, C. B., Melia, K. R., & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature*, *345*(6277), 716-718.

Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby, C. R., & Kawahara, R. (2006). Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *30*(6), 1155-1158.

Mitra, R., & Sapolsky, R. M. (2008). Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. *Proceedings of the National Academy of Sciences*, *105*(14), 5573-5578.

- Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(26), 9371-9376.
- Miyamoto, J., Tsuji, M., Takeda, H., Nawa, H., & Matsumiya, T. (2000). Pretreatment with diazepam suppresses the reduction in defensive freezing behavior induced by fluvoxamine in the conditioned fear stress paradigm in mice. *European Journal of Pharmacology*, *409*(1), 81-84.
- Miyamoto, J., Tsuji, M., Takeda, H., Ohzeki, M., Nawa, H., & Matsumiya, T. (2004). Characterization of the anxiolytic-like effects of fluvoxamine, milnacipran and risperidone in mice using the conditioned fear stress paradigm. *European Journal of Pharmacology*, *504*(1-2), 97-103.
- Mogg, K., Bradbury, K. E., & Bradley, B. P. (2006). Interpretation of ambiguous information in clinical depression. *Behaviour Research and Therapy*, *44*(10), 1411-1419.
- Moghaddam, B. (1993). Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: Comparison to hippocampus and basal ganglia. *Journal of Neurochemistry*, *60*(5), 1650-1657.
- Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *The Journal of Neuroscience*, *17*(8), 2921-2927.
- Moghaddam, B., Bolinao, M. L., Stein-Behrens, B., & Sapolsky, R. (1994). Glucocorticoids mediate the stress-induced extracellular accumulation of glutamate. *Brain Research*, *655*, 251-254.

- Molteni, R., Calabrese, F., Bedogni, F., Tongiorgi, E., Fumagalli, F., Racagni, G., & Riva, M. A. (2006). Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions. *The International Journal of Neuropsychopharmacology*, 9(3), 307-317.
- Monroe, S. M., & Simons, A. D. (1991). Diathesis-stress theories in the context of life-stress research: implications for the depressive disorders. *Psychological Bulletin*, 110, 406-425.
- Monsey, M. S., Boyle, L. M., Zhang, M. L., Nguyen, C. P., Kronman, H. G., Ota, K. T., Duman, R. S., Taylor, J. R., & Schafe, G. E. (2014). Chronic corticosterone exposure persistently elevates the expression of memory-related genes in the lateral amygdala and enhances the consolidation of a Pavlovian fear memory. *PLoS One*, 9(3), e91530.
- Monteggia, L. M., Luikart, B., Barrot, M., Theobald, D., Malkovska, I., Nef, S., Parada, L. F., & Nestler, E. J. (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biological Psychiatry*, 61(2), 187-197.
- Montezinho, L. P., Miller, S., Plath, N., Jensen, N. H., Karlsson, J. J., Witten, L., & Mørk, A. (2010). The effects of acute treatment with escitalopram on the different stages of contextual fear conditioning are reversed by atomoxetine. *Psychopharmacology (Berlin)*, 212(2), 131-143.
- Morales-Medina, J. C., Sanchez, F., Flores, G., Dumont, Y., & Quirion, R. (2009). Morphological reorganization after repeated corticosterone administration in the hippocampus, nucleus accumbens and amygdala in the rat. *Journal of Chemical Neuroanatomy*, 38, 266-272.
- Morgan, C. J., & Curran, H. V. (2012). Ketamine use: A review. *Addiction*, 107(1), 27-38.

- Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: Contribution of medial prefrontal cortex. *Neuroscience Letters*, *163*(1), 109-113.
- Morgan, M. A., Shulkin, J., & LeDoux, J. E. (2003). Ventral medial prefrontal cortex and emotional preservation: The memory for prior extinction training. *Behavioural Brain Research*, *146*(1-2), 121-130.
- Morimoto, M., Morita, N., Ozawa, H., Yokoyama, K., & Kawata, M. (1996). Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study. *Neuroscience Research*, *26*(3), 235-269.
- Mørk, A., Montezinho, L. P., Miller, S., Trippodi-Murphy, C., Plath, N., Li, Y., Gulienello, M., & Sanchez, C. (2013). Vortioxetine (Lu AA21004), a novel multimodal antidepressant, enhances memory in rats. *Pharmacology, Biochemistry, and Behavior*, *105*, 41-50.
- Moryl, E., Danysz, W., & Quack, G. (1993). Potential antidepressive properties of amantadine, memantine and bifemelane. *Pharmacology & Toxicology*, *72*(6), 394-397.
- Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and contextual stimuli. *Behavioral Neuroscience*, *111*(4), 683-691.
- Müller, M. B., Lucassen, P. J., Yassouridis, A., Hoogendijk, W. J., Holsboer, F., & Swaab, D. F. (2001). Neither major depression nor glucocorticoid treatments affects cellular integrity of the human hippocampus. *The European Journal of Neuroscience*, *14*(10), 1603-1612.
- Murray, F., Smith, D. W., & Hutson, P. H. (2008). Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. *European Journal of Pharmacology*, *583*(1), 115-127.

- Murrough, J. W., Perez, A. M., Pillemer, S., Stern, J., Parides, M. K., aan het Rot, M., Collins, K. A., Mathew, S. J., Charney, D. S., & Iosifescu, D. V. (2013). Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biological Psychiatry*, *74*(4), 250-256.
- Musazzi, L., Milanese, M., Farisello, P., Zappettini, S., Tardito, D., Barbiero, V. S., Bonifacino, T., Mallei, A., Baldelli, P., Racagni, G., Raiteri, M., Benfenati, F., Bonanno, G., & Popoli, M. (2010). Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. *PLoS One*, *5*(1), e8566.
- Nedelescu, H., Kelso, C. M., Lázaro-Muñoz, G., Purpura, M., Cain, C. K., LeDoux, J. E., & Aoki, C. (2010). Endogenous GluR1-containing AMPA receptors translocate to asymmetric synapses in the lateral amygdala during the early phase of fear memory formation: an electron microscopic immunocytochemical study. *The Journal of Comparative Neurology*, *518*(23), 4723-4739.
- Neschat-Doost, H. T., Taghavi, M. R., Moradi, A. R., Yule, W., & Dalgleish, T. (1998). Memory for emotional trait adjectives in clinically depressed youth. *Journal of Abnormal Psychology*, *107*(4), 642-650.
- Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron*, *34*, 13-25.
- Nibuya, M., Morinobu, S., & Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *The Journal of Neuroscience*, *15*(11), 7539-7547.
- Nibuya, M., Nestler, E. J., & Duman, R. S. (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *The Journal of Neuroscience*, *16*(7), 2365-2372.

- Nibuya, M., Takahashi, M., Russell, D. S., & Duman, R. S. (1999). Repeated stress increases catalytic TrkB mRNA in rat hippocampus. *Neuroscience Letters*, *267*(2), 81-84.
- Nitschke, J. B., Heller, W., Etienne, M. A., & Miller, G. A. (2004). Prefrontal cortex activity differentiates processes affecting memory in depression. *Biological Psychology*, *67*(1-2), 125-143.
- Nitta, A., Fukumitsu, H., Kataoka, H., Nomoto, H., & Furukawa, S. (1997). Administration of corticosterone alters intracellular localization of brain-derived neurotrophic factor-like immunoreactivity in the rat brain. *Neuroscience Letters*, *226*(2), 115-118.
- Nolen-Hoeksema, S. (2000). The role of rumination in depressive disorders and mixed anxiety/depressive symptoms. *Journal of Abnormal Psychology*, *109*(3), 504-511.
- Nosyreva, E., Szabla, K., Autry, A. E., Ryazanov, A. G., Monteggia, L. M., & Kavalali, E. T. (2013). Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *The Journal of Neuroscience*, *33*(16), 6990-7002.
- Nowak, G., Trullas, R., Layer, R. T., Skolnick, P., & Paul, I. A. (1993). Adaptive changes in the N-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid. *The Journal of Pharmacology and Experimental Therapeutics*, *265*(3), 1380-1386.
- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., & Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*, *307*(5950), 462-465.
- O'Doherty, D. C., Chitty, K. M., Saddiqui, S., Bennett, M. R., & Lagopoulos, J. (2015). A systematic review and meta-analysis of magnetic resonance imaging measurement of structural volumes in posttraumatic stress disorder. *Psychiatry Research*, *232*(1), 1-33.

- O'Donovan, S., Dalton, V., Harkin, A., & McLoughlin, D. M. (2014). Effects of brief pulse and ultrabrief pulse electroconvulsive stimulation on rodent brain and behaviour in the corticosterone model of depression. *International Journal of Neuropsychopharmacology*, *17*, 1477-1486.
- Okamoto, N., Nakai, T., Sakamoto, K., Nagafusa, Y., Higuchi, T., & Nishikawa, T. (2010). Rapid antidepressant effect of ketamine anesthesia during electroconvulsive therapy of treatment-resistant depression: comparing ketamine and propofol anesthesia. *The Journal of ECT*, *26*(3), 223-227.
- Ongür, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(22), 13290-13295.
- Oye, I., Paulsen, O., & Maurset, A. (1992). Effects of ketamine on sensory perception: Evidence for a role of N-methyl-D-aspartate receptors. *The Journal of Pharmacology and Experimental Therapeutics*, *260*(3), 1209-1213.
- Papciak, J., Popik, P., Fuchs, E., & Rygula, R. (2013). Chronic psychosocial stress makes rats more 'pessimistic' in the ambiguous-cue interpretation paradigm. *Behavioural Brain Research*, *256*, 305-310.
- Pape, H., & Paré, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiological Reviews*, *90*, 419-463.
- Papouin, T., & Oliet, S. H. (2014). Organization, control and function of extrasynaptic NMDA receptors. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1654), 20130601.

- Papp, M., & Moryl, E. (1993). New evidence for the antidepressant activity of MK-801, a non-competitive antagonist of NMDA receptors. *Polish Journal of Pharmacology*, 45(5-6), 549-553.
- Papp, M., & Moryl, E. (1994). Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. *European Journal of Pharmacology*, 263(1-2), 1-7.
- Papp, M., & Moryl, E. (1996). Antidepressant-like effects of 1-aminocyclopropanecarboxylic acid and D-cycloserine in an animal model of depression. *European Journal of Pharmacology*, 316(2-3), 145-151.
- Paré, D., & Smith, Y. (1993). The intercalated cell masses project to the central and medial nuclei of the amygdala in cats. *Neuroscience*, 57(4), 1077-1090.
- Parker, K. J., Schatzberg, A. F., & Lyons, D. M. (2003). Neuroendocrine aspects of hypercortisolism in major depression. *Hormones and Behavior*, 43(1), 60-66.
- Patterson, S. L., Abel, T., Deuel, T. A., Martin, K. C., Rose, J. C., & Kandel, E. R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron*, 16(6), 1137-1145.
- Paul, I. A., Nowak, G., Layer, R. T., Popik, P., & Skolnick, P. (1994). Adaptation of the N-methyl-D-aspartate receptor complex following chronic antidepressant treatments. *The Journal of Pharmacology and Experimental Therapeutics*, 269(1), 95-102.
- Pavlidis, C., Nivón, L. G., & McEwen, B. S. (2002). Effects of chronic stress on hippocampal long-term potentiation. *Hippocampus*, 12(2), 245-257.

- Pavlidis, C., Ogawa, S., Kimura, A., & McEwen, B. S. (1996). Role of adrenal steroid mineralocorticoid and glucocorticoid receptors in long-term potentiation in the CA1 field of hippocampal slices. *Brain Research*, 738(2), 229-235.
- Peluso, M. A., Glahn, D. C., Matsuo, K., Monkul, E. S., Najt, P., Zamarripa, F., Li, J., Lancaster, J. L., Fox, P. T., Gao, J. H., & Soares, J. C. (2009). Amygdala hyperactivation in untreated depressed individuals. *Psychiatry Research*, 173(2), 158-161.
- Perrine, S. A., Ghodoussi, F., Michaels, M. S., Sheikh, I. S., McKelvey, G., & Galloway, M. P. (2014). Ketamine reverses stress-induced depression-like behavior and increased GABA levels in the anterior cingulate: an 11.7 T 1H-MRS study in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 51, 9-15.
- Perrot-Sinal, T. S., Gregus, A., Boudreau, D., & Kalynchuk, L. E. (2004). Sex and repeated restraint stress interact to affect cat odor-induced defensive behavior in adult rats. *Brain Research*, 1027(1-2), 161-172.
- Peters, J., Dieppa-Perea, L. M., Melendez, L. M., & Quirk, G. J. (2010). Induction of fear extinction with hippocampal-infralimbic BDNF. *Science*, 328, 1288-1290.
- Phelps, L. E., Brutsche, N., Moral, J. R., Luckenbaugh, D. A., Manij, H. K., & Zarate, C. A. (2009). Family history of alcohol dependence and initial antidepressant response to an N-methyl-D-aspartate antagonist. *Biological Psychiatry*, 65(2), 181-184.
- Phillips, M. L., Drevets, W. C., Rauch, S. L., & Lane, R. (2003). Neurobiology of emotion perception II: Implications for major psychiatric disorders. *Biological Psychiatry*, 54(5), 515-528.
- Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience*, 106(2), 274-285.

- Pietersen, C. Y., Bosker, F. J., Doorduyn, J., Jongasma, M. E., Postema, F., Haas, J. V., Johnson, M. P., Koch, T., Vladusich, T., & den Boer, J. A. (2007). An animal model of emotional blunting in schizophrenia. *PLoS One*, *2*(12), e1360.
- Pietersen, C. Y., Bosker, F. J., Postema, F., Fokkema, D. S., Korf, J., & den Boer, J. A. (2006). Ketamine administration disturbs behavioural and distributed neural correlates of fear conditioning in the rat. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *30*, 1209-1218.
- Pitkänen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Annals of the New York Academy of Sciences*, *911*, 369-391.
- Pitman, D. L., Ottenweller, J. E., & Natelson, B. H. (1988). Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: chronic stress and habituation. *Physiology & Behavior*, *43*(1), 47-55.
- Pittenger, C., & Duman, R. S. (2008). Stress, depression, and neuroplasticity: A convergence of mechanisms. *Neuropsychopharmacology Reviews*, *33*, 88-109.
- Pittenger, C., Sanacora, G., & Krystal, J. H. (2007). The NMDA receptor as a therapeutic target in major depressive disorder. *CNS & Neurological Disorders - Drug Targets*, *6*, 101-115.
- Platt, J. E., & Stone, E. A. (1982). Chronic restraint stress elicits a positive antidepressant response on the forced swim test. *European Journal of Pharmacology*, *82*(3-4), 179-181.
- Pollard, I., White, B. M., Bassett, J. R., & Cairncross, K. D. (1975). Plasma glucocorticoid elevation and desynchronization of the estrous cycle following unpredictable stress in the rat. *Behavioral Biology*, *14*(1), 103-108.

- Popik, P., Kos, T., Sowa-Kućma, M., & Nowak, G. (2008). Lack of persistent effects of ketamine in rodent models of depression. *Psychopharmacology (Berlin)*, *198*(3), 421-430.
- Popova, D., Ágústsdóttir, A., Lindholm, J., Mazulis, U., Akamine, Y., Castrén, E., & Karpova, N. N. (2014). Combination of fluoxetine and extinction treatments forms a unique synaptic protein profile that correlates with long-term fear reduction in adult mice. *European Neuropsychopharmacology*, *24*(7), 1162-1174.
- Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatment. *European Journal of Pharmacology*, *47*(4), 379-391.
- Porsolt, R. D., Le, P. M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730-732.
- Price, R. B., Nock, M., K., Charney, D. S., & Mathew, S. J. (2009). Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biological Psychiatry*, *66*(5), 522-526.
- Przegaliński, E., Tatarczyńska, E., Dereń-Wesołek, A., & Chojnacka-Wojcik, E. (1997). Antidepressant-like effects of a partial agonist at strychnine-insensitive glycine receptors and a competitive NMDA receptor antagonist. *Neuropharmacology*, *36*(1), 31-37.
- Pugh, C. R., Fleshner, M., & Rudy, J. W. (1997a). Type II glucocorticoid receptor antagonists impair contextual but not auditory-cue fear conditioning in juvenile rats. *Neurobiology of Learning and Memory*, *67*(1), 75-79.
- Pugh, C. R., Tremblay, D., Fleshner, M., & Rudy, J. W. (1997b). A selective role for corticosterone in contextual-fear conditioning. *Behavioral Neuroscience*, *111*(3), 503-511.

- Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology Reviews*, *33*, 56-72.
- Quirk, G. J., Likhtik, E., Pelletier, J. G., & Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. *The Journal of Neuroscience*, *23*(25), 8800-8807.
- Quirk, G. J., Russo, G. K., Barron, J. L., & Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *The Journal of Neuroscience*, *20*(16), 6225-6231.
- Radley, J. J., Sisti, H. M., Hao, J., Rocher, A. B., McCall, T., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2004). Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience*, *125*(1), 1-6.
- Raio, C. M., Brignoni-Perez, E., Goldman, R., & Phelps, E. A. (2014). Acute stress impairs the retrieval of extinction memory in humans. *Neurobiology of Learning and Memory*, *112*, 212-221.
- Raison, C. L., & Miller, A. H. (2003). When not enough is too much: The role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *American Journal of Psychiatry*, *160*(9), 1554-1565.
- Rajkowska, G., Halaris, A., & Selemon, L. D. (2001). Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biological Psychiatry*, *49*(9), 741-752.
- Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dille, G., Pittman, S. D., Meltzer, H. Y., Overholser, J. C., Roth, B. L., & Stockmeier, C. A. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biological Psychiatry*, *45*(9), 1085-1098.

- Rasmussen, K. G., Lineberry, T. W., Galardy, C. W., Kung, S., Lapid, M. I., Palmer, B. A., Ritter, M. J., Schak, K. M., Sola, C. L., Hanson, A. J., & Frye, M. A. (2013). Serial infusions of low-dose ketamine for major depression. *Journal of Psychopharmacology*, 27(5), 444-450.
- Rasmusson, A. M., Shi, L., & Duman, R. (2002). Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. *Neuropsychopharmacology*, 27(2), 133-142.
- Rau, V., & Fanselow, M. S. (2009). Exposure to a stressor produces a long lasting enhancement of fear learning in rats. *Stress*, 12(2), 125-133.
- Ravinder, S., Burghardt, N. S., Brodsky, R., Bauer, E. P., & Chattarji, S. (2013). A role for the extended amygdala in the fear-enhancing effects of acute selective serotonin reuptake inhibitor treatment. *Translational Psychiatry*, 3, e209.
- Ray, B., Gaskins, D. L., Sajdyk, T. J., Spence, J. P., Fitz, S. D., Shekhar, A., & Lahiri, D. K. (2011). Restraint stress and repeated corticotrophin-releasing factor receptor activation in the amygdala both increase amyloid- β precursor protein and amyloid- β peptide but have divergent effects on brain-derived neurotrophic factor and pre-synaptic proteins in the prefrontal cortex of rats. *Neuroscience*, 184, 139-150.
- Rentesi, G., Antoniou, K., Marselos, M., Fotopoulus, A., Alboycharali, J., & Konstandi, M. (2010). Long-term consequences of early maternal deprivation in serotonergic activity and HPA function in adult rat. *Neuroscience Letters*, 480(1), 7-11.
- Rescorla, R. A., & Heth, C. D. (1975). Reinstatement of fear to an extinguished conditioned stimulus. *Journal of Experimental Psychology: Animal Behavioral Processes*, 1(1), 88-96.

- Reul, J. M. H. M., & de Kloet, E. R. (1985). Two receptor systems for corticosterone in the rat brain: microdistribution and differential occupation. *Endocrinology*, *117*(6), 2505-2511.
- Rey, M., Carlier, E., Talmi, M., & Soumireu-Mourat, B. (1994). Corticosterone effects on long-term potentiation in mouse hippocampal slices. *Neuroendocrinology*, *60*(1), 36-41.
- Reznikov, L. R., Grillo, C. A., Piroli, G. G., Pasumarthi, R. K., Reagan, L. P., & Fadel, J. (2007). Acute stress-mediated increases in extracellular glutamate levels in the rat amygdala: Differential effects of antidepressant treatment. *The European Journal of Neuroscience*, *25*(10), 3109-3114.
- Ricken, R., Adil, M., Lange, C., Krusche, E., Stamm, T. J., Gaus, S., Koehler, S., Nase, S., Bschor, T., Richter, C., Steinacher, B., Heinz, A., Rapp, M. A., Borgwardt, S., Hellweg, R., & Lang, U. E. (2013). *Journal of Clinical Psychopharmacology*, *33*(6), 806-809.
- Robinson, O. J., & Sahakian, B. J. (2008). Recurrence in major depressive disorder: a neurocognitive perspective. *Psychological Medicine*, *38*(3), 315-318.
- Rocher, C., Spedding, M., Munoz, C., & Jay, T. M. (2004). Acute stress-induced changes in hippocampal/prefrontal circuits in rats: Effects of antidepressants. *Cerebral Cortex*, *14*(2), 224-229.
- Rodrigues, S. M., & Sapolsky, R. M. (2009). Disruption of fear memory through dual-hormone gene therapy. *Biological Psychiatry*, *65*(5), 441-444.
- Rodrigues, S. M., LeDoux, J. E., & Sapolsky, R. M. (2009). The influence of stress hormones on fear circuitry. *Annual Review of Neuroscience*, *32*, 289-313.
- Rodrigues, S. M., Schafe, G. E., & LeDoux, J. E. (2001). Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. *The Journal of Neuroscience*, *21*(17), 6889-6896.

- Rodríguez Echandia, E. L., Gonzalez, A. S., Cabrera, R., & Fraccia, L. N. (1988). A further analysis of behavioral and endocrine effects of unpredictable stress. *Physiology & Behavior*, *43*(6), 789-795.
- Rodríguez Manzanares, P. A., Isoardi, N. A., Carrer, H. F., & Molina, V. A. (2005). Previous stress facilitates fear memory, attenuates GABAergic inhibition, and increases synaptic plasticity in the rat basolateral amygdala. *The Journal of Neuroscience*, *25*(28), 8725-8734.
- Rogan, M. T., Stäubli, U. V., & LeDoux, J. E. (1997). AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. *The Journal of Neuroscience*, *17*(15), 5928-5935.
- Roiser, J. P., Elliott, R., & Sahakian, B. J. (2012). Cognitive mechanisms of treatment in depression. *Neuropsychopharmacology*, *37*(1), 117-136.
- Romanski, L. M., & LeDoux, J. E. (1992). Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *The Journal of Neuroscience*, *12*(11).
- Romanski, L. M., Clugnet, M. C., Bordi, F., & LeDoux, J. E. (1993). Somatosensory and auditory convergence in the lateral nucleus of the amygdala. *Behavioral Neuroscience*, *107*(3), 444-450.
- Romero, N., Sanchez, A., & Vazquez, C. (2014). Memory biases in remitted depression: The role of negative cognitions at explicit and automatic processing levels. *Journal of Behavioral Therapy & Experimental Psychiatry*, *45*(1), 128-135.
- Roosendaal, B., & McGaugh, J. L. (1997). Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. *Neurobiology of Learning and Memory*, *67*(2), 176-169.

- Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory, and the amygdala. *Nature Reviews: Neuroscience*, *10*(6), 423-433.
- Rosas-Vidal, L. E., Do-Monte, F. H., Sotres-Bayon, F., & Quirk, G. J. (2014). Hippocampal-prefrontal BDNF and memory for fear extinction. *Neuropsychopharmacology*, *39*, 2161-2169.
- Rosen, J. B., Hitchcock, J. M., Miserendino, M. J., Falls, W. A., Campeau, S., & Davis, M. (1992). Lesions of the perirhinal cortex but not the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. *The Journal of Neuroscience*, *12*(12), 4624-4633.
- Royer, S., & Paré, D. (2002). Bidirectional synaptic plasticity in intercalated amygdala neurons and the extinction of conditioned fear responses. *Neuroscience*, *115*(2), 455-462.
- Rumpel, S., LeDoux, J., Zador, A., & Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. *Science*, *308*(5718), 83-88.
- Russo-Neustadt, A., Beard, R. C., & Cotman, C. W. (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology*, *21*(5), 679-682.
- Rygula, R., Papciak, J., & Popik, P. (2013). Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. *Neuropsychopharmacology*, *38*(11), 2188-2196.
- Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., Agerman, K., Haapasalo, A., Nawa, H., Aloyz, R., Ernfors, P., & Castrén, E. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *The Journal of Neuroscience*, *23*(1), 349-357.

- Sachar, E. J. (1975). Twenty-four hour cortisol secretory patterns in depressed and manic patients. *Progressive Brain Research*, 42, 381.
- Sachar, E. J. (1976). Neuroendocrine dysfunction in depressive illness. *Annual Review of Medicine*, 27, 389-396.
- Sachar, E. J., & Baron, M. (1979). The biology of affective disorders. *Annual Review of Neuroscience*, 2, 505-517.
- Sah, P., Westbrook, R. F., & Lüthi, A. (2008). Fear conditioning and long-term potentiation in the amygdala: What really is the connection? *Annals of the New York Academy of Sciences*, 1129, 88-95.
- Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, 62(1), 63-77.
- Sanacora, G., Zarate, C. A., Krystal, J. H., & Manji, H. K. (2008). Targeting the glutamatergic system to develop novel, improve therapeutics for mood disorders. *Nature Reviews: Drug Discovery*, 7(5), 426-437.
- Sananes, C. B., & Davis, M. (1992). N-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behavioral Neuroscience*, 106(1), 72-80.
- Sandi, C., Merino, J. J., Cordero, M. I., Touyarot, K., & Venero, C. (2001). Effects of chronic stress on contextual fear conditioning and the hippocampal expression of the neural cell adhesion molecule, its polysialylation, and L1. *Neuroscience*, 102(2), 329-339.
- Sandi, C., Venero, C., & Guaza, C. (1996). Novelty-related rapid locomotor effects of corticosterone in rats. *The European Journal of Neuroscience*, 8(4), 794-800.

- Santini, E., Muller, R. U., & Quirk, G. J. (2001). Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *The Journal of Neuroscience*, *21*(22), 9009-9017.
- Santos, J. M., Martinez, R. C., & Brandão, M. L. (2006). Effects of acute and subchronic treatments with fluoxetine and desipramine on the memory of fear in moderate and high-intensity contextual conditioning. *European Journal of Pharmacology*, *542*(1-3), 121-128.
- Satoh, E., & Shimeki, S. (2010). Acute restraint stress enhances calcium mobilization and glutamate exocytosis in cerebrocortical synaptosomes from mice. *Neurochemical Research*, *35*(5), 693-701.
- Savitz, J., & Drevits, W. C. (2009). Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. *Neuroscience & Biobehavioral Reviews*, *33*(5), 699-771.
- Schaefer, H. S., Putnam, K. M., Benca, R. M., & Davidson, R. J. (2006). Event-related functional magnetic resonance imaging measures of neural activity to positive social stimuli in pre- and post-treatment depression. *60*(9), 974-986.
- Scheller, M., Bufler, J., Hertle, I., Schneck, H. J., Franke, C., & Kochs, E. (1996). Ketamine blocks currents through mammalian nicotinic acetylcholine receptor channels by interaction with both the open and closed state. *Anesthesia and Analgesia*, *83*(4), 830-836.
- Schmidt, H. D., & Duman, R. S. (2010). Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology*, *35*(12), 2378-2391.

- Schönenbeg, M., Reichwald, U., Domes, G., Badke, A., & Hautzinger, M. (2005). Effects of peritraumatic ketamine medication on early and sustained posttraumatic stress symptoms in moderately injured accident victims. *Psychopharmacology*, *182*, 420-425.
- Schroeder, B. W., & Shinnick-Gallagher, P. (2005). Fear learning induces persistent facilitation of amygdala synaptic transmission. *The European Journal of Neuroscience*, *22*(7), 1775-1783.
- Seale, J. V., Wood, S. A., Atkinson, H. C., Bate, E., Lightman, S. L., Ingram, C. D., Jessop, D. S., & Harbuz, M. S. (2004a). Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *Journal of Neuroendocrinology*, *16*(6), 516-524.
- Seale, J. V., Wood, S. A., Atkinson, H. C., Harbuz, M. S., & Lightman, S. L. (2004b). Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. *Journal of Neuroendocrinology*, *16*(12), 989-998.
- Sears, C. R., Thomas, C. L., LeHuquet, J. M., & Johnson, J. C. S. (2010). Attentional biases in dysphoria: An eye-tracking study of the allocation and disengagement of attention. *Cognition and Emotion*, *24*(8), 1349-1368.
- Seib, L. M., & Wellman, C. L. (2003). Daily injections alter spine density in rat medial prefrontal cortex. *Neuroscience Letters*, *337*, 29-32.
- Sen, S., Duman, R., & Sanacora, G. (2008). Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biological Psychiatry*, *64*(6), 527-532.
- Shansky, R. M. & Morrison, J. H. (2009). Stress-induced dendritic remodeling in the medial prefrontal cortex: Effects of circuit, hormones and rest. *Brain Research*, *1293*, 108-113.

- Shansky, R. M., Hamo, C., Hof, P. R., Lou, W., McEwen, B. S., & Morrison, J. H. (2010). Estrogen promotes stress sensitivity in a prefrontal cortex-amygdala pathway. *Cerebral Cortex*, *20*(11), 2560-2567.
- Sheline, Y. I., Barch, D. M., Donnelly, J. M., Ollinger, J. M., Snyder, A. Z., & Mintun, M. A. (2001). Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biological Psychiatry*, *50*(9), 651-658.
- Sheline, Y. I., Gado, M. H., & Kraemer, H. C. (2003). Untreated depression and hippocampal volume loss. *American Journal of Psychiatry*, *160*(8), 1516-1518.
- Sheline, Y. I., Sanghavi, M., Mintun, M. A., & Gado, M. H. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *The Journal of Neuroscience*, *19*(12), 5034-5043.
- Shen, H., Igarashi, H., Imamura, N., Matsuki, N., & Nomura, H. (2013). N-methyl-D-aspartate receptors and protein synthesis are necessary for reinstatement of conditioned fear. *Neuroreport*, *24*(14), 763-767.
- Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., Nakazato, M., Watanabe, H., Shinoda, N., Okada, S., & Iyo, M. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biological Psychiatry*, *54*(1), 70-75.
- Shimizu, E., Tang, Y. P., Rampon, C., & Tsien, J. Z. (2000). NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. *Science*, *290*(5494), 1170-1174.

- Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S., & Duman, R. S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *The Journal of Neuroscience*, 22(8), 3251-3261.
- Shors, T. J., Seib, T. B., Levine, S., & Thompson, R. F. (1989). Inescapable versus escapable shock modulates long-term potentiation in the rat hippocampus. *Science*, 244(4901), 224-226.
- Si, X., Miguel-Hidalgo, J. J., O'Dwyer, G., Stockmeier, C. A., & Rajkowska, G. (2004). Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. *Neuropsychopharmacology*, 29(11), 2088-2096.
- Sierra-Mercado, D., Corcoran, K. A., Lebrón-Milad, K., & Quirk, G. J. (2006). Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction. *The European Journal of Neuroscience*, 24(6), 1751-1758.
- Skolnick, P. (1999). Antidepressants for the new millennium. *European Journal of Pharmacology*, 375(1-3), 31-40.
- Skórzewska, A., Bidziński, A., Lehner, M., Turzyńska, D., Wisłowska-Stanek, A., Sobolewska, A., Szyndler, J., Maciejak, P., Taracha, E., & Płaźnik, A. (2006). The effects of acute and chronic administration of corticosterone on rat behavior in two models of fear responses, plasma corticosterone concentration, and c-Fos expression in the brain structures. *Pharmacology, Biochemistry and Behavior*, 85, 522-534.
- Slavich, G. M., Monroe, S. M., & Gotlib, I. H. (2011). Early parental loss and depression history: Associations with recent life stress in major depressive disorder. *Journal of Psychiatric Research*, 45, 1146-1152.

- Slavich, G. M., O'Donovan, A., Epel, E. S., & Kemeny, M. E. (2010). Black sheep get the blues: a psychobiological model of social rejection and depression. *Neuroscience & Biobehavioral Reviews*, 33(7), 1169-1175.
- Slipczuk, L., Tomaiuolo, m., Garagoli, F., Weisstaub, N., Katche, C., Bekinschtein, P., & Medina, J. H. (2013). Attenuating the persistence of fear memory storage using a single dose of antidepressant. *Molecular Psychiatry*, 18(1), 7-8.
- Smith, D. J., Bouchal, R. L., deSanctis, C. A., Monroe, P. J., Amedro, J. B., Perrotti, J. M., & Crisp, T. (1987). Properties of the interaction between ketamine and opiate binding sites in vivo and in vitro. *Neuropharmacology*, 26(9), 1253-1260.
- Smith, M. A., Makino, S., Kvetnansky, R., & Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *The Journal of Neuroscience*, 15(3), 1768-1777.
- Song, L., Che, W., Min-Wei, W., Murakami, Y., & Matsumoto, K. (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacology, Biochemistry*, 83(2), 186-193.
- Sonino, N., & Fava, G. A. (2002). Erratum to "CNS drugs in Cushing's disease: pathophysiological and therapeutic implications for mood disorders." [Prog. Neuro-Psychol. Bio. Psychiatry, 26, 763 (2002)]. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 26, 1011-1018.
- Sotres-Bayon, F., Bush, D. E. A., & LeDoux, E. (2007). Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. *Neuropsychopharmacology*, 32, 1929-1940.

- Sotres-Bayon, F., Diaz-Mataix, L., Bush, D. E. A., & LeDoux, J. E. (2009). Dissociable roles for the ventromedial prefrontal cortex and amygdala in fear extinction: NR2B contribution. *Cerebral Cortex*, 19, 474-482.
- Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., & Quirk, G. J. (2012). Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron*, 76, 804-812.
- Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F. X., & Paula-Barbosa, M. M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience*, 97(2), 253-266.
- Spennato, G., Zerbib, C., Mondadori, C., & Garcia, R. (2008). Fluoxetine protects hippocampal plasticity during conditioned fear stress and prevents fear learning potentiation. *Psychopharmacology (Berlin)*, 196(4), 583-589.
- Starkman, M. N., Giordani, B., Gebarski, S. S., Berent, S., Schork, M. A., & Schteingart, D. E. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry*, 46, 1595-1602.
- Starkman, M. N., Schteingart, D. E., & Schork, M. A. (1986). Cushing's syndrome after treatment: changes in cortisol and CTH levels, and amelioration of the depressive syndrome. *Psychiatry Research*, 19, 177-188.
- Stein-Behrens, B. A., Elliott, E. M., Miller, C. A., Schilling, J. W., Newcombe, R., & Sapolsky, R. M. (1992). Glucocorticoids exacerbate kainic acid-induced extracellular accumulation of excitatory amino acids in the rat hippocampus. *Journal of Neurochemistry*, 58(5), 1730-1735.

- Stein-Behrens, B. A., Lin, W. J., & Sapolsky, R. M. (1994). Physiological elevations of glucocorticoids potentiate glutamate accumulation in the hippocampus. *Journal of Neurochemistry*, *63*(2), 596-602.
- Sterner, E. Y., & Kalynchuk, L. E. (2010). Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: Relevance to depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *34*, 777-790.
- Stone, E. A., & Lin, Y. (2008). An anti-immobility effect of exogenous corticosterone in mice. *European Journal of Pharmacology*, *580*(1-2), 135-142.
- Stone, J. M., Dietrich, C., Edden, R., Mehta, M. A., De Simmoni, S., Reed, L. J., Krystal, J. H., Nutt, D., & Barker, G. J. (2012). Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. *Molecular Psychiatry*, *17*(7), 664-665.
- Strunk, D. R., & Adler, A. D. (2009). Cognitive biases in three prediction tasks: a test of the cognitive model of depression. *Behaviour Research and Therapy*, *47*(1), 34-40.
- Strunk, D. R., Lopez, H., & DeRubeis, R. J. (2006). Depressive symptoms are associated with unrealistic negative predictions of future life events. *Behaviour Research and Therapy*, *44*(6), 861-882.
- Stuhrmann, A., Suslow, T., & Dannlowski, U. (2011). Facial emotion processing in major depression: a systematic review of neuroimaging findings. *Biology of Mood & Anxiety Disorders*, *1*(1), 1-17.
- Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: review and meta-analysis. *The American Journal of Psychiatry*, *10*, 1552-1562.

- Surguladze, S., Brammer, M. J., Keedwell, P., Giampietro, V., Young, A. W., Travis, M. J., Williams, S. C., & Phillips, M. L. (2005). A differential pattern of neural response toward sad versus happy facial expressions in major depressive disorder. *Biological Psychiatry*, *57*(3), 201-209.
- Suslow, T., Konrad, C., Kugel, H., Rumstadt, D., Zwitterlood, P., Schöning, S., Ohrmann, P., Bauer, J., Pyka, M., Kersting, A., Arolt, V., Heindel, W., & Dannlowski, U. (2010). Automatic mood-congruent amygdala responses to masked facial expressions in major depression. *Biological Psychiatry*, *67*(2), 155-160.
- Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, Satoshi. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *The Journal of Neuroscience*, *24*(20), 4787-4795.
- Takahashi, T., Morinobu, S., Iwamoto, Y., & Yamawaki, S. (2006). Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats. *Psychopharmacology (Berlin)*, *189*(2), 165-173.
- Takei, N., Inamura, N., Kawamura, M., Namba, H., Hara, K., Yonezawa, K., & Nawa, H. (2004). Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *The Journal of Neuroscience*, *24*(44), 9760-9769.
- Taliaz, D., Stall, N., Dar, D. E., & Zangen, A. (2010). Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Molecular Psychiatry*, *15*(1), 80-92.
- Tao, X., Finkbeiner, S., Arnold, D. B., Shaywitz, A. J., & Greenberg, M. E. (1998). Ca²⁺ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron*, *20*, 709-726.

- Tata, D. A., & Anderson, B. J. (2010). The effects of chronic glucocorticoid exposure on dendritic length, synapse numbers and glial volume in animal models: implications for hippocampal volume reductions in depression. *Physiology & Behavior*, *99*, 186-193.
- Tata, D. A., Marciano, V. A., & Anderson, B. J. (2006). Synapse loss from chronically elevated glucocorticoids: relationship to neuropil volume and cell number in hippocampal area CA3. *Journal of Comparative Neurology*, *498*(3), 363-374.
- Taylor, J. L., & John, C. H. (2004). Attentional and memory bias in persecutory delusions and depression. *Psychopathology*, *37*(5), 233-241.
- Thase, M. E., Haight, B. R., Richard, N., Rockett, C. B., Mitton, M., Modell, J. G., VanMeter, S., Harriett, A. E., & Wang, Y. (2005). Remission rates following antidepressant therapy with bupropion or selective serotonin reuptake inhibitors: a meta-analysis of original data from 7 randomized controlled trials. *The Journal of Clinical Psychiatry*, *66*(8), 974-981.
- Thome, J., Sakai, N., Shin, K., Steffen, C., Zhang, Y. J., Impey, S., Storm, D., & Duman, R. S. (2000). cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. *The Journal of Neuroscience*, *20*(11), 4030-4036.
- Thompson, B. L., Erickson, K., Schulkin, J., & Rosen, J. B. (2004). Corticosterone facilitates retention of contextually conditioned fear and increases CRH mRNA expression in the amygdala. *Behavioural Brain Research*, *149*(2), 209-215.
- Tiraboschi, E., Tardito, D., Kashahara, J., Moraschi, S., Pruneri, P., Gennarelli, M., Racagni, G., & Popoli, M. (2004). Selective phosphorylation of nuclear CREB by fluoxetine is linked to activation of CaM kinase IV and MAP kinase cascades. *Neuropsychopharmacology*, *29*(10), 1831-1840.

- Tops, M., van der Pompe, G., Baas, D., Mulder, L. J., Den Boer, J. A., Meijman, T. F., & Korf, J. (2003). Acute cortisol effects on immediate free recall and recognition of nouns depend on stimulus valance. *Psychophysiology*, *40*(2), 167-173.
- Toro, C. T., Hallak, J. E., Dunham, J. S., & Deakin, J. F. (2006). Glial fibrillary acidic protein and glutamine synthetase in subregions of prefrontal cortex in schizophrenia and mood disorder. *Neuroscience Letters*, *404*(3), 276-281.
- Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Riz, L., Norquist, G., Howland, R. H., Lebowitz, B., McGrath, P. J., Shores-Wilson, K., Biggs, M. M., Balasubramani, G. K., Fava, M., & STAR*D STudy Team. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. *The American Journal of Psychiatry*, *163*(1), 28-40.
- Trullas, R., & Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *European Journal of Pharmacology*, *185*(1), 1-10.
- Tully, K., Li, Y., Tsvetkov, E., & Bolshakov, V. Y. (2007). Norepinephrine enables the induction of associative long-term potentiation at thalamo-amygdala synapses. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(35), 14146-14150.
- Ulloa, J. L., Castañeda, P., Berrios, C., Diaz-Veliz, G., Mora, S., Bravo, J. A., Araneda, K., Menares, C., Morales, P., & Fiedler, J. L. (2010). Comparison of the antidepressant sertraline on differential depression-like behaviors elicited by restraint stress and repeated corticosterone administration. *Pharmacology Biochemistry & Behavior*, *97*(2), 213-221.
- Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress response. *Nature Reviews*, *10*(6), 397-409.

- Ushijima, K., Morikawa, T., To, H., Higuchi, S., & Ohdo, S. (2006). Chronobiological disturbances with hyperthermia and hypercortisolism induced by chronic mild stress in rats. *Behavioural Brain Research*, *173*(2), 326-330.
- van Tol, M. J., van der Wee, N. J., van den Heuvel, O. A., Nielen, M. M., Demenescu, L. R., Aleman, A., Renken, R., van Buchem, M. A., Zitman, F. G., & Veltman, D. J. (2010). regional brain volume in depression and anxiety disorders. *Archives of General Psychiatry*, *67*(10), 1002-1011.
- VanElzaker, M. B., Dahlgren, M. K., Davis, F. C., Dubois, S., & Shin, L. M. (2013). From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiology of Learning and Memory*.
- Veilleux-Lemiux, D., Castel, A., Carrier, D., Beaudry, F., & Vachon, P. (2013). Pharmacokinetics of ketamine and xylazine in young and old Spague-Dawley rats. *Journal of the American Association for Laboratory Animal Service*, *52*(5), 567-570.
- Venero, C., & Borrell, J. (1999). Rapid glucocorticoid effects on excitatory amino acid levels in the hippocampus: A microdialysis study in freely moving rats. *European Journal of Neuroscience*, *11*(7), 2465-2473.
- Viau, V., & Meaney, M. J. (1991). Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology*, *129*(5), 2503-2511.
- Victor, T. A., Furey, M. L., Fromm, S. J., Ohman, A., & Drevets, W. C. (2010). Relationship between amygdala responses to masked faces and mood state and treatment in major depressive disorder. *Archives of General Psychiatry*, *67*(11), 1128-1138.
- Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S. L., & Quirk, G. J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learning and Memory*, *13*, 728-733.

- Videbech, P., & Ravnkilde, B. (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. *American Journal of Psychiatry*, *161*(11), 1957-1966.
- Virgin, C. E., Ha, T. P., Packan, D. R., Tombaugh, G. C., Yang, S. H., Horner, H. C., & Sapolsky, R. M. (1991). Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: Implications for glucocorticoid neurotoxicity. *Journal of Neurochemistry*, *57*(4), 1422-1428.
- Vogel, W. H., & Jensh, R. (1988). Chronic stress and plasma catecholamine and corticosterone levels in male rats. *Neuroscience Letters*, *87*(1-2), 183-188.
- Vyas, A., Bernal, S., & Chattarji, S. (2003). Effects of chronic stress on dendritic arborization in the central and extended amygdala. *Brain Research*, *965*(1-2), 290-294.
- Vyas, A., Jadhav, S., & Chattarji, S. (2006). Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. *Neuroscience*, *143*(2), 387-393.
- Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *The Journal of Neuroscience*, *22*(15), 6810-6818.
- Vyas, A., Pillai, A. G., & Chattarji, S. (2004). Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. *Neuroscience*, *128*(4), 667-673.
- Walker, D. L., & Davis, M. (2000). Involvement of NMDA receptors within the amygdala in short- versus long-term memory for fear conditioning as assessed with fear-potentiated startle. *Behavioral Neuroscience*, *114*(6), 1019-1033.
- Wang, C. C., & Wang, S. J. (2009). Modulation of presynaptic glucocorticoid receptors on glutamate release from rat hippocampal nerve terminals. *Synapse*, *63*(9), 745-751.

- Wang, C., Liu, F., Patterson, T. A., Paule, M. G., & Slikker, W. (2013). Preclinical assessment of ketamine. *CNS Neuroscience & Therapeutics*, *19*, 448-453.
- Wang, X., Chen, Y., Zhou, X., Liu, F., Zhang, T., & Zhang, C. (2012). Effects of propofol and ketamine as combined anesthesia for electroconvulsive therapy in patients with depressive disorder. *The Journal of ECT*, *28*(2), 128-132.
- Watanabe, Y., Gould, E., & McEwen, B. S. (1992b). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research*, *588*(2), 341-345.
- Watanabe, Y., Gould, E., Daniels, D. C., Cameron, H., & McEwen, B. S. (1992a). Tianeptine attenuates stress-induced morphological changes in the hippocampus. *European Journal of Pharmacology*, *222*(1), 157-162.
- Weinstock, M., Razin, M., Schorer-Apelbaum, D., Men, D., & McCarty, R. (1998). Gender differences in sympathoadrenal activity in rats at rest and in response to footshock stress. *International Journal of Developmental Neuroscience*, *16*(3-4), 289-295.
- Wellman, C. L. (2001). Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *Journal of Neurobiology*, *49*(3), 245-253.
- Wilber, A. A., Walker, A. G., Southwood, C. J., Farrell, M. R., Lin, G. L., Rebec, G. V., & Wellman, C. L. (2011). Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction. *Neuroscience*, *174*, 115-131.
- Wilkinson, P. O., & Goodyer, I. M. (2011). Childhood adversity and allostatic overload of the hypothalamic-pituitary-adrenal axis: a vulnerability model for depressive disorders. *Development and Psychopathology*, *23*(4), 1017-1037.

- Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berlin)*, *134*(4), 319-329.
- Willner, P. (2005). Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology*, *52*(2), 90-110.
- Willner, P., Scheel-Krüger, J., & Belzung, C. (2013). The Neurobiology of depression and antidepressant action. *Neuroscience and Biobehavioral Reviews*, *37*, 2331-2371.
- Woolley, C. S., Gould, E., & McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Research*, *531*(1-2), 225-231.
- World Health Organization. (2012). Depression: A global public health concern. Retrieved November 11, 2015, from http://www.who.int/mental_health/management/depression/who_paper_depression_wfmh_2012.pdf
- Wu, T. C., Chen, H. T., Chang, H. Y., Yang, C. Y., Hsiao, M. C., Cheng, M. L., & Chen, J. C. (2013). Mineralocorticoid receptor antagonist spironolactone prevents chronic corticosterone induced depression-like behavior. *Psychoneuroendocrinology*, *38*, 871-883.
- Xu, L., Anwyl, R., & Rowan, M. J. (1997). Behavioural stress facilitates the induction of long-term depression in the hippocampus. *Nature*, *387*(6632), 497-500.
- Yamada, S., Yamamoto, M., Ozawa, H., Riederer, P., & Saito, T. (2003). Reduced phosphorylation of cyclic AMP-responsive element binding protein in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Journal of Neural Transmission*, *110*(6), 671-680.

- Yan, W. J., Tan, Y. C., Xu, J. C., Tang, X. P., Zhang, C., Zhang, P. B., & Ren, Z. Q. (2015). Protective effects of silibinin and its possible mechanism of action in mice exposed to chronic unpredictable mild stress. *Biomolecules & Therapeutics (Seoul)*, 23(3), 245-250.
- Yang, C. H., Huang, C. C., & Hsu, K. S. (2005). Behavioral stress enhances hippocampal CA1 long-term depression through the blockade of the glutamate uptake. *The Journal of Neuroscience*, 25(17), 4288-4293.
- Yang, C. h., Shi, H. S., Zhu, W. L., Wu, P., Sun, L. L., Si, J. J., Liu, M. M., Zhang, Y., Suo, L., & Yang, J. L. (2012). Venlafaxine facilitates between-session extinction and prevents reinstatement of auditory-cue conditioned fear. *Behavioural Brain Research*, 230(1), 268-273.
- Yau, S. Y., Lau, B. W. M., Tong, J. B., Wong, R., Ching, Y. P., Qiu, G., Tang, S. W., Lee, T. M. C., & So, K. F. (2011). Hippocampal neurogenesis and dendritic plasticity support running-improved spatial learning and depression-like behaviour in stressed rats. *PLoS ONE*, 6(9), e24263.
- Yau, S. Y., Li, A., Zhang, E. D., Christie, B. R., Xu, A., Lee, T. M., & So, K. F. (2014). Sustained running in rats administered corticosterone prevents the development of depressive behaviors and enhances hippocampal neurogenesis and synaptic plasticity without increasing neurotrophic factor levels. *Cell Transplantation*, 23(4-5), 481-492.
- Yeh, S. H., Mao, S. C., Lin, H. C., & Gean, P. W. (2006). Synaptic expression of glutamate receptor after encoding of fear memory in the rat amygdala. *Molecular Pharmacology*, 69(1), 299-308.
- Yilmaz, A., Schulz, D., Aksoy, A., & Canbeyli, R. (2002). Prolonged effect of an anesthetic dose of ketamine on behavioral despair. *Pharmacology, Biochemistry, and Behavior*, 71(1-2), 341-344.

- Yoshii, A., & Constantine-Paton, M. (2007). BDNF induces transport of PSD-95 to dendrites through PI3K-AKT signaling after NMDA receptor activation. *Nature Neuroscience*, *10*(6), 702-711.
- Yoshii, A., & Constantine-Paton, M. (2010). Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Developmental Neurobiology*, *70*(5), 304-322.
- Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., Alkondon, M., Yuan, P., Pribut, H. J., Sing, N. S., Dossou, K. S., Fang, Y., Huang, X. P., Mayo, C. L., Wainer, I. W., Albuquerque, E. X., Thompson, S. M., Thomas, C. J., Zarate, C. A. Jr., & Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, *533*(7604), 481-486.
- Zarate, C. A., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., Charney, D. S., & Manji, H. K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*, *63*, 856-864.
- Zetterström, T. S., Pei, Q., & Grahame-Smith, D. G. (1998). Repeated electroconvulsive shock extends the duration of enhanced gene expression for BDNF in rat brain compared with a single administration. *Brain Research: Molecular Brain Research*, *57*(1), 106-110.
- Zhang, J. C., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., Han, M., & Hahimoto, K. (2015a). Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. *Psychopharmacology (Berlin)*, *232*(23), 4325-4335.
- Zhang, L., Zhou, W., Ji, Y., Li, Y., Zhao, N., Chen, H., Xue, R., Mei, X., Zhang, Y., Wang, H., & Li, Y. (2015b). Anxiolytic effects of ketamine in animal models of posttraumatic stress disorder. *Psychopharmacology*, *232*, 663-672.

- Zhang, W., & Rosenkranz, J. A. (2013). Repeated restraint stress enhances cue-elicited conditioned freezing and impairs acquisition of extinction in an age-dependent manner. *Behavioural Brain Research, 248*, 12-24.
- Zhao, Y., Ma, R., Shen, J., Su, H., Xing, D., & Du, L. (2008a). A mouse model of depression induced by repeated corticosterone injections. *European Journal of Pharmacology, 581*(1-2), 113-120.
- Zhao, Y., Shen, J., Su, H., Li, B., Xing, D., & Du, L. (2008b). Chronic corticosterone injections induce a decrease of ATP levels and sustained activation of AMP-activated protein kinase in hippocampal tissues of male mice. *Brain Research, 1191*, 148-156.
- Zheng, X., Deschaux, O., Lavigne, J., Nachon, O., Cleren, C., Moreau, J., & Garcia, R. (2013). Prefrontal high-frequency stimulation prevents sub-conditioning procedure-provoked, but not acute stress-provoked, reemergence of extinguished fear. *Neurobiology of Learning and Memory, 101*, 33-38.
- Zhong, M., Wang, X., Xiao, J., Yi, J., Zhu, X., Liao, J., Wang, W., & Yao, S. (2011). Amygdala hyperactivation and prefrontal hypoactivation in subjects with cognitive vulnerability to depression. *Biological Psychiatry, 88*(2-3), 233-242.
- Zhu, X., Gao, R., Liu, Z., Cheng, Z., Qi, Y., Fan, C., & Yu, S. Y. (2016). Ginsenoside Rg1 reverses stress-induced depression-like behaviors and BDNF expression within the prefrontal cortex. *The European Journal of Neuroscience, 13255*.
- Zink, M., Vollmayr, B., Gebicke-Haerter, P. J., & Henn, F. A. (2010). Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. *Neuropharmacology, 58*(2), 465-473.

- Zobel, A. W., Nickel, T., Künzel, H. E., Ackl, N., Sonntag, A., Ising, M., & Holsboer, F. (2000). Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *Journal of Psychiatric Research, 34*, 171-181.
- Zobel, A., Yassouridis, A., Frieboes, R. M., & Holsboer, F. (1999). Cortisol response to the combined dexamethasone-CRH test predicts medium-term outcome in patients with remitted depression. *The American Journal of Psychiatry, 156*, 949-951.
- Zorawski, M., & Killcross, S. (2002). Posttraining glucocorticoid receptor agonist enhances memory in appetitive and aversive Pavlovian discrete-cue conditioning paradigms. *Neurobiology of Learning and Memory, 78*(2), 458-464.
- Zorawski, M., Blanding, N. Q., Kuhn, C. M., & LaBar, K. S. (2006). Effects of stress and sex on acquisition and consolidation of human fear conditioning. *Learning & Memory, 13*(4), 441-450.
- Zorawski, M., Cook, C. A., Kuhn, C. M., & LaBar, K. S. (2005). Sex, stress, and fear: Individual differences in conditioned learning. *Cognitive, Affective & Behavioral Neuroscience, 5*(2), 191-201.