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The Effects of Ketamine on Cognitive Bias in a Stress Vulnerable Chick Strain

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THE EFFECTS OF KETAMINE ON COGNITIVE BIAS IN A STRESS VULNERABLE CHICK STRAIN

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ABSTRACT

Cognitive bias is a phenomenon that presents in individuals suffering from anxiety and depression where anxious individuals tend to adopt a more pessimistic interpretation of ambiguous aversive stimuli and depressed individuals tend to adopt both a more pessimistic interpretation of ambiguous aversive stimuli as well as a less optimistic interpretation of ambiguous appetitive stimuli. Such biases have been pharmacologically reversed using anxiolytics and antidepressants. The chick anxiety-depression model has observed more pessimistic-like and less optimistic-like behavior in approach/avoidant runway performance to ambiguous aversive and ambiguous appetitive stimuli, respectively. Further, both types of cognitive biases have been reversed in a White Leghorn strain using the antidepressant imipramine. One goal of the current study was to examine whether cognitive biases of more pessimism and less optimism would manifest in a pattern reflecting the stress vulnerability and resiliency in Black Australorp and Production Red strains, respectively. Non-isolated and isolated (90 min) chicks were tested in a straight alley maze under an ambiguous appetitive (75c:25o) and an ambiguous aversive (25c:75o) stimulus cue with start and goal latency and distance traveled as the dependent measures. Less optimistic-like behavior and more pessimistic-like behaviors were observed under the 75c:25o and 25c:75o stimulus cues, respectively. Interestingly, stress vulnerability on cognitive bias in BAs presented primarily in non-isolated conditions.

A second goal of the current study was to examine if cognitive biases in BAs could be reversed in a manner that parallels the strain's differential drug responsivity, whereby ketamine

would reverse and imipramine would fail to reverse cognitive bias. Non-isolated and isolated (90 min) chicks received an administration of either a physiological saline vehicle, 10.0 mg/kg of imipramine and 10.0 mg/kg of ketamine prior to maze testing which followed the same procedure as Experiment 1. Imipramine and ketamine failed to produce a significant antidepressant effect on DVoc rates in isolated chicks. Consistent with the inability to detect a significant ketamine effect, more pessimistic-like behavior was not reversed under the 25c:75o stimulus cue. Surprisingly, not only did the 75c:25o stimulus cue fail to show less optimistic-like behavior, but the observed effects were in the opposite direction. The absence of a ketamine effect may be due to experimental procedures necessary to quantify cognitive bias.

Collectively, the current study identified cognitive biases of more pessimism and less optimism in a stress-vulnerable Black Australorp and a stress-resilient Production Red strain. Surprisingly, the most robust strain difference presented between non-isolated conditions. These findings strengthen homologies between clinical populations by providing validative support to the identification of a stress-vulnerable and stress-resilient strain which further validate the chick anxiety-depression model as a neuropsychiatric simulation.

DEDICATION

I would like to dedicate my dissertation to my mother; my best friend and my biggest fan.

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INTRODUCTION

Anxiety and Depressive Disorders

Anxiety and depression are two very common and detrimental mental health disorders. Anxiety affects approximately 40 million Americans, ages 18 years and older, while depression affects 14.8 million Americans (National Institute of Mental Health, 2009a) and 150 million people worldwide (World Health Organization, 2003). Further, with comorbidity rates ranging from 50 to 90%, many patients suffer from a concurrent presentation of anxiety and depression (Kessler, Berglund, Demleer, Jin, Merikangas, & Walters, 2005*;* Kessler, McGonagle, Zhao, Nelson, Hughes, & Eshleman, 1994; Rivas-Vazquez, Saff-Biller, Ruiz, Blais, & A. Rivas-Vazquez, 2004). Approximately 85% of people suffering from depression will present symptoms of anxiety and 90% of people suffering from anxiety will present symptoms of depression resulting in even further debilitation (Gorman, 1996-1997).

Depression is the primary cause of disability in the United States for ages 15-44 (National Institute of Mental Health, 2009a) and is the third largest contributing factor to the global burden of disease (World Health Organization, 2003). In addition to the toll on the quality of life, mental disorders produce immense financial strains associated with treatments, reduced productivity, increased incarceration, and increased mortality rates that not only the patient, but also society must endure (Surgeon General, 1999; World Health Organization, 2003). In the United States, there is an estimated $193 billion annual income deficit for all mental disorders (Kessler et al., 2008; National Institute of Mental Health, 2009b); $53 million is

estimated from depression alone (Greenburg et al., 1996). However, a more imperative issue is the limited efficacy of standard monoamine based pharmacotherapuetics as evidenced by the long delay of therapeutic action and undesirable side effects (Krishnan, 2004; Nelson, 2004; Rosenbaum & Tollefson, 2004).

Monoamine Theory of Depression

Current mainstay pharmacotherapeutic options for the treatment of depression include the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). Although each of these drug classes is chemically diverse, has varying degrees of success and possesses their own side effect profiles, they all increase biogenic monoamine levels (dopamine, serotonin and/or norepinephrine), supporting the original monoamine hypothesis of depression.

This original hypothesis stated that depression results from a depletion of biogenic monoamine levels in the brain and antidepressants work to stabilize those levels by inhibiting the metabolism or the reuptake of monoamines, thus increasing availability at the synapse (Elhwuegi, 2004; Schildkraut, 1965) Evidence to support the monoamine hypothesis of depression came from the depressive side effects of reserpine, an antihypertensive medication, (Harris, 1957; Muller, Pryer, Gibbons, & Orgain, 1955) and tetrabenazine, an antihyperkinetic, (Jankovic & Beach, 1997; Lingjaerde, 1963). Each of these therapeutic compounds depletes presynaptic stores of norepinephrine (NE), serotonin (5-HT), and dopamine (DA) (Schildkraut, 1965; Coppen, 1967). In contrast, an antituberculosis medication, iproniazid, which produced relatively euphoric side effects (Crane, 1957; Goldberg, 1964), was shown to increase NE and DA levels by inhibiting monoamine oxidase (MAO), a metabolizing enzyme (Bondy, 2002).

The monoamine oxidase inhibitors (MAOIs) were the first class of antidepressants (Boland & Keller, 2004) developed to relieve symptoms of depression by elevating 5-HT, NE and DA by inhibiting MAO (Bondy, 2002; Elhwuegi, 2004). However, MAOIs' potential for lethal side effects greatly limits their use (Boland & Keller, 2004). Taking MAOIs in combination with foods or beverages containing tyramine produce adverse reactions (Baldessarini, 1990; Krishnan, 2004) including hypertension and death (Krishnan, 2004). MAOIs can also react negatively with several medications such as some types of birth control pills, mild pain relievers (e.g., Tylenol or Advil) or herbal supplements (Treatment of Anxiety Disorders, 2009). Nevertheless, MAOIs possess similar efficacy rates compared to the tricyclic antidepressants (TCAs) (Baldessarini, 1990).

The TCAs were the first widely used antidepressants (Nelson, 2004). One classic TCA, imipramine, which inhibits the reuptake of 5-HT and NE, possesses antidepressant efficacy rates of approximately 65% (Nelson, 2004) and also produces anxiolytic effects (Diniz, dos Reis, de Castro, Medalha, & Viana, 2011). However, a disadvantage of the TCAs is the delay of onset which may be up to 2 or 3 weeks (Baldessarini, 1990). Another disadvantage is high treatment dropout rates (Boland & Keller, 2004), caused by adverse side effects including postural hypotension, dry mouth, sedation, urinary retention, constipation (Nestler, Hyman, & Malenka, 2009), dizziness, weight gain (Treatment of Anxiety Disorders, 2009), cardiovascular effects, hepatic effects and overdose (Nelson, 2004). In an attempt to increase efficacy rates and decrease the side effect profile of the existing TCAs, novel therapeutics began to focus on selectively increasing CNS levels of specific monoamines.

Although the selective serotonin reuptake inhibitors (SSRIs) do possess more tolerable side effects (Treatment of Anxiety Disorders, 2009) and lower dropout rates in treatment

programs (Boland & Keller, 2004) they fail to produce significantly higher efficacy rates compared to TCAs (Kasper & Moller, 1922; Rosenbaum & Tollefson, 2004). However, similar to the TCAs, SSRIs also show efficacy in the treatment of anxiety disorders (Davidson & Connor, 2004). For example, Plewes, Koke, & Sayler (1997) found that fluoxetine alleviated symptoms of anxiety and depression in patients with a comorbid presentation of the disorders and the efficacy was comparable to TCAs. In contrast to these benefits, some SSRIs present varying degrees of symptom alleviation and varying onsets of therapeutic action ranging from 10-42 days (Rosenbaum & Tollefson, 2004). The side effects produced by the SSRIs include nausea and nervousness. Fluoxetine, in particular, produces agitation, anxiety, tremors, sleep disturbances (Treatment of Anxiety Disorders, 2009), nausea, vomiting, dizziness, lethargy, sensory and sleep disturbances, flulike symptoms and fatigue. However, the main complaint among patients is the sexual dysfunction side effect that frequently accompanies SSRI use (Rosenbaum & Tollefson, 2004).

The original monoamine hypothesis of depression, previously described, has suffered many criticisms: 1) other compounds, such as amphetamine and cocaine, increase monoamine levels but fail to show antidepressants effects, 2) individuals respond differently to each class of antidepressants and 3) increases in monoamine levels at the synapse occur within hours after administration, but the onset of therapeutic effects takes several weeks (Elwuegi, 2004). Subsequent to continued research, the monoamine hypothesis has now undergone two additional iterations. The second iteration stated that continuous administration of antidepressants modulated receptor sensitivity and/or density thereby producing therapeutic effects (Charney, Menkes, & Heninger, 1981; Elhwuegi, 2004; Friedhof & Miller, 1983). The third iteration of the monoamine hypothesis states that antidepressants produce downstream increases in neurotrophic

factor expression (Pittenger & Duman, 2008) and increase synaptic plasticity, the latter producing therapeutic effects (Campbell & Macqueen, 2004; Duman & Aghajanian, 2012). These observations fostered the development of the neurotrophin theory of depression.

Neurotrophin Theory of Depression

The neurotrophin theory of depression states that cell loss and decreased synaptic connectivity in mood and emotion circuitry produces depressive symptomology (Duman & Aghajanian, 2012). Consistent with this hypothesis, postmortem and brain imaging studies of depressed patients have shown a decrease in cortical and limbic region volume, specifically the prefrontal cortex (PFC) and hippocampus, (MacQueen & Frod, 2011; Price & Drevets, 2010), as well as a decrease in the number of synapses in and between these regions (Perlman et al., 2012). Similarly, rodents have shown cell loss and a reduction in synapse number in these brain regions after repeated exposure to chronic unpredictable stress (Li et al., 2011). Stress, which precipitates depression in humans, decreases brain-derived neurotrophic factor (BDNF) (Krishnan & Nestler, 2008) which is necessary for the production and sustainability of neurons and synaptic connections (Dunman & Voleti, 2012).

Monoamine based antidepressants are currently thought to produce therapeutic effects by ultimately increasing BDNF expression (Pittenger & Duman, 2008**)** and neurogenesis (Campbell & Macqueen, 2004) which explains the slow onset of action of these compounds. In contrast, ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, produces rapid (within hours) and long lasting (7-10 days) (Berman et al., 2000; Zarate et al., 2006) antidepressant effects. By inhibiting NMDA receptors (NMDAR), ketamine promotes glutamate activity which ultimately increases BDNF functioning and thus antidepressant effects (N, Li, 2011). However, due to ketamine's psychotomimetic effects and high abuse potential it is limited in its use as a

pharmacotherapeutic (Duman & Aghajanian, 2012). To further advance the understanding of the neurotrophin theory of depression and novel pharmacological therapies associated with it, current research heavily relies on the development, validation and utilization of animal models.

Animal Models

Advancements in the understanding of any clinical syndrome mainly rely on two types of animal models, screening assays and simulations. According to Willner (1991a), screening assays evaluate and compare the drug action of novel compounds with known compounds to identify potential clinical uses (e.g., antidepressant or anxiolytic effects). Since they are solely concerned with observing a positive or negative outcome analogous to clinical trials, screening assays must adhere to predictive validity, the degree to which a model can accurately predict the performance of novel or known drug actions based on the outcome observed. Simulations are intended to mimic a disorder by assessing behaviors relative to a particular species and disorder to determine the etiology, physiological foundations, and responses to drug treatments. For a simulation to correctly model a clinical disorder, it is to adhere not only to predictive validity but also to construct and face validity. Construct validity is the theoretical rationale upon which the model is founded and asks a variety of questions narrowing down to, "Does the model correctly measure the characteristics associated with the human disorder." Face validity evaluates the similarity between the model and the actual human disorder in terms of the etiology, physiology, symptomatology and treatment effects. The simulation and the disorder should have as many similarities as possible and the face validity is subject to change as new information arises (Willner, 1991a). In addition to being characterized by these three types of validity, animal models should also adhere to the principles of generalizability and reproducibility (Miczek & de

Wit, 2008; van der Staay, 2006). The utilization of sound animal models has led to significant advances in our knowledge and treatment of anxiety and depression (Willner, 1991a).

Traditional animal models of anxiety induce anxiety-like symptoms using conflict and/or conditioned or unconditioned responses to threat. Anxiety models also use exploratory paradigms such as the open field test, elevated plus maze, holeboard and light-dark box (for reviews see, Bourin, Petit-Demoulie`re, Dhonnchadha & Hascoäet 2007; Ladner, 1991). Such tests are designed to measure avoidance, the latency period to perform the task, which is increased with stress-inducing stimuli or situations, and reduced after the administration of anxiolytic drugs (Green & Hodges, 1991). Most animal models of depression employ either stress (e.g., chronic mild stress), learned helplessness (e.g., forced swim test) or separation-isolation paradigms to engender depressive-like characteristics in animals (for review see, Willner, 1991b). Animal models of depression attempt to mirror the characteristics of the human disorder, to examine the drug-induced behavioral changes and the neurochemical effects (McArthur & Borsini, 2006). Animal models help us to understand and treat anxiety and depression by identifying of the disorders' multifaceted symptom profiles and determining the etiological foundations. These findings initiate the development of more target specific drug treatments and facilitate research of the physiological responses to such treatments (Ladner, 1991). However, despite these advances, many criticisms of animal models have been recently raised.

Kalueff, Wheaton, and Murphy (2007) offer a number of criticisms of current rodent-based models of anxiety and depression. First, animal models provide questionable within- and between-laboratory reliability owing to varying results. A second criticism of animal models is the constraints of species-specific behaviors through artificial environments (e.g., exploratory

paradigms); which often restrict the environment so any movements may be due solely to the environment, not necessarily innate behaviors (Whishaw, Gharbawie, Clark, & Lehmann, 2006). Other criticisms include an over emphasis on either internal genetic (e.g., strain differences) or external epigenetic (e.g., environmental) factors. Lastly, these authors as well as Frazer & Morilak (2005) suggest that animal models should attempt to simulate the multi-syndromal aspect of anxiety and depressive disorders (Frazer & Morilak, 2005; Kalueff et al., 2007) due to the increasing amount of data showing high comorbidity rates between anxiety and depressive disorders (Gorman, 1996-1997; Kessler et al., 1994, 2005; Rivas-Vazquez et al., 2004).

Because the comorbid presentation rates of anxiety and depression are so prevalent (Gorman, 1996-1997; Kessler et al., 1994, 2005; Rivas-Vazquez et al., 2004), the two disorders are now suggested to be on a single continuum (Kasper, 2001). The anxiety-depression continuum theory states that anxiety and depression are different temporal facets due to repeated stressors with anxiety-like symptoms preceding depression-like symptoms (Kasper, 2001). However, current animal models of anxiety and depression examine the two disorders separately, so as to mirror the classification by the Diagnostic and Statistical Manual of Mental Disorders-IV (American Psychiatric Association, 1994). Owing to the recent data showing high comorbidity rates, current animal models are not producing adequate information to treat anxiety and/or depression. To resolve this lack of sufficient animal models, Kalueff et al., (2008, 2007) suggest a "hybridization" of anxiety and depression models.

Chick Anxiety-Depression Model

Sufka et al. (2006) proposed such a "hybrid" model to examine the anxiety-depression continuum theory by combining two paradigms that measured the same behavioral response, distress vocalizations, to a social isolation stressor in domestic fowl chicks (Lehr, 1989;

Panksepp, 2003, Panksepp, Meeker, & Bean, 1980; Panksepp, Vilberg, Bean, Coy, & Kastin, 1978). This "hybrid" model, the chick anxiety-depression continuum model, involves isolating chicks from conspecifics and measuring the distress vocalizations (DVocs) over a 2-hour test session which reveals both an anxiety-like phase and a depression-like phase within a single paradigm (Sufka et al., 2006). Within the first 5 min of isolation, DVocs rates are relatively high which is indicative of an anxiety-like (panic-like) state whereby chicks attempt to reestablish social contact. In the next 20-25 min of isolation, DVoc rates display a steady decline which is characterized as a transitional period. In the final 30-120 min of isolation, DVocs reach a plateau of approximately 50% of the initial rate which is characteristic of a depression-like state (i.e., behavioral despair).

In addition, the anxiety- and depression-like phases can be pharmacologically dissociated by administering diverse compounds possessing anxiolytic and antidepressant effects. Compounds with anxiolytic effects (e.g., chlordiazepoxide, clonidine, and imipramine) attenuate the high DVoc rates during the anxiety-like phase, whereas compounds with antidepressant effects (e.g., imipramine, maprotiline, and fluoxetine) attenuate the reduction in DVoc rates during the depression-like phase (Sufka et al., 2006; Warnick, Huang, Acevedo & Sufka, 2009). Further, common stress and depression biomarkers present within the model and include elevated corticosterone and interleukin-6 (IL-6) levels (Sufka et al., 2006; Warnick et al., 2009). A recent study that efficacy screened 7 compounds targeting novel CNS sites, each of which previously passed antidepressant screening in rodent models, yielded a somewhat different profile than those early pre-clinical screens. The chick anxiety-depression model identified prasterone, ketamine, mifepristone, CGP36742 and DOV216,303 as possessing antidepressant properties while memantine and antalarmin did not (Sufka et al., 2009). Interestingly, this pattern

of effects is in line with early clinical trial outcomes and illustrates the predictive validity of the model by correctly detecting efficacy of some compounds while avoiding two false positives (Belanoff et al., 2002; Schechter et al., 2005; Wolkowitz et al., 1999; Zarate et al., 2006a; Zarate et al., 2006b). Collectively, these results begin to provide validative support for the chick model as a screening assay and a neuropsychiatric simulation.

Recent research has provided further support for the chick model as a simulation by examining two important facets of anxiety and depression, environmental influences on and genetic vulnerability to stress. To address the former, Kim and Sufka (2011) sought to observe the effects of environmental enrichment on isolation induced distress vocalizations (DVocs) in the chick model. The 6 day environmental manipulation consisted of four conditions: 6d enriched, 6d non-enriched, 3d enriched to 3d non-enriched (early) or 3d non-enriched to 3d enriched (late). On day 7, chicks were tested in isolation for 120 min and DVoc rates were collected. In general, chicks in all conditions displayed relatively high DVoc rates in the first 2-3 min of isolation, indicative of an anxiety-like state, followed by a 50% decline and plateau of DVoc rates in the last 30-120 min of isolation, indicative of a depression-like state. Interestingly, environmental enrichment did not reveal a difference in DVocs rates in the anxiety- or depression-like phase between conditions; however, it did reveal a difference in latency for each condition to reach the depression-like phase. DVoc rates were transformed into onset to depression phase threshold latencies by calculating the time point at which each chick's DVoc rate/min from the anxiety-like phase (2-3 min) had declined by 25, 50, 75 and 95% into the rate/min of the depression-like phase (30-120 min). All enrichment conditions reached the 25 and 50% thresholds at similar time points; however by the 75% and 95% thresholds, differences in latency to reach the depression-like phase emerged. Late and continuous enrichment revealed

longer latencies to reach the 95% threshold than early enrichment and continuous non-enrichment. These results are consistent with the notion that an enriched environment, relative to an impoverished environment, can help mitigate the effects of stress related disorders which has also been observed in rodent models (Brenes Saenz, Villagra, Fornaguera Trias, 2006; Cirulli, Berry, Bonsignore, Capone, D'Andrea, Aloe et al., 2010) and in humans (Akhtar-Danesh & Landeen, 2007; Arborelius, Owens, Plotsky, & Nemeroff, 1999).

To examine genetic vulnerability to stress, Hymel, Loria, Salmeto, White and Sufka (under review) tested nine different chick strains selected based on feather pigmentation diversity which is related to physiological and behavioral differences in aves (Lee and Keeler, 1951; Karlsson, Kerje, Anderson and Jensen, 2010; Karlsson, Mormede, Kerje, and Jensen, 2011). Strains included Ameraucana, Barred Rock, Black Australorp, Buff, White Leghorn 236, New Hampshire Red, Production Red, Rhode Island Red and Silver Laced Wyandotte in the chick model. Chicks were tested in either an isolated condition or non-isolated condition (with two conspecifics and two mirrors) for 90 min and collected DVoc rates to observe possible differences in onset to depression phase threshold latencies at 25, 50, 75 and 95% into the depression-like phase. In general, chicks in the non-isolated condition displayed relatively low DVoc rates throughout the test session, despite some variability in initial rates. Chicks in the isolated condition displayed relatively high DVoc rates in the first 3 min, indicative of an anxiety-like state, which declined by approximately 50% within 10-25 min in all strains and remained stable thereafter, indicative of a depression-like state. In order to determine strain differences in stress vulnerability that would account for different base rates of DVocs, DVoc rates were transformed into onset to depression phase thresholds by calculating the time point at which each chick's DVoc rate/min from the anxiety-like phase (0-3 min) had declined by 25, 50,

75 and 95% into the rate/min of the depression-like phase (30-90 min). At the 25% threshold, no strain differences were observed; however, by the 50% threshold three distinct groups emerged whereby seven of the strains clustered into one homogenous group. To examine threshold latency differences between strains, 9 planned contrasts were performed to compare a single strain to the aggregate mean of the remaining 8 strains. These contrasts revealed two separate strains that differed from the aggregate mean; the Production Red strain displayed a longer latency at the 50% threshold and the Black Australorp strain displayed shorter latencies at all four thresholds. Of the remaining seven strains, the Silver Laced Wyandotte displayed depression threshold latencies that best represent an intermediate stress response.

Interestingly, some strain variability in DVoc rate was observed under the non-isolated condition which was hypothesized to be an overall strain vulnerability or resiliency to the experimental procedure. To examine this, correlations between each strain's' mean DVoc rates in non-isolated chicks in the first 3 min block and depression threshold latency in isolated chicks were conducted at each threshold. Moderate to robust correlations were observed at all four depression thresholds. This observation provides convergent validity of strain mediated stress vulnerability and resiliency in the chick model.

Further enhancing the validity of the chick anxiety-depression model requires strengthening the amount of connections made between the model and the clinical presentation of anxiety and depression (Miczek & de Wit, 2008; Panksepp, 2003; van der Staay, 2006). An approach to this type of enhancement is through quantifying behavioral endophenotypes which are defined as "a set of behavioral and/or physiologic characteristics that accompany a basic process that is altered in relation to the illness that is being studied" (Bakshi & Kalin, 2002). The use of endophenotypes must occur in a top-down fashion whereby the characteristics that make

up the human clinical disorder are translated and understood within the natural behavior of the model species (i.e., domestic chicks) (van der Staay, 2006). Humans suffering from anxiety and/or depression have demonstrated the endophenotype of cognitive disturbances and being able to quantify similar kinds of cognitive disturbances in the chick anxiety-depression model can strengthen this paradigm as a neuropsychiatric simulation (Kalueff & Murphy, 2007).

Cognitive Bias

Cognitive bias is a phenomenon that presents in individuals suffering from anxiety and/or depression in which cognitive disturbances elicit negative interpretations of ambiguous stimuli and/or events. More specifically, anxiety is associated with increased negative expectations of future events, known as more pessimism, whereas depression is associated with both increased negative expectations and also decreased positive expectations of future events, known as less optimism (Wright & Bower 1992; MacLeod & Byrne 1996). Further, both anxious and depressed individuals make more negative interpretations of themselves (Beck, 1963 as cited in Clark & Beck, 1999) and negative interpretations of the future (Butler & Mathews, 1983, 1987) and/or current events (Schwarz & Clore, 1983) for themselves and others (Alloy & Ahrens, 1987). In his cognitive model of psychopathology, Beck (1976) proposed that such cognitive disturbances are not only a product of, but may also play a role in maintaining anxiety and depression due to the constant processing of negative information and negative recurring thoughts. Therefore understanding the behavioral processes of such cognitive disturbances may help to reduce the severity of anxiety and/or depression.

In a study that clearly showed the cognitive biases associated with anxiety and depression, Miranda & Mennin (2007) assessed participants' predictions of future events. Participants completed a questionnaire which contained positive and negative future events for

which they were to indicate “yes” or “no” the event was likely to occur to them and how certain they were. Both anxious and depressed individuals were more pessimistic in their beliefs about negative events occurring to them; however only depressed individuals were less optimistic in their beliefs of positive events occurring to them (Miranda & Mennin, 2007). These results are consistent with a study by MacLeod & Byrne (1996) in which anxious individuals showed an increase in expectations of negative events and depressed individuals showed both an increase in expectations of negative and decreased expectations of positive events for events occurring presently, in a week, or in 5-10 years.

Several paradigms have been used to measure the effects of cognitive bias in anxious and depressed individuals including interference tasks, attentional probe tasks and homophone tasks (for review see, Mathews & MacLeod, 1994; Mogg & Bradley, 2005). The most common cognitive interference task is a modified version of the Stroop Task wherein the words presented in colored ink are either neutral or threat-related words (e.g., collapse, death, and failure), and the participant is to respond with the color of the word rather than the content. Anxious individuals displayed longer latencies for the color-naming of threat-related words as compared to neutral words; indicating that the threat-related words create a greater cognitive interference relative to the neutral words (Matthews and Macloed, 1985). Whereas depressed individuals displayed greater latencies when color-naming negative self-descriptive or negative socially-related words relative to neutral words (Mogg & Bradley, 2005) (Mathews & MacLeod, 1994); they also displayed greater latencies when color-naming depressed-related words relative to neutral- or manic-related words (Gotlib & McCann, 1984).

To assess cognitive bias in the attention of anxious and depressed individuals, visual probe tasks are utilized. In these tasks, word pairs, one negative and one neutral word, are

presented on a computer screen and followed by a small dot probe presented in the area where either the negative or neutral word had appeared. Anxious individuals displayed faster probe detection of dots presented in the negative word location as compared to the neutral word location, suggesting that anxious individuals allocate more attention to the negative stimuli whereas controls tended to shift attention away from the negative probes. In contrast, depressed individuals did not display differences in probe detection latencies presented in either the negative or neutral word location **(**MacLeod, Mathews, & Tata, 1986).

Cognitive biases in anxiety and depression can also be observed using the homophone task wherein a previously recorded audio tape presents ambiguous homophones differing in spelling and emotional valance, either threat-related or neutral words (e.g., die/dye or guilt/gilt). Anxious individuals (Eysenck, MacLeod, & Mathews, 1987; Mathews, Richards, & Eysenck, 1989) as well as depressed individuals (Mogg, Bradbury, & Bradley, 2006) reported a higher number of threat-related rather than neutral homophones when compared to non-anxious controls.

Interestingly cognitive bias is sensitive to a variety of therapies effecting mood disturbances including pharmacotherapies. Weinstein & Nutt (1995) reported that before treatment, anxious individuals displayed longer response latencies for emotional words on the modified Stroop task as compared to a recovered anxious, depressed and control group. After treatment with SSRIs (serotonin selective reuptake inhibitors), antidepressants also known to have anxiolytic properties, the previously anxious patients no longer significantly differed from the control group; suggesting that cognitive bias is responsive to pharmacological treatments (Weinstein & Nutt, 1995). In a similar study, Mogg, Baldwin, Brodrick, & Bradley (2004) observed a reversal of symptoms of cognitive bias in anxiety with SSRI treatment using the

homophone task. After SSRI administration, anxious individuals displayed not only lower levels of anxiety, but also less negative interpretation bias on the task. Further, cognitive bias processes decreased as a function of treatment improvement; the more efficacious the treatment, the fewer negative interpretations presented (Mogg et al., 2004). Though these findings seem promising, not all of the drug classes produced similar results.

Golombok et al. (1991) was unable to observe a reversal of cognitive bias on the modified Stroop task upon administration of the benzodiazepine anxiolytics. Although the benzodiazepines did appear to reduce anxiety and create an overall slowing of latencies for the task, there were no improvements in the negative interpretation biases. Golombok et al. (1991) concluded that the benzodiazepines only ameliorate an anxious mood, not the cognitive disturbances associated with anxiety. In a similar study, Stewart, Westra, Thompson, & Conrad (2000) wanted to observe the effects of naturalistic benzodiazepine use (i.e., taken on an "as needed basis") on cognitive bias within a variety of anxiety disorders (e.g., generalized anxiety disorder and post-traumatic stress disorder) to assess possible tolerance effects on the cognitive impairments produced by benzodiazepines and also to test the theory that benzodiazepines increase attention to threat-related stimuli. Consistent with the results from Golombok et al. (1991), individuals currently taking benzodiazepines did not reveal any improvements in the negative interpretation bias as compared to the medication nonusers. Further, the benzodiazepine users displayed greater attention to threat cues than the medication nonusers suggesting that the benzodiazepines do increase attention to threat-related stimuli (Stewart et al., 2000).

A study by Harmer et al. (2009) examined the effects of a single dose of reboxetine, a selective norepinephrine reuptake inhibitor, or a placebo on negative affective bias in depressed

individuals and healthy controls. Three hours after drug administration all participants were tested using a facial expression recognition task and an emotional categorization and memory task. The administration of reboxetine did not produce changes in mood or anxiety in either patients or controls. The facial expression recognition task which required individuals to identify the correct emotional expression (e.g., happiness, surprise, sadness, fear and anger) revealed that before reboxetine administration depressed individuals were less accurate in recognizing facial expressions of happiness and surprise compared to the controls, indicative of cognitive bias. This effect was reversed by reboxetine which increased the perception of happy facial expressions in depressed individuals. In a separate task, depressed individuals displayed longer response latencies for positive self-referential characteristics relative to negative self-referential characteristics compared to controls, indicative of a negative bias when judging one's personality. This effect was reversed by reboxetine which shortened response latencies for positive self-referential characteristics in depressed individuals. Further, depressed individuals had the worst recall of personality characteristics, especially those that were positive on the emotional memory task. This effect was reversed by reboxetine which improved recall of the positive self-referential characteristics (Harmer et al., 2009).

It is interesting to note that the phenomenon of cognitive bias in humans has also been examined in non-human animals such as rhesus macaques, dogs, rats, and avians subjected to various stressors (Bateson & Matheson, 2007; Bethell, Semple, Holmes, & MacLarnon, 2007; Brilot, Normandale, Parkin, & Bateson, 2009; Burman, Parker, Paul, & Mendl, 2009; Harding, Paul, & Mendl, 2004; Matheson, Asher, & Bateson, 2008; for review see Mendl, Burman, Parker, & Paul, 2004). For example, Bateson & Matheson (2007), trained European Starlings on a go/no-go task to differentiate between two visual stimuli, colored cardboard lids, representing

appetitive or aversive outcomes (e.g., white lids concealed a palatable mealworm; black lids concealed an aversive tasting mealworm). Prior to testing, the housing conditions were manipulated from an enriched to an impoverished environment, (inducing a more pessimistic-like state) or from an impoverished to an enriched environment (inducing a less optimistic-like state). At testing, starlings were exposed to ambiguous colored lids, intermediate shades of grey between black and white. Starlings that were switched from an enriched to an impoverished environment were less likely to flip the intermediate grey lids than those that were switched from an impoverished to an enriched environment (i.e., more pessimistic-like behavior after a decline in environment).

Matheson et al. (2008) utilized a similar paradigm to assess the effects of chronic enriched or standard housing environments on cognitive bias. In this study, starlings were trained to differentiate two temporal stimuli (e.g., 2 versus 10 second light stimuli) for an instant or delayed food reward and at test were exposed to a range of ambiguous temporal durations within the 2 to 10 second range. Compared to starlings housed in an enriched environment that were more likely to classify the ambiguous stimuli as being associated with an instant food reward, those in standard housing were less likely to do so. The behavior of starlings housed in standard cages reflects less optimism associated with depression-like states.

More recent studies of cognitive bias have examined behavioral responses to ecologically-relevant stimuli that are likely to produce similar approach-avoidant responses, but without extensive training. Brilot et al. (2009) used variations of eyespots, which are naturally aversive to many avian species, in conjunction with neutral or threatening, anxiety producing, calls to assess cognitive biases in starlings. Immediately after playing a particular call they recorded the starlings' behavior in front of either eyespots, ambiguous eyespots, or no eyespots.

Although there was no interaction between the anxiety states induced by different calls and responses to the various eyespot stimuli, the eyespots did reveal to be generally aversive to starlings and therefore an accurate assessment of anxiety and approach behavior.

To measure approach-avoidant behaviors in domestic fowl chicks, Salmeto et al. (2011) used a straight-alley maze, a paradigm used to quantify chick social reinstatement (Marin, Freytes, Guzman, & Jones, 2001) with start and goal latencies as the dependent measure. Various stimulus cues were located at the goal which served as the approach-avoidant manipulation. The stimulus cues were a silhouette of a conspecific chick (or mirror), a silhouette of a horned owl, a natural predator to the chick, and three intermediate ambiguous silhouettes with varying degrees of characteristics between the two (e.g., 75c:25o, 50c:50o, 25c:75o). In Experiment 1, non-stressed chicks displayed start latencies that were unaffected by the various stimulus cues, whereas goal latencies were longer under cues with greater owl silhouette characteristics.

These results reveal that the range of stimulus cues produce the necessary approach/avoidant behavior to examine cognitive bias under anxiety- and depressive-like states. One interesting finding was that chicks displayed longer goal latencies under the Chick stimulus cue than for the mirror cue in the pre-test session. Therefore, the second experiment replaced the Chick stimulus for the mirror to promote more life-like characteristics than that of a still image to allow for the most approach behavior.

Experiment 2 utilized the same procedure with the introduction of an initial isolation manipulation to induce either an anxiety-like state (5 min isolation) or a depression-like state (60 min isolation). In the social condition, start latencies were unaffected by the various stimulus cues, which is consistent with Experiment 1 results. In the anxiety-like condition, start latencies were significantly longer under the stimulus cues with greater aversive characteristics (e.g.,

50c:50o, 25c:75o, and Owl) relative to the social condition. These results reflect the cognitive bias of more pessimism, which is an increased avoidant behavior to ambiguous aversive stimuli. In the depression-like condition, start latencies were significantly longer under the stimuli with greater aversive characteristics, as well as greater appetitive characteristics (e.g., Chick and 75c:25o) relative to the social condition. These results reflect the cognitive bias of more pessimism, as well as less optimism, which is a decreased approach behavior to ambiguous appetitive stimuli.

However, the goal latencies did not produce such clear results. Relative to the social condition, goal latencies in the anxiety-like and depression-like conditions were significantly longer under the 50c:50o and the Owl stimulus cues (i.e., more pessimism), but not the 25c:75o stimulus cue. In addition, goal latencies in the depression-like conditions were significantly longer in the Chick cue (i.e., less optimism), but not in the 75c:25o stimulus cue. These results may be due to a ceiling effect imposed by the 5 min test session criteria as many of the chicks did approach the cues to varying degrees but did not reach the goal line. Collectively, these observations reveal that a runway test to ambiguous appetitive and aversive cues can assess both types of cognitive biases within a single paradigm. In addition, the chick model produced results that are consistent with how cognitive bias presents in the human clinical literature, wherein more pessimism is present in the anxiety-like state and both more pessimism and less optimism are present in the depression-like state.

In a follow up study, Hymel & Sufka (2012) sought to determine whether cognitive bias in the chick anxiety-depression model was similarly sensitive to the pharmacological reversal observed in humans. More specifically to examine whether imipramine, an antidepressant which also possess anxiolytic effects, reversed more pessimistic-like behavior under ambiguous

aversive cues in both anxiety- and depression-like states and reversed less optimistic-like behavior under ambiguous appetitive cues in the depression-like state. In addition, if clonidine, a non-benzodiazepine anxiolytic, reversed more pessimistic-like behavior in the anxiety-like state. The experimental procedures were replicated from Salmeto et al. (2011) experiment 2 (described above) with the added administration of imipramine, clonidine or a vehicle 15 min prior to the 60 min isolation stress procedure. The observed pattern of DVoc rates were consistent with previous studies wherein chicks initially produced relatively high DVoc rates in the anxiety-like phase which were reduced by approximately 50% in the depression-like phase (Sufka et al., 2006). In addition, clonidine attenuated DVocs in the anxiety-like phase (Warnick et al., 2006), whereas imipramine prevented the onset of behavioral despair (Sufka et al., 2006; Warnick et al., 2009). Consistent with previous findings from Salmeto et al. (2011), chicks in the anxiety-like phase displayed more pessimistic-like behavior on runway performance under ambiguous aversive cues, and chicks in the depression-like phase displayed both more pessimistic-like and less optimistic-like behavior on runway performance under ambiguous aversive and appetitive cues, respectively. Further, more pessimistic-like and less optimistic-like behavior was reversed by imipramine in the depression-like phase. However, more pessimistic-like behavior was not reversed by clonidine in the anxiety-like phase; clonidine appeared to have sedative effects on runway performance which appeared to be related to the sedative nature of the compound (Dahmani et al., 2010; Feltenstein et al., 2004; Warnick et al., 2006).

Strain Vulnerability and Antidepressant Drug Sensitivity

More recent research sought to examine antidepressant drug responses in the resilient Production Reds (PR) versus the vulnerable Black Australorp (BA) (White, personal communication, June 2013). Dose response functions for imipramine were tested in PRs and

BAs. Results showed that imipramine produced both anxiolytic and antidepressant effects in the PRs but had no effect on either phase in the BAs. Interestingly, dose response functions for ketamine showed the opposite pattern. Ketamine produced antidepressant effects in the vulnerable BAs, but had no effect on the depression phase in the PRs. The authors suggest that the BAs may represent a viable antidepressant screening assay for treatment resistant depression.

Given that cognitive bias represents a core symptom in anxiety and depressive disorders and that the BAs represent a model of stress vulnerability that is sensitive to ketamine, it follows that enhanced model validity would be provided with evidence showing: a) BAs present CB to a greater degree than PRs and b) that ketamine, but not imipramine, attenuates CB in BAs.

METHODS

Subjects

Cockerels (Gallus gallus; Black Australorp and Production Red; Ideal Poultry Cameron, TX, USA) were received 2-days post hatch and housed in 34 × 57 × 40 cm stainless steel cages with 12–13 chicks per cage. Chicks were removed and briefly handled daily to minimize experimenter-related stress. Food (Purina Start and Grow, St Louis, Missouri, USA) and water was available ad libitum through one quart gravity-fed feeders (Murray MacMurray; Model 4BGFJ) and waterers (Murray MacMurray; Model 4YQW0). Room temperature was maintained at 29 ± 1 °C and overhead illumination was maintained on a 12-h light-dark cycle.

Apparatus

Straight alley maze

The apparatus consisted of a 50 x 30 x 10 cm arena made of opaque high-density polyethylene material that contained a straight alley maze adjacent to a holding arena (see Fig. 1). The straight alley maze consisted of a 10 x 10 x 10 cm start box with a guillotine door that opened up to a 40 x 10 x 10 cm runway with either an 8 x 10 cm mirror or various 8 x 10 cm stimulus cues placed at its end (detailed below). The runway contained markings in 5 cm units that permitted a measure of distance traveled. A 40 x 20 x 10 cm holding arena housed 12 conspecifics throughout the test session and permitted the testing of chicks under non-isolated treatment conditions. These conspecifics remained out of view during maze testing. However, once chicks reach the goal, full view of the arena was permitted through a 20 x 10 cm clear

Plexiglas wall. Pine bedding was placed throughout the arena floor and food and water was available ad libitum in 200 ml stainless steel cups.

Morphed Stimulus Conditions

Morpheus Photo Morpher v3.01 Professional for Mac (Morpheus Software, LLC) was used to produce 'morphed' images that blended elements of a chick and a horned owl silhouette, an ecologically aversive stimulus which has previously shown increased avoidant behavior in this paradigm (Hymel & Sufka, 2012;p Salmeto et al., 2011). From these images, the software mapped a series of approximately 200 dots onto each photos to match the location of the dots between the images. This allowed for 100 morphed frames linking the start (chick) and end (owl) photos. Within this series two 'key' frames were defined: 75% chick and 25% owl, and 25% chick and 75% owl were used (75c:25o and 25c:75o) (See Fig. 2A). For the current study the 75c:25o morph, which in previous studies was morphed using a white leghorn chick, was pigmented to resemble the strains being tested (See Fig. 2B) The pixelated edges of the images were smoothed out and the images were adjusted so that they were all approximately the same size and fit on an 8 x 10 cm stimulus card. The images were saved as jpeg files, printed and taped at the end of the runway during testing.

Isolation Apparatus

A six-unit test apparatus containing Plexiglas viewing chambers (25 x 25 x 22 cm) situated in sound-attenuating enclosures was used to collect isolation-induced distress vocalizations. The units were illuminated using 25W light bulbs and ventilated by an 8-cm diameter rotary fan (Model FP- 108AXS1; Commonwealth Industrial Corp. Taipei, Taiwan). Miniature video cameras (Model PC60XP; SuperCircuit, Liberty Hill, Texas, USA) mounted at floor level in the corner of the enclosures and routed through a multiplexer (Model PC47MC;

SuperCircuit) allowed for animal observation. Distress vocalizations were collected via microphones [Model 3-675-001 (modified); Lafayette Instruments, Lafayette, Indiana, USA] mounted on the rear wall of the Plexiglas chamber, routed through sound-activating relays (Model 3-675- 001 (modified for AC current); Lafayette Instruments, Lafayette, Indiana, USA] mounted on the ceiling of the Plexiglas chamber and routed through a USB interface via custom designed software.

EXPERIMENT 1 METHODS

Procedure

Experiment 1 was conducted to examine whether runway behaviors of non-isolated Black Australorp (BA) and Production Red (PR) chicks manifest in a pattern similar to White Leghorn strain used in previous cognitive bias studies. In this experiment, chicks were tested across ages 4-6 days post hatch. In the first trial, 4 days post hatch, 12 cagemate conspecifics were placed into the holding arena. After a 5 min adaptation period, chicks were individually tested in the maze under the mirror. Each chick was placed into the start box for 15 sec after which the guillotine door was raised. Dependent measures were start and goal latencies and farthest distance traveled. Start latency was defined as the time it took to step completely outside the start box. Goal latency was defined as the time to cross a defined mark located 10 cm away from the mirror. Because all test sessions were terminated at 5 min, the farthest distance traveled (cm) from the start box was measured to account for possible differences between chicks that completed the straight alley maze and those that did not. Chicks were placed back into the holding arena until all were tested. Randomized group assignment for Trial 2 was based on goal latencies from this test session. Trial 2 was conducted at either 5 or 6 days post hatch. Since this experiment sought to observe runway behaviors in non-isolated chicks (straight out of homecage), procedures and dependent measures were as described for the first trial except chicks were tested under either the mirror, 75c:25o, 25c:75o or 0c:100o stimulus cue conditions in the runway.

Statistical Analysis

To assess for significant runway differences under the various stimulus cue conditions for each strain, two one-way MANCOVAs were conducted with mean start and goal latencies and mean distance traveled as the dependent variables and with pre-goal latency as a covariate to account for baseline performance. Due to large variances in runway scores, square root transformations were performed on each variable prior to analysis. Unadjusted means and standard deviations for start and goal latencies and distance traveled under each stimulus cue condition for Black Australorp and Production Red strains are presented in Table 1. A priori planning to assess runway differences across stimulus cue conditions for each strain individually set the MANCOVA p-value at $p > 0.025$ (Keppel & Wickens, 2007). Given the significance of the MANCOVA, univariate main effects were examined. Post-hoc analyses were conducted using Fisher's least significant difference tests.

EXPERIMENT 1 RESULTS

Black Australorp Strain

The effects of various stimulus cue conditions on mean start and goal latencies and mean distance traveled for non-isolated Black Australorp chicks are presented in Fig. 3 A, B and C, respectively. In general, mean start latencies$^{x^{1/2}}$ were relatively short (e.g., chicks left the start box in under 5 sec$^{x^{1/2}}$; where √300 sec = 17.32 sec); however, the 25c:75o cue was longer compared to the mirror and the 75c:25o cue. Mean goal latencies$^{x^{1/2}}$ generally increased with greater amounts of owl silhouette in the stimulus cue morphs. Mean goal latencies$^{x^{1/2}}$ for the 75c:25o, 25c:75o and 0c:100o cues were longer compared to the mirror. In addition, mean goal latencies$^{x^{1/2}}$ for the 25c:75o and 0c:100o cues were longer compared to the 75c:25o cue. In general, mean distance traveled$^{x^{1/2}}$ was approximately 5 cm$^{x^{1/2}}$ (where √30 cm = 5.48 cm), indicating that most chicks completed the maze or approached the stimulus cue.

Consistent with these observations, a one-way MANCOVA revealed a significant multivariate main effect for stimulus cue, Wilks' $\lambda = 0.411$, $F(9,102.37) = 5.018$, $p < 0.001$, partial eta squared = 0.257. Power to detect the effect was 0.983. Given the significance of the overall test, the univariate main effects were examined. Significant univariate main effects for stimulus cue condition were obtained for mean start latency$^{x^{1/2}}$ $F(3,44) = 3.31$, $p < 0.05$, as well as for mean goal latency$^{x^{1/2}}$ $F(3,44) = 14.02$, $p < 0.001$. Fisher's LSD pairwise comparisons revealed a significantly longer mean start latency$^{x^{1/2}}$ for the 25c:75o stimulus cue compared to the

mirror and the 75c:25o cue (ps < 0.05). Mean goal latencies^{x½} for the 75c:25o, 25c:75o and 0c:100o cues were significantly longer compared to the mirror (ps < 0.005). Further, mean goal latency^{½} for the 0c:100o cue was significantly longer compared to the 75c:25o cue (p < 0.01).

Production Red Strain

The effects of various stimulus cue conditions on mean start and goal latencies and mean distance traveled for non-isolated Production Red chicks are presented in Fig. 4 A, B and C, respectively. In general, mean start latencies ^{x½} were relatively short (e.g., chicks left the start box in under 5 sec^{x½}; where √300 sec = 17.32 sec); however, the 25c:75o and 0c:100 cues were longer compared to the mirror and the 75c:25o cue. Mean goal latencies^{x½} generally increased with greater amounts of owl silhouette in the stimulus cue morphs. Mean goal latencies^{x½} for the 75c:25o, 25c:75o and 0c:100o cues were longer compared to the mirror. In general, mean distance traveled^{x½} was approximately 5 cm$^{x ½}$ (where √30 cm = 5.48 cm), indicating that most chicks completed the maze or approached the stimulus cue.

Consistent with these observations, a one-way MANCOVA revealed a significant multivariate main effect for stimulus cue, Wilks' λ = 0.535, F(9,95.07) = 3.100, p < 0.005, partial eta squared = 0.188. Power to detect the effect was 0.847. Given the significance of the overall test, the univariate main effects were examined. A significant univariate main effect for stimulus cue was obtained for mean goal latency ^{x½} F(3,41) = 9.681, p < 0.001. Fisher's LSD pairwise comparisons revealed a significantly longer mean goal latency^{x½} for the 75c:25o, 25c:75o and 0c:100o cues compared to the mirror (ps < 0.001).

EXPERIMENT 1 DISCUSSION

Experiment 1 was conducted to examine whether runway behaviors of non-isolated Black Australorp (BA) and Production Red (PR) chicks manifest in a pattern similar to the White Leghorn (WLH) strain used in previous cognitive bias studies. Non-isolated chicks were tested in the straight alley maze to one of four stimulus cue conditions (mirror, 75c:25o, 25c:75o or 0c:100o). However, since the original cues were morphed using a WLH chick image, the 75c:25o was pigmented to match the coloration of the BA and PR strains. Dependent measures were start and goal latency and distance traveled. In general, mean start latency and mean distance traveled were unaffected by the varying stimulus cue conditions for both BAs and PRs which is consistent with previous studies using the WLH Leghorn strain (Hymel & Sufka, 2012; Salmeto et al., 2011). Interestingly, the BAs displayed a significantly longer mean start latency for the 25c:75o cue compared to the mirror, a pattern that was also present in the PRs although not significantly, suggesting that mean start latency, which has shown varying sensitivity previously, may be a sensitive measure for non-isolated chicks of different strains. Mean goal latency for both strains generally increased as a function of the aversive characteristics of the stimulus cue conditions, although the BAs displayed a clearer pattern which parallels that of the WLH strain (Salmeto et al., 2011). These replicated responses seen under the range of appetitive to aversive stimulus cue conditions suggest that cognitive bias should present in a manner parallel to the WLH Strain. In addition, it showed that the pigmented BA and PR 75c:25o

stimulus cues produce the same behavioral effect as the WLH 75c:25o did for the WLH strain and can as the ambiguous appetitive stimulus cues.

EXPERIMENT 2 METHODS

Procedure

Experiment 2 was conducted to examine whether cognitive biases of more pessimism and less optimism would manifest in a pattern reflecting the stress vulnerability and resiliency in Black Australorp and Production Red strains, respectively. Trial 1 was conducted at 4 days post hatch using the same experimental procedures as Experiment 1 Trial 1. Trial 2 was conducted at either 5 or 6 days post hatch and consisted of a 2 x 2 (strain by isolation stress) experimental design that included non-isolated and isolated stress conditions for each strain. Separate groups of the aforementioned treatment conditions were tested under either an ambiguous appetitive cue (75c:25o morph) to model less optimism or an ambiguous aversive cue (25c:75o morph) to model more pessimism. Non-isolated chicks were taken from their homecage and placed in the maze arena for a 5 min adaption period. Chicks were then individually tested in maze and remained in the arena throughout testing of the isolated conditions. Isolated chicks were tested in staggered groups of 3 in the isolation apparatus for 90 min. More specifically, 3 chicks were placed into the top row of the isolation apparatus to begin testing and 8 min later 3 additional chicks were placed into the bottom row of the isolated apparatus for a total run time of 98 min. The staggered groups provided sufficient time for a 5 min maze test of 3 chicks at a time and ensured all chicks were isolated for a total of 90 min. After isolation testing, the staggered groups of chicks were then transported from the isolation apparatus in a 2 quart opaque plastic container and tested immediately in the maze. Dependent measures were start and goal latencies and

farthest distance traveled (See Ex. 1 Trial 2). Chicks were returned to their home cage after testing.

Statistical Analysis

To examine strain differences on distress vocalization (DVoc) rates, all DVocs were transformed into a rate/min function and collapsed across 3 min blocks. To examine strain differences between the anxiety- and depression-like phases, a 2-way within/between ANOVA was conducted on the anxiety- (i.e., first 3 min/3) and depression-like phase (i.e., 30-90 min/60) rates. Post-hoc analyses were conducted using Fisher's least significant difference tests.

To examine strain differences in onset to the depression-like phase, time points at which each chick's DVoc rate/min from the anxiety-like phase declined by 25, 50, 75 and 95% of the rate/min of the depression-like phase were calculated. To elaborate, DVoc rates were compared minute to minute over consecutive 3 min blocks to determine the time point at which the average rate of that block was at or below these four thresholds; the middle time point of that block was operationally defined as the onset latency into 25, 50, 75 or 95% of the depression-like phase. Using these four thresholds, a 2-way Within/between ANOVA was conducted to examine strain differences in onset to the depression-like phase. Post-hoc analyses were conducted using Fisher's least significant difference tests.

Because a unique pattern of isolation stress and strain were predicted on each dependent measure (i.e., start and goal latency and distance traveled) that would vary under each stimulus cue condition, a 2 (strain) x 2 (isolation stress condition) MANCOVA was performed for each stimulus cue with pretest goal latency data as a covariate to account for baseline performance. These a prior planned comparisons incorporated a Bonferroni correction procedure which individually set the *p*-value at $p = < 0.025$ for significance to examine univariate effects (Keppel

& Wickens, 2007). Post-hoc analyses were conducted using Fisher's least significant difference test.

EXPERIMENT 2 RESULTS

Distress Vocalizations and Onset to Depression Thresholds

The effects of isolation stress on distress vocalization (DVoc) rates for the Black Australorp and Production Red strains across the 90 min test session are presented in panels A and B of Figure 5, respectively. In general, each strain displayed relatively high DVoc rates within the first 3 min of isolation; indicative of an anxiety-like (panic-like) state. Then, over the next 10-25 min each strain's DVoc rates declined by approximately 50% of their initial high rate and remained relatively stable in the final 60 min of isolation, indicative of a depression-like state (i.e., behavioral despair). However, BA DVoc rates began to decline and reached their plateau more quickly than PR DVoc rates as evidenced by shorter onset to depression phase threshold latencies (see Fig. 6).

Consistent with these observations a 2-way within/between ANOVA on DVoc rates revealed a significant main effect of time $F(1,82) = 554.89$, $p < 0.001$, as well as a time x strain interaction that approached significance $F(1,82) = 3.92$, $p = 0.051$. However, the ANOVA failed to reveal a significant main effect of strain $F(1,82) = 2.46$, $p =$ n.s. Fisher's LSD pairwise comparisons revealed significantly lower DVoc rates in the depression-like phase compared to the anxiety-like phase in each strain (ps < 0.001). In addition, a separate 2-way within/between ANOVA on depression phase thresholds revealed a significant main effect of time, $F(3,246) = 73.02$, $p < 0.001$, a significant main effect of strain $F(1,82) = 5.81$, $p < 0.05$. However the ANOVA failed to reveal a significant interaction $F(3,246) = 1.02$, $p =$ n.s. Fisher's LSD pairwise

comparisons revealed the BA strain displayed significantly shorter depression onset threshold latencies compared to the PR strain (ps < 0.05).

Ambiguous Appetitive Stimulus Cue (75c:25o Morph)

The effects of strain and isolation stress condition on mean start and goal latencies and mean distance traveled under the ambiguous appetitive stimulus cue are presented in panels A, B and C of Figure 7, respectively. In non-isolated conditions of each strain, mean start latencies were relatively short (e.g., chicks left the start box under approximately 5 sec for BAs and 12 sec for PRs) and mean goal latencies were relatively long (e.g., chicks reached the goal under approximately 1 ½ min for BAs and 2 min for PRs). Mean distance traveled for non-isolated conditions of each strain was approximately 29 cm, indicating that most chicks either completed the maze or approached the stimulus cue. Isolated conditions of each strain displayed longer mean start latencies relative to non-isolated controls. In addition, isolated conditions of each strain displayed similar mean goal latency and mean distance traveled relative to non-isolated controls. Lastly, non-isolated and isolated conditions of the BA strain displayed mean start and goal latencies were somewhat shorter compared to non-isolated and isolated conditions of the PR strain.

Consistent with these observations, a 2 x 2 MANCOVA revealed a multivariate effect for strain that approached significance, Wilks' $\lambda = 0.918$, $F(3, 107) = 3.18$, $p < 0.027$ (partial eta squared = 0.082 and power to detect the effect = 0.617), as well as a significant multivariate effect for stress, Wilks' $\lambda = 0.771$, $F(3, 107) = 10.60$, $p < 0.001$ (partial eta squared = 0.229 and power to detect the effect = 0.997). However the MANCOVA failed to reveal a significant multivariate interaction Wilks' $\lambda = 0.980$, $F(3, 107) = 0.71$, p = n.s. (partial eta squared = 0.020 and power to detect the effect = 0.126), where $p < 0.025$ is considered significant. Given the

significant multivariate effect for stress, the univariate main effects were examined. A significant univariate main effect was obtained for mean start latency $F(1, 109) = 20.31$, $p < 0.001$. For isolated conditions of each strain, Fisher's LSD pairwise comparisons revealed significantly longer mean start latencies relative to non-isolated controls (ps < 0.001).

Ambiguous Aversive Stimulus Cue (25c:75o Morph)

The effects of strain and isolation stress condition on mean start and goal latencies and mean distance traveled under the ambiguous aversive stimulus cue are presented in panels A, B and C of Figure 8, respectively. In non-isolated conditions of each strain, mean start latencies were relatively short (e.g., chicks left the start box under approximately 10 sec for BAs and 40 sec for PRs) and goal latencies were relatively long (e.g., chicks reached the goal in approximately 2 min for BAs and 3 ½ min for PRs). Mean distance traveled for non-isolated conditions of each strain was approximately 27 ½ cm, indicating that most chicks either completed the maze or approached the stimulus cue. Isolated conditions of each strain displayed longer mean start and goal latencies and shorter mean distance traveled relative to non-isolated controls. Lastly, non-isolated and isolated conditions of the BA strain displayed shorter mean start and goal latencies and somewhat longer mean distance traveled compared to the non-isolated and isolated conditions of the PR strain.

Consistent with these observations, a 2 x 2 MANCOVA revealed a significant multivariate effect for stress, Wilks' $\lambda = 0.693$, $F(3, 103) = 15.20$, $p < 0.001$ (partial eta squared = 0.307 and power to detect the effect = 1.00), as well as a significant multivariate effect for strain Wilks' $\lambda = 0.890$, $F(3, 103) = 4.26$, $p < 0.01$ (partial eta squared = 0.110 and power to detect the effect = 0.773). However, the MANCOVA failed to reveal a significant multivariate interaction Wilks' $\lambda = 0.982$, $F(3, 103) = 0.638$, p = n.s. (partial eta squared = 0.113 and power

to detect the effect = 0.018) , where $p < 0.025$ is considered significant. Given the significant multivariate effect for stress and strain, the univariate main effects were examined. Univariate analyses on stress revealed a significant main effect for mean start latency $F(1, 105) = 24.05$, $p < 0.001$, mean goal latency $F(1, 105) = 24.91$, $p < 0.001$, and mean distance traveled $F(1, 105) = 35.83$, $p < 0.001$. For isolated conditions of each strain, Fisher's LSD pairwise comparisons revealed significantly longer mean start and goal latencies and significantly shorter mean distances traveled relative to non-isolated controls (ps < 0.001). Univariate analyses on strain revealed a main effect for mean start latency that approached significance $F(1, 105) = 3.86$, $p = 0.052$, a significant main effect for mean goal latency $F(1, 105) = 11.63$, $p < 0.005$, and main effect for distance traveled that approached significance $F(1, 105) = 3.01$, $p = 0.086$. For non-isolated and isolated conditions of the BA strain, Fisher's LSD pairwise comparisons revealed significantly shorter mean goal latencies compared to the non-isolated and isolated conditions of the PR strain (ps < 0.005).

EXPERIMENT 2 DISCUSSION

Experiment 2 was conducted to examine whether cognitive biases of more pessimism and less optimism would manifest in a pattern reflecting the stress vulnerability and resiliency in Black Australorp and Production Red strains, respectively. Non-isolated and isolated (90 min) chicks were tested in a straight alley maze to either an ambiguous appetitive (75c:25o) or ambiguous aversive (25c:75o) stimulus cue. Distress vocalizations served as the dependent behavioral measure for isolated chicks and start and goal latency and distance traveled served as the dependent measures for the maze.

Distress Vocalizations and Onset to Depression Thresholds

The pattern of distress vocalizations across strains were consistent with previous studies of isolation stress wherein chicks in the anxiety-like phase (i.e., first 3-min) displayed high DVocs which was followed by approximately a 50% reduction of DVocs in the depression-like phase (i.e., last 60 min) (Hymel et al., under review; Loria et al., 2013; Sufka 2006). Strain differences were not detected on overall DVoc rates, but strain vulnerability and resiliency were revealed in onset of depression threshold latencies. The BA strain displayed shorter onset latencies compared to the PR strain. These findings are consistent with Hymel et al. (under review) and Loria et al., (2013) showing that the BA strain is more vulnerable to isolation stress and enters into behavioral despair more quickly than the PR strain.

Ambiguous and Appetitive Stimulus Cues (75c:25o and 25c:75o Morphs)

Runway performance for non-isolated BA and PR chicks under both cues is consistent with Experiment 1 and previous findings using the White Leghorn strain wherein most chicks leave the start box under approximately 30 sec and reach the goal line under approximately 2 min (distance traveled being approximately 30 cm). The isolated BAs and PRs displayed longer mean start latencies under the 75c:25o and 25c:75o stimulus cue conditions relative to non-isolated controls. These findings are consistent with previous studies showing that cognitive bias in a depression-like state presents as less optimistic-like and more pessimistic-like behavior, respectively (Hymel & Sufka, 2012; Salmeto et al., 2011). Although not previous observed, both strains displayed more pessimistic-like behavior on mean goal latency as evidenced by isolated conditions displaying longer latencies relative to non-isolated controls under only the 25c:75o stimulus cue. Another interesting finding was that more pessimistic-like, but not less optimistic-like, behavior was observed for both strains on distance traveled as evidenced by isolated conditions displaying shorter distances traveled relative to non-isolated controls under only the 25c:75o stimulus cue. In Hymel & Sufka (2012) distance traveled was added since goal latency failed to be sensitive measure of cognitive bias in isolated chicks most likely due to the 5 min test criteria (Salmeto et al., 2011). That mean goal latency was sensitive for the 25c:75o and that distance traveled was insensitive for the 75c:25o may reflect the appetitive/aversive nature of the stimulus cues. The BA and PR 75c:25o cues were designed based on strain pigmentation, isolated chicks may be responding to cue colors and/or the features, although runway performance in non-isolated conditions is consistent with Experiment 1. Other test parameters such as the 5 min test criteria and maze length may also have affected runway performance.

Although the only significant strain difference between isolated conditions presented on mean goal latency under the 25c:75 stimulus cue condition, a pattern of strain effects that reflected the BAs' stress-vulnerability was detected in baseline runway performance and in non-isolated conditions (i.e., stress vulnerable BAs displayed faster runway performance, in general). After controlling for chicks that did not complete the maze within the 5 min test criteria, the BA strain displayed significantly shorter goal latencies than the PR strain ($p = 0.029$). Interestingly, Marin et al., (2001) showed that chicks exposed to a crush cage stressor displayed significantly shorter latencies to complete a 160 cm runway towards cagemate conspecifics relative to non-isolated controls. These findings suggest stressed chicks attempt to reinstate social contact more quickly than non-stressed controls.

Although pretest chicks are non-isolated (straight out of homecage), they do experience significant stress from being taken from their homecage and placed into the maze. In addition, chicks are colored marked for identification on their chests due to their black plumage. Correct identification from above may become difficult causing the experimenter to pick a chick up several times. That chicks are likely experiencing some measurable amount of stress is consistent with the differences in maze performance. Such differences in stress vulnerability and resiliency differences are consistent with Loria et al. (2013). In this study, subsequent to stress, hippocampal BDNF levels in the vulnerable BA strain increased during 90 min of isolation which then decreased thereafter; whereas, hippocampal BDNF levels in the resilient PRs remained stable throughout 120 min of isolation. These findings provide convergent validity to the identification of a stress vulnerable and resilient strain within the chick anxiety-depression model.

Two possibilities may have led to the absence of a more robust strain effect in the isolated conditions. The first may be the test parameters such as the 5 min test cut off and 30 cm maze length. Previous studies utilized a 160 cm straight alley maze for 10 min test session (Marin et al., 2001). In addition, observing stress and/or comfort behaviors while in the runway may provide a more fine grained measure of the chick's level of stress. Besides latencies, other observable behaviors include defecation, freezing, distress calling, time spent standing, ambulation, preening, jumping and pecking, among others. However, these behaviors may also be increased or decreased with repeated exposure to a novel environment and may also differ between strains (Jones, 1977). Perhaps increasing the maze test parameters would allow for a more fined grained analysis of stress responsivity to observe strain differences.

The second may be due to the 90 min isolation test preceding the maze test. Onset to depression phase threshold latencies show that by approximately 10-12 min the BA strain is approximately 75% into their behavioral despair state. Maze testing after 90 min of isolation when both strains are well into behavioral despair may diminish possible differences that appear when strain vulnerability and resiliency is most detectable.[1]

[1] A follow up study examining 10 min isolation stress on cognitive bias did not reveal any significant effects of cognitive bias nor strain differences. However it illustrates that ample time into behavioral despair is necessary to detect cognitive disturbances in chicks.

EXPERIMENT 3 METHODS

Procedure

Experiment 3 was conducted to examine whether cognitive biases observed in the Black Australorp strain could be reversed in a manner that parallels the strain's differential drug responsivity, whereby imipramine would fail and ketamine would succeed in reversing cognitive biases, respectively. Trial 1 was conducted using the same experimental procedures as Experiment 1 Trial 1. Trial 2 consisted of a 2 x 3 (isolation stress by drug treatment) experimental design that included a non-isolated and isolated stress condition for either physiological saline as a vehicle, 10.0 mg/kg of imipramine or 10.0 mg/kg of ketamine drug treatment condition. Drug probes were administered intraperitoneally (IP) 15 min before placement into the isolation apparatus. As before, these treatment conditions were tested under either an ambiguous appetitive cue (75c:25o morph) to model less optimism or an ambiguous aversive cue (25c:75o morph) to model more pessimism). Taken from their homecage in groups of 3, non-isolated chicks were weighed, administered one of the drug probes and placed into the arena for a 105 min injection-to-test time interval and then tested in the maze. This 105 min interval matched the injection-to-maze-test interval for the 90 min isolated conditions. Non-isolated chicks remained in the arena throughout the testing of isolated conditions. Isolated chicks were tested in staggered groups of 3 (see Experiment 2). Following weighing, drug administration and a 90 min isolation test, isolated chicks were transported from the isolation apparatus in a 2 quart opaque plastic container and tested immediately in the maze. Dependent

measures were start and goal latency and distance traveled (See Ex. 1 Trial 1). Chicks were returned to their home cage after testing.

Statistical Analysis

To examine various drug treatment effects on distress vocalization (DVoc) rates, all DVocs were transformed into a rate/min function and collapsed across 3 min blocks. To examine drug treatment effects on anxiety- and depression-like phases, a 3-way Within/between ANOVA was conducted on the anxiety- (i.e., first 3 min/3) and depression-like phase (i.e., 30-90 min/60). Post-hoc analyses were conducted using Fisher's least significant difference tests. Because a unique pattern of isolation stress and drug treatment were predicted under each dependent measure (i.e., start and goal latency and distance traveled) that would vary each stimulus cue condition, a 2 (isolation stress) x 3 (drug treatment) MANCOVA was performed for each stimulus cue with pretest goal latency data as a covariate to account for baseline performance. These a prior planned comparisons incorporated a Bonferroni correction procedure which individually set the *p*-value at $p = < 0.025$ for significance to examine univariate effects (Keppel & Wickens, 2007). Chicks that did not reach at least 80 DVocs in the anxiety-like phase or that had an increase in DVoc rates from the anxiety- to the depression-like state were discarded (n = 21/237chicks). Post-hoc analyses were conducted using Fisher's least significant difference test.

EXPERIMENT 3 RESULTS

Distress Vocalizations

The effects of drug treatment conditions on distress vocalization (DVoc) rates across the 90 min test session are presented in panels A and B of Figure 9, respectively. Vehicle treated chicks displayed relatively high DVoc rates within the first 3 min of isolation, indicative of an anxiety-like (panic-like) state. Then, DVoc rates declined by approximately 50% of the initial high rate during and remained relatively stable in the final 60 min of isolation (i.e., 30-90 min); indicative of a depression-like state (i.e., behavioral despair). All drug treatment conditions appeared to enter into behavioral despair in similar patterns. However, in general, in the imipramine treated condition, DVocs rates were somewhat higher compared to the vehicle and ketamine treated conditions.

Consistent with these observations a 2-way within/between ANOVA on DVoc rates revealed significant main effect of time $F(1,94) = 170.95$, $p < 0.001$, as well as a time x drug treatment interaction that approached significance $F(2,94) = 2.86$, $p = 0.06$. However, the ANOVA failed to reveal a significant main effect of drug treatment $F(2, 94) = 644.31$, p = n.s. For each drug treatment, Fisher's LSD pairwise comparisons revealed significantly lower DVoc rates in the depression-like phase compared to the anxiety-like phase (ps < 0.001).

Ambiguous Appetitive Stimulus Cue (75c:25o Morph)

The effects of isolation stress and various drug treatment conditions on mean start and goal latency and mean distance traveled under the ambiguous appetitive stimulus cue are

presented in panels A, B and C of Figure 10, respectively. In non-isolated conditions of each drug treatment, mean start latencies were relatively short (e.g., chicks left the start box under approximately 10 sec) and mean goal latencies were relatively long (e.g., chicks reached the goal under approximately 2 ½ min). Mean distance traveled for non-isolated conditions of each drug treatment was approximately 29 cm, indicating that most chicks either completed the maze or approached the stimulus cue. Isolated conditions of each drug treatment displayed mean start latencies that were similar to non-isolated controls. Surprisingly, isolated conditions of each drug treatment displayed shorter mean goal latencies and longer mean distance traveled relative to non-isolated controls.

Consistent with these observations, a 2 x 3 MANCVOA revealed a significant multivariate effect for stress, Wilks' $\lambda = 0.870$, $F(3, 96) = 4.77$, $p < 0.005$ (partial eta squared = 0.130 and power to detect the effect = 0.825). However, the MANCOVA failed to reveal a significant multivariate effect for drug treatment, Wilks' $\lambda = 0.940$, $F(6, 192) = 1.004$, p = n.s. (partial eta squared = 0.030 and power to detect the effect = 0.286), and also failed to reveal a significant multivariate interaction Wilks' $\lambda = 0.963$, $F(6, 192) = 0.606$, p = n.s. (partial eta squared = 0.019 and power to detect the effect = 0.158), where $p < 0.025$ is considered significant. Given the significant multivariate effect for stress, the univariate main effects were examined. A significant univariate main effect was obtained for mean goal latency $F(1,98) = 7.80$, $p < 0.01$ and for mean distance traveled $F(1,98) = 4.20$, $p < 0.05$.

Ambiguous Aversive Stimulus Cue (25c:75o Morph)

The effects of isolation stress and various drug treatment conditions on mean start and goal latency and mean distance traveled under the ambiguous aversive stimulus cue are presented in panels A, B and C of Figure 11, respectively. In non-isolated conditions of each

drug treatment, mean start latencies were relatively short (e.g., chicks left the start box in approximately 15 sec) and mean goal latencies were relatively long (e.g., chicks reached the goal under approximately 3 min). Mean distance traveled for non-isolated conditions of each drug treatment was approximately 27 ½ cm, indicating that most chicks either completed the maze or approached the stimulus cue. In general, isolated conditions of each drug treatment displayed longer mean start latencies and shorter mean distance traveled relative to non-isolated controls. In addition, isolated conditions for vehicle and imipramine treated chicks displayed longer mean goal latencies relative to non-isolated controls.

Consistent with these observations, a 2 x 3 MANCOVA revealed a significant multivariate effect for stress, Wilks' $\lambda = 0.829$, $F(3, 100) = 6.882$, $p < 0.001$ (partial eta squared = 0.171 and power to detect the effect = 0.951). However, the MANCOVA failed to reveal a significant multivariate effect for drug treatment, Wilks' $\lambda = 0.874$, $F(6, 200) = 2.327$, p = n.s. (partial eta squared = 0.065 and power to detect the effect = 0.707), and also failed to reveal a significant multivariate interaction Wilks' $\lambda = 0.951$, $F(6, 200) = 0.844$, p = n.s. (partial eta squared = 0.025 and power to detect the effect = 0.232), where $p < 0.025$ is considered significant. Given the significant multivariate effect for stress, the univariate main effects were examined. A significant univariate main effect was obtained for mean start latency $F(1,102) = 20.25$, $p < 0.001$, mean goal latency $F(1,102) = 4.045$, $p < 0.05$ and approached significance for mean distance traveled $F(1,102) = 5.468$, $p = 0.051$.

EXPERIMENT 3 DISUCSSION

Experiment 3 was conducted to examine whether cognitive biases observed in the Black Australorp (BA) strain could be reversed in a manner that parallels the strain's differential drug responsivity, whereby imipramine would fail and ketamine would succeed in reversing cognitive biases, respectively. Chicks received either physiological saline as a vehicle, imipramine or ketamine and were placed into the maze (non-isolated condition) or into the isolation apparatus (isolated condition). After 90 min, non-isolated and isolated chicks were tested in a straight alley maze to either an ambiguous appetitive (75c:25o) or an ambiguous aversive (25c:75o) stimulus cue. Distress vocalizations served as the dependent behavioral measure for isolated chicks and start and goal latency and distance traveled served as the dependent measures for the maze.

Distress Vocalizations

The pattern of distress vocalization (DVoc) rates in the vehicle condition were replicated from Experiment 1 and are consistent with previous studies of isolation stress wherein chicks in the anxiety-like phase (i.e., first 3-min) displayed high DVocs which was followed by approximately a 50% reduction of DVocs in the depression-like phase (i.e., last 60 min) (Hymel et al., under review; Sufka, 2006). However, inconsistent with previous studies showing that ketamine, but not imipramine attenuated the onset of behavioral despair by elevating DVoc rates in the depression-like phase (White, personal communication, June 2013), both ketamine and imipramine failed to show a significant elevation of DVoc rates compared to the vehicle treated

condition. The Black Australorp strains' insensitivity to imipramine at 10.0 mg/kg is consistent with previous findings showing a failed antidepressant effect of imipramine at 5.0, 10.0 and 15 mg/kg. However, the insensitivity of ketamine at 10.0 mg/kg is inconsistent with previous findings showing an antidepressant effect of ketamine at 5.0, 10.0 and 15.0 mg/kg (White, personal communication, April 2013). The inability to produce a significant ketamine effect may be due to several procedural factors that differed between the current study and previous study.

First, due to the large experimental design, only one dose of ketamine was tested in the current study. Although the doses were selected from previous studies showing antidepressant effects at 10.0 mg/kg (White, personal communication, April 2013), the nature and procedures of the current study may have necessitated either a higher or a lower dose of ketamine to produce significant antidepressant effects. Second, typical protocols do not require daily handling. To quantify cognitive bias, handling is necessary to reduce experimenter related stress on runway performance (Hymel & Sufka, 2012; Salmeto et al., 2011). However, handling may have altered the chicks' stress and drug responsivity.

Interestingly, neonatal handling in rats has shown to decrease emotivity in an open field test as measured by a decrease in defecation in an open field test relative to non-handled controls. In addition, neonatal handling also reduced vulnerability to learned helplessness as measured by decreased failure to avoid conditioned foot shocks in a shuttle-box relative to non-handled controls (Tejedor-Real, Costela, and Gibert-Rahola, 1998). Consistent with these findings a separate study showed that handled male rats displayed decreased immobility in a chronic, but not acute, forced swimming test relative to non-handled controls. Interestingly, handled female rats displayed increased immobility in a chronic, but not acute, forced swimming

test relative to non-handled controls. Further, neonatal handling decreased basal plasma Corticosterone levels in males and females (Papaioannou, Gerozissis, Prokopiou, Bolaris and Stylianopoulou, 2002). These findings reveal that handling can alter biological mechanisms associated with stress responsivity.

Handling has also shown to alter drug responsivity. A study by File, Andrews, Wu, Zharkovsky and Zangrossi (1992) showed that Chlordiazepoxide produced an anxiolytic effect in handled and non-handled rats in an elevated plus maze test by increasing the time spent in the open arms. However, prior exposure to the maze altered drug responsivity between handled and non-handled conditions. Handled rats with one prior exposure to the maze failed to show a Chlordiazepoxide effect; whereas, non-handled rats with one prior exposure did show a significant Chlordiazepoxide effect. These finding suggest that rat's prior exposure to not only the experimenter but also the test apparatus alters drug responsivity. Stress and drug responsivity did not appear to be altered by handling in the White Leghorn strain (Hymel & Sufka, 2012); however, handling alterations in the BA strain may reflect the strains' vulnerable nature.

Third, to minimize handling, the typical injection and testing protocol places squads of 6 into the isolation chambers simultaneously. The current study necessitated squads of 3 since only 3 at a time could be tested in the maze. However, this double handling protocols. Experimenter related stress (Feltenstein, Ford, Freeman, & Sufka, 2002) and flock reduction (Marx, Leppelt & Ellendorff, 2001) have shown to increase stress responses in chicks. Although measures were taken to reduce experimental stress, chicks may still experience a significant amount of stress as evidenced by clonidine's anxiolytic effect and imipramine's anxiolytic and antidepressant effect in socially tested chicks (2 conspecifics and two mirrors) (Hymel, 2010). In addition, altering isolating tests, simultaneously tested chicks entering behavioral despair with those in the panic-

like state. Chicks exhibit DVocs to attempt to reinstate social contact (Gallup & Suarez, 1980). Although the test apparatus media material attenuates sound between chambers, given the communicative nature of DVocs chicks were most likely influenced by the calls of adjacent chicks therefore altering DVoc rates for the anxiety-like phase and onset to depression threshold lattices, respectively.

Lastly, transportation differences likely contributed to outcome variation. Each shipment was 2 days; however, the previous study shipped during winter months and the current study shipped during spring months. Cheng & Jefferson (2008) showed that following transportation stress, two chick strains selected for varying commercial productivity and survivability displayed differences in feeding, comforting behaviors, adrenal gland size, Corticosterone, 5-HT levels and 5-HT1A receptor expression. As an added factor, in chickens cold stress may alter cellular immunity and corticosterone levels in a duration dependent manner (Hangalapura, Nieuwland, Buyse, Kemp, & Parmentier, 2004; Hangalapura, Nieuwland, de Vries Reilingh, van den Brand, Kemp, & Parmentier, 2004). Collectively, shipping differences in combination with all the procedural differences (previously discussed) are presumably the reason for alterations in ketamine's drug responsivity observed between the two experiments.

Ambiguous Appetitive Stimulus Cue (75c:25o Morph)

Runway performance for non-isolated vehicle treated Black Australorp chicks is consistent with Experiment 2 and previous findings using the White Leghorn strain wherein most chicks leave the start box under approximately 30 sec and reach the goal line in approximately 2 min (distance traveled being approximately 30 cm). In addition, no significant drug treatment effects were observed on runway performance between non-isolated conditions. In general, isolated conditions did not reveal any significant differences on mean start latencies, but did

reveal significantly shorter mean goal latencies and significantly longer mean distances traveled relative to non-isolated. In addition, no significant drug treatment effects were observed on runway performance between isolated conditions which is consistent with the finding that imipramine and ketamine failed to prevent the onset of behavioral despair.

Interestingly, less optimistic-like behavior under the 75c:25o stimulus cue failed to be replicated from Experiment 2 and previous studies as evidenced by the isolated vehicle condition displaying increased, rather than decreased, runway performance relative to the non-isolated vehicle condition. In addition, all isolated drug treatment conditions displayed increased performance on mean goal latency and mean distance traveled. In previous studies, cognitive bias of less optimistic-like behavior presented as decreased approach behavior to ambiguous appetitive stimuli (i.e., longer start and goal latencies and shorter distance traveled compared to the non-isolated vehicle condition). It is unfortunate that less optimism was not observed; however, it is surprising and unclear as to why the isolated drug treatment conditions revealed runway performance on mean goal latency and mean distance traveled in the opposite direction.

These findings may possibly be interpreted as the isolated drug treatment conditions showing an increased stress response relative to non-isolated controls. Chicks were exposed to the maze during the pretest and may have experienced social reinstatement learning whereby the 75c:25o cue was appetitive enough to increase isolated chick's need for social reinstatement during testing. However, these findings are inconsistent with findings from Experiment 1 and 2 and previous studies (Hymel & Sufka, 2012; Salmeto et al., 2011).

Ambiguous Aversive Stimulus Cue (75c:25o Morph)

Runway performance for non-isolated vehicle treated Black Australorp chicks is consistent with Experiment 2 and previous findings using the White Leghorn strain wherein most

chicks leave the start box under approximately 30 sec and reach the goal line under approximately 3 min (distance traveled being approximately 30 cm). In addition, no significant drug treatment effects were observed on runway performance between non-isolated conditions. In general, isolated drug treatment conditions did reveal significantly longer mean start latencies, significantly longer mean goal latencies and somewhat shorter mean distances traveled relative to non-isolated controls. However, no significant drug treatment effects were observed on runway performance between isolated conditions which is consistent with the finding that imipramine and ketamine failed to prevent the onset of behavioral despair. In previous studies, more pessimistic-like behavior presented as increased avoidant behavior to ambiguous aversive stimuli (i.e., longer start and goal latencies and shorter distance traveled compared to the non-isolated vehicle condition). The current study accurately replicated more pessimism from Experiment 2 and previous studies as evidenced by the isolated vehicle condition; however, it failed to show a pharmacological reversal.

It is interesting to note that mean goal latency was shown to be a sensitive measure for the 25c:75o in Experiment 2 and 3. Although mean goal latency is a sensitive measure of runway performance in non-isolated chicks, the current findings are inconsistent with previous studies showing that mean goal latency failed to reveal cognitive bias in non-isolated conditions (Hymel & Sufka, 2012; Salmeto, 2011).

GENERAL DISCUSSION

Cognitive bias is a phenomenon that presents in individuals suffering from anxiety and/or depression in which cognitive disturbances elicit negative interpretations of ambiguous stimuli and/or events. More specifically, anxiety is associated with more pessimistic judgments, whereas depression is associated with both more pessimistic judgments and less optimistic judgments (Wright & Bower 1992; MacLeod & Byrne, 1996). Interestingly, cognitive bias has been shown to be sensitive to a variety of therapies affecting mood disturbances including Cognitive Behavioral Therapy (CBT) and Cognitive Bias Modification (CBM) (Bowler et al., 2012; Mobini, Reynolds & Mackintosh, 2013; Segal & Gemar, 1997) and a variety of pharmacotherapies. Following the administration of citrolpam, a serotonin selective reuptake inhibitor (SSRI), the negative interpretation biases observed in anxious individuals were ameliorated (Mogg et al., 2004; Weinstein & Nutt, 1995). Further, a single dose of reboxetine, a norepinephrine selective reuptake inhibitor (NSRI), reversed the negative biases observed in depressed individuals (Harmer et al., 2009).

Cognitive bias has previously been examined in the chick anxiety-depression continuum model using a measure of approach/avoidant behavior to a range of appetitive to aversive stimulus cues in a straight alley maze (Salmeto et al., 2011). Similar to clinical findings, cognitive bias is sensitive to pharmacological reversal in the chick model. Imipramine was able to reverse the cognitive biases of less optimism and more pessimism under the ambiguous

appetitive (mirror and 75:25c) and ambiguous aversive (25c:75o and 0c:100o) stimulus cues, respectively in a White Leghorn strain (Hymel & Sufka, 2012).

Consistent with clinical literature, the current study identified cognitive biases of more pessimism and less optimism in a stress-vulnerable Black Australorp and a stress-resilient Production Red strain. Surprisingly, the most robust strain difference presented between non-isolated conditions. The isolated conditions may have displayed an all-or-none effect on cognitive bias due to the 90 min isolation stressor. Interestingly, Mogg et al, (2004) observed that in anxious individuals cognitive bias decreased as a function of SSRI treatment improvement; the more efficacious the treatment, the fewer negative interpretations presented. Altering isolation test time and increasing runway parameters may allow for a more fine grained analysis to detect strain vulnerability and resiliency on cognitive bias and should be explored further.

Further, the current study was unable to detect a significant ketamine effect for reasons unknown. Although necessary to detect cognitive bias, several procedures differed between the current and previous study showing ketamine's antidepressant effects. From these, most likely candidate in mediating ketamine's sensitivity is handling. Handling has been shown to alter stress and drug responsivity (File et al., 1992; Papaioannou et al., 2002; Tejedor-Real et al., 1998;) and may do so in a dose dependent manner. In contrast, handling has also shown to increase stress (Feltenstein, Ford, Freeman, & Sufka, 2002). The effect of handling on stress and pharmacological sensitivity should be further explored dependent of the cognitive bias paradigm. Once the optimal dose of ketamine is identified, cognitive bias reversal with ketamine should be further explored. Collectively, by providing validative support to the identification of a stress-vulnerable and stress-resilient chick strain, the current study strengthens homologies between

clinical population of depression and this model. These findings further validate the chick anxiety-depression model as a neuropsychiatric simulation.

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VITA

University of Mississippi
Department of Psychology

A. EDUCATIONAL HISTORY

Master of Arts Degree
University of Mississippi
November 2010
Thesis Title: *Pharmacological Reversal of Cognitive Bias in the Chick Anxiety-Depression Model*

Bachelor of Arts Degree
University of Mississippi
May 2008
Major: Psychology; Minor: Philosophy

B. PROFESSIONAL POSITIONS

Research Assistant, Psychopharmacology Laboratory, University of Mississippi. 2008-13.
Duties: IACUC Compliance, URA Training and Supervision, Research Design and Scheduling, Data Collection, Analysis and Presentation and Manuscript Preparations
Supervisor: Kenneth J. Sufka, Ph. D., Professor of Psychology and Pharmacology

Teaching Assistant, Quantitative Methods in Psychology I and II. 2010-11.
Duties: Attend lectures, grade homework and office hours
Supervisor: Nicolaas Prins, Ph. D., Associate Professor of Perception

Graduate Instructor, University of Mississippi. 2010-12.
Duties: Presentation and examination of course material; PSY 319: Brain and Behavior
Supervisor: Karen Sabol, Ph. D., Associate Professor of Psychology and Pharmacology
Supervisor: Kenneth J. Sufka, Ph. D., Professor of Psychology and Pharmacology
Teaching Assistant, Introduction to Psychology 2011-12.
Duties: Attend lectures, supervise student learning sessions and office hours
Supervisor: Kenneth Sufka, Ph. D., Professor of Psychology and Pharmacology
Supervisor: Todd A. Smitherman, Ph.D., Assistant Professor of Psychology

Graduate Instructor, University of Mississippi. 2012-13.
Duties: Presentation and examination of course material; PSY 201: Introduction to Psychology
Supervisor: Kenneth J. Sufka, Ph. D., Professor of Psychology and Pharmacology

C. PROFESSIONAL ACTIVITIES

Society for Neuroscience Student Member, 2008-2013.

National Association of Graduate-Professional Students
National Director of Outreach, 2011-2012.

Faculty Senate Administrative Assistant
Senate Chair: Kenneth J. Sufka, Ph.D., 2008-2010.
Senate Chair: Robert B. Albritton, Ph.D., 2010-2011.

Graduate Student Council Member
Representing the Experimental Psychology Program, 2009-2010.
Representing the Psychology Department, 2010-2011.
Secretary, 2011-2012.
Vice President, 2012-2013.

University Standing Committees: Graduate Council and Student Affairs, 2012-2013.
Representing the Graduate Student Council

Associated Student Body
Representing the Graduate Student Body, 2010-2011.

Gamma Beta Phi Honors Society, 2009-2013.

American Psychological Association Campus Representative, Fall 2011.

Graduate Mentor for incoming graduate students, Fall 2011.

American Foundation for Suicide Prevention and Psi Chi's "Out of the Darkness" Suicide Prevention Walk Volunteer, 2011.

D. PUBLICATIONS

Hymel KA, Loria MJ, Salmeto AL, White SW, Sufka KJ (under review). Strain vulnerability and resiliency in the chick anxiety-depression model. Physiology and Behavior.

Loria MS, White SW, Robbins SA, Salmeto AL, Hymel KA, Murthy SN, Manda, P, Sufka KJ (in press). Brain-derived neurotrophic factor response in vulnerable and resilient genetic lines in the chick anxiety-depression model. Behavioural Brain Research.

Hymel KA, Sufka KJ, (2011). Pharmacological reversal of cognitive bias in the chick anxiety depression model. Neuropharmacology, 62, 161-166.

Salmeto AL, Hymel KA, Carpenter EC, Brilot BO, Bateson M, Sufka KJ (2011) Cognitive bias in the chick anxiety-depression model. Brain Research, 1373, 124-130.

Hymel KA, Salmeto AL, Kim EH, Sufka KJ (2010). Development and validation of the chick anxiety depression continuum model. In JE Warnick, AV Kalueff (Eds.), Translational Neuroscience and its Advancement of Animal Research: Advancement, Challenges, and Research Ethics, Nova Science Publishers, pp. 83-110.

E. CONFERENCE PRESENTATIONS

Salmeto AL, Hymel KA, Loria MJ, White SW, Sufka KJ (June 2013). Strain Vulnerability and Resiliency in the chick anxiety-depression model. 20th Annual International "Stress and Behavior" Neuroscience and Biopsychiatry Conference, New Orleans, LA.

Loria MJ, White SW, Robbins SA, Salmeto, AL, Hymel KA, Manda P, Murthy SN, Sufka KJ (June 2013). BDNF response in vulnerable and resilient lines in the chick anxiety-depression model. 20th Annual International "Stress and Behavior" Neuroscience and Biopsychiatry Conference, New Orleans, LA.

Hymel KA, Loria MJ, Salmeto AL, White SW, Sufka KJ (October 2012). The sky is falling: Strain vulnerability and resiliency in the chick anxiety-depression model. Society for Neuroscience. New Orleans, LA.

Sufka KJ, Hymel KA, Smitherman TA (February 2012). Supplemental peer instruction: Improving course material mastery. Annual Conference on the First Year Experience, San Antonio, TX.

Hymel KA, Sufka KJ, (November, 2011). Pharmacological reversal of cognitive bias in the chick anxiety-depression model. Neuroscience and Behavior Research Day, The University of Mississippi Medical Center, Jackson, MS.

Hymel KA, Sufka KJ, (November, 2011). Pharmacological reversal of cognitive bias in the chick anxiety-depression model. Society for Neuroscience. Washington, DC.

Salmeto AL, Hymel KA, Carpenter EC, Brilot BO, Bateson M, Sufka KJ (October, 2009) Cognitive bias in the chick anxiety-depression model. Society for Neuroscience. Chicago, IL.