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Measurement Of Anhedonia In The Chick Anxiety-Depression Model

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MEASUREMENT OF ANHEDONIA IN THE CHICK ANXIETY-DEPRESSION MODEL

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ABSTRACT

Anhedonia, the loss of pleasure in previously pleasurable activities, is one of the cardinal features of depression. To further validate the chick anxiety-depression model, the current study aimed at quantifying anhedonia as well its reversal with pharmaceuticals. The first goal was to identify a measure to quantify the display of anhedonia in chicks following exposure to an isolation stressor, the chick anxiety-depression model. All experiments involved a baseline and test measurement either after removal from the home cage (No Test) or exposure to the isolation apparatus with conspecifics and mirrors (Social) or individually (Isolated). Experiment 1 used a straight maze with start and goal latency serving as the dependent measures. Isolated chicks expressed delays in start latency compared to No Test chicks, which is interpreted as anhedonia-like behavior. Experiment 2 used a modified sucrose preference task to assess sucrose preference and the number of drinking events for water and sucrose. Consistent with the rodent literature, results showed decreased sucrose preference in Isolated chicks compared to No Test chicks. Experiment 3 involved measurement of behavior in a dust bath apparatus. Contrary to predictions, limited behavior was observed in the apparatus.

The second goal of the present study was to be able to reverse the display of anhedonia-like behavior, as measured in the straight alley maze, with pharmaceutical manipulation. Experiment 4 involved administration of vehicle, 10 or 15 mg/kg Imipramine prior to exposure to the isolation apparatus followed by testing in the straight alley maze. Both 10 and 15 mg/kg Imipramine were shown to alleviate the onset of behavioral despair in the isolation test. The 15 mg/kg Imipramine dose also alleviated the display of anhedonia in the straight alley maze.

Experiment 5 involved the administration of vehicle, 5 or 10 mg/kg Ketamine prior to exposure to the isolation apparatus followed by testing in the straight alley maze. Neither 5 nor 10 mg/kg Ketamine were able to alleviate the onset of behavioral despair or anhedonia as assessed in the straight alley maze.

DEDICATION

I would like to dedicate my dissertation to my husband who has supported me through every step of this journey.

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INTRODUCTION

Depression impacts 6.7% of individuals within the United States in a given year and is the most common mental disorder in the U.S. (NIMH, 2014). Depression is characterized by a general feeling of sadness, hopelessness, loss of motivation, loss of interest in previously pleasurable activities, and problems concerned with sleep, attention, and eating habits (Diagnostic and Statistics Manual-IV-TR). Depression impacts the individual, their family and society as a whole as indicated by the prediction that it will be the second leading cause of disability by 2020 (Murray and Lopez, 1997). Current treatment strategies include behavioral therapy (Wuthrich & Rapee, 2013) and pharmaceutical treatment (Dombrovskiet al., 2007; Quitkin et al. 2002). One of the first pharmaceutical strategies is often selective serotonin reuptake inhibitors (SSRIs) but 28-55% of patients do not respond to this treatment. In addition, the onset of symptom relief can be slow and others continue to experience residual symptoms (Nutt et al., 2007). As such, researchers continue to explore the etiology, biology and symptomology of depression as well as assessments of new treatment strategies.

Like many clinical syndromes, insights often come from animal models. Within the field of psychopharmacology, animal models fall into one of three different classes: behavioral bioassays, simulations and screening tests. Specifically behavioral bioassays measure brain-behavior relations, while simulations attempt to replicate a clinical syndrome in animals and screenings assess the efficacy of pharmaceutical therapies (Willner, *Behavioural Models in Psychopharmacology*).Within animal models of depression, simulations seem to best address the multifaceted nature of this disorder and include separation models, stress models and brain

damage models. Separation models, in general, involve isolating an animal from its conspecifics which elicits an initial stage of protest followed by a state of despair. These behaviors have been observed in a wide variety of species including non-human primates, hamsters, rats and chicks.

Stress models represent the largest group of animal models of depression, and involve assessments of behaviors that follow the implementation of stressors such as constraint, isolation, shock and others. One example is learned helplessness which involves exposing an animal to an uncontrollable stressor leading to decreased performance of the animal in learning and appetitive tasks, decreased locomotor activity, decreased appetite and decreased aggression. Another stress model is behavioral despair, which involves exposure to a task that initially leads an animal to display frenzied behaviors aimed at escape followed by immobility, which is characterized as the animal giving up. Additionally, chronic unpredictable stress (CUS) exposes animals to procedures such as electric shocks and immobilization which leads to decreased exploration in an open field task and elevated plasma corticosteroid levels. A variant on this is chronic unpredictable mild stress (CUMS) which involves the application of mild stressors such as food deprivation and changes in temperature over a period of weeks leading animals to display decreased reward sensitivity. Finally, some propose using brain damage such as olfactory bulbectomy to investigate depression (Willner, *Animal Models of Depression*).

Though rodent stress models have shown some efficacy in investigations of depression, in the last 5-10 years criticisms of the quality of current animal models of depression have been raised and broadly include inabilities to detect novel therapeutics and narrow symptom focus. A limit to novel compound detection is related to the creation of animal models based on responsivity to current pharmaceuticals that mirrors existing models. Additionally, despite depression being shown to be multi-syndromal, many models focus upon only one or a few of

the symptoms of depression. This multi-syndromal nature is best exemplified in the tripartite model which suggests that depression involves abnormalities in negative affect (ex; cognitive disturbances), positive affect (ex; anhedonia) and physiological hyperarousal (ex; shortness of breath) (Frazer and Morilak, 2005).

Considerable symptom overlap between anxiety and depression has been observed in clinical populations (Nutt, 200). To address this, hybridization of animal models of depression and animal models of anxiety is suggested (Kalueff et al., 2007). This not only addresses the time-course related nature of anxiety and depression, but reduces the number of purpose bred animals in the investigation of anxiety and depression.

Due to differences in behavioral repertoires, an animal simulation cannot be homologous to the human clinical picture, but it can model isomorphic features of the disorder. As the number of symptoms the simulation mimics increases the construct validity of the simulation is also enhanced (Miczek and de Wit, 2008). One strategy to validate a good animal model simulation is endophenotypic mapping. This involves assessments of behavioral, physiological, biochemical, endocrinological and/or neuroanatomical characteristics of the disease state under investigation. An ideal animal simulation should be an isomorph of the human clinical syndrome, where both show similar but species typical responses. The more connections of behavior, biology, pharmaceutical response and cognitive changes that can be made between the disease as expressed in humans and the disease as expressed in animals the stronger the animal simulation. Further, it should be possible to use information gained in an animal simulation and extend it to other species including humans (van der Staay, 2006).

A novel simulation that addresses these concerns involves the separation of chicks from conspecifics to produce both anxiety and depression-like states (Sufka et al. 2006). Specifically,

separation initially produces high distress vocalization (DVoc) rates characteristic of an anxiety-like state (i.e., panic; Warnick et al. 2006) that is followed by lower DVoc rates characteristic of a depression-like state (i.e., behavioral despair; Lehr 1989). These phases can be pharmacologically dissociated in that diverse compounds possessing anxiolytic effects (e.g., chlordiazepoxide, clonidine, imipramine) attenuate the high DVoc rates during the anxiety-like phase while compounds possessing antidepressant effects (e.g., imipramine, maprotiline, fluoxetine and ketamine) attenuate the reduction in DVoc rates during the depression-like phase (Sufka et al. 2006; Warnick et al. 2009; see also Lehr 1989). Common stress and depression biomarkers are present in the model and include elevated corticosterone and interleukin-6 (IL-6) levels (Sufka et al. 2006; Warnick et al. 2009). Another study screened the efficacy of seven compounds that had previously passed antidepressant screening in rodent models, but yielded different results that were more in line with early clinical trial outcomes. This illustrates the predictive validity of the model by correctly detecting efficacy of five compounds and avoiding two false positives of the rodent models (Sufka et al. 2009;Wolkowitz et al. 1999; Zarate et al. 2006;Belanoff et al., 2002; Schechter et al., 2005).

More recent research (Salmeto et al., 2011) has demonstrated homologies with the display of cognitive bias in anxiety and depression, where in clinical populations anxious individuals tend to adopt a more pessimistic-like interpretation of ambiguous aversive stimuli whereas depressed individuals tend to adopt a less optimistic-like interpretation of ambiguous appetitive stimuli (Wright & Bower 1992; MacLeod & Byrne 1996). Within the chick anxiety-depression paradigm results mirrored the clinical literature. Specifically, chicks in an anxiety-like state displayed more pessimism to ambiguous appetitive stimuli and chicks in a depressive-like state displayed both more pessimism and less optimism to ambiguous aversive stimuli. The

reversal of cognitive bias with pharmaceuticals was explored by Hymel and Sufka (2011), showing that administration of Imipramine to chicks exposed to the depression-like phase was able to reverse the cognitive biases of less optimism and more pessimism.

Environmental enrichment has also been related to the display of depression. To investigate this in the chick anxiety-depression model (Kim and Sufka, 2011) the chick home cage environment was altered. Chicks were either housed in a standard non-enriched environment or an enriched environment or a mix of the two. When tested in the isolation apparatus, chicks housed in continuous enrichment and chicks housed in early non-enrichment followed by enrichment displayed delays in depression onset latencies compared to chicks housed in continuous non-enrichment and those exposed to early enrichment followed by non-enrichment.

Although many connections between the animal model and the disease state have been demonstrated, areas that still need to be explored involve genetic mapping of the various strains, analysis of biomarkers and the biological basis for drug sensitivity/insensitivity. Another homology that could be demonstrated between the model and the clinical syndrome is the symptom of anhedonia, which is one of the defining features of depression (Diagnostic and Statistics Manual-IV-TR).

In order to investigate anhedonia in the chick model, it is necessary to consider how anhedonia has been modeled/measured in humans and animals. One measure of anhedonia in humans is the Fawcett and Clark Pleasure scale which consists of sentences that describe pleasurable situations and asks people to rate the degree of pleasure they would experience if that situation were to actually occur (Fawcett et al., 1983a). A non-survey strategy to study anhedonia explored by Amsterdam et al. (1987) involves analysis of sweet taste preference in people

suffering from depression where results showed depressed patients rated the stimuli as more pleasant compared to ratings of control participants. Similarly Berlin et al. (1998) had depressed and control participants taste five sucrose solutions of different concentrations and rate the pleasantness of each. Despite the lack of significant difference in hedonic response to sucrose between the groups, mean perceived concentration for sweet taste was shown to be higher in depressed patients, indicating a higher perception threshold for sweet taste despite a lack of difference in hedonic responses. A similar strategy was explored by Chentsova-Dutton and Hanley (2010) and assessed hedonic response (HR) to food to differentiate the type of reward deficits observed in individuals suffering from depression and/or anhedonia. Participants completed reports of anticipated HR to six potential study tasks, experienced HR to seven food samples and recall HR taken the next day. Results showed that all participants reported a greater anticipatory HR to the chocolate tasting than their experienced HR and that their recalled HR was lower than both (Chentsova-Dutton & Hanley, 2010). It is suggested that the deficits in pleasure response of MDD patients can be observed at a higher cognitive level of appraisal but the basic ability for pleasure reactions remains intact. This postulation is consistent with theories of anhedonia which highlight an undervaluation of reward as opposed to a global flattening of pleasure responses (Dichter et al., 2010).

One objective laboratory measure to assess hedonic capacity is that of Pizzagalli et al. (2009) and assesses the impact of reinforcement history through use of a probabilistic reward task. Overall study results showed that MDD patients were impaired in their ability to integrate prior reinforcement information into their current behavior (Pizzagalli et al., 2009). Another more specific measure is that of Treadway et al. (2009) and involves an adapted rat model of reward wanting (Salamone et al., 1994) for use in human participants, in order to assess the

decreased motivation to pursue reward that is another characteristic of anhedonia. Specifically, participants completed the Effort-Expenditure for Rewards Task (EEfRT) which involves being presented with a choice to complete an easy or difficult task where the difficult task is rewarded with a larger monetary payout. Participants were not guaranteed to receive a reward upon successful completion of the task and instead were provided information as to the probability of the task resulting in a reward prior to beginning the task. The relationship between task choices and questionnaire anhedonia measures showed that individuals with higher levels of trait and state anhedonia displayed a reduction in hard task choices. Further, it was shown that anhedonia level was the best predictor of hard-task choices for trials in which the uncertainty of reward and payoff were high. Similar to the results of Pizzagali et al. (2009), it was also shown that outcomes of recent previous trials were more influential on the decisions of whether or not to complete the hard task in participants with higher levels of trait anhedonia (Treadway et al., 2009). The fact that the EEfRT model was created based upon an animal model to assess anhedonia shows how utilization of both humans and animals in the study of anhedonia is important for its understanding.

The classic rodent model for the assessment of anhedonia involves the sweet taste preference task where the amount of sucrose solution consumed is compared to the amount of water consumed. It is suggested that lower consumption of the sucrose solution is indicative of a state resembling anhedonia (Hayase, 2011; Paul et al., 2000). Hayase (2011) utilized the sucrose test to investigate mice exposed to immobilization stress (IM). Results showed that IM lead to a significant attenuation in preference for the sucrose solution compared to control mice. Further, upon exposure to drugs that inhibit the reuptake of the monoamines, sucrose consumption levels were returned to those of control mice. Similarly, Delgado y Palacios et al. (2011) exposed rats

to the chronic mild stress model (CMS), which is used to induce depression-like symptoms. Next, all rats were exposed to a sucrose solution twice a week for three weeks and split into two groups, matched upon their baseline sucrose intake. One group was left in standard housing while the other was exposed to mild stressors for a period of 8 weeks. Sucrose test results showed that even after 1 week of exposure to CMS the rats could be divided into anhedonic and resilient types and this difference remained throughout the stress protocol. Further, control rats not exposed to CMS consumed significantly more of the sucrose solution than the anhedonic group but there was no significant difference in sucrose consumption between the control rats and the resilient group (Delgado y Palacios et al., 2011).

In another study, a breeding paradigm that selects for rats that display helpless behavior (cLH) and those that show resilience to learned helplessness (cNLH) assessed intake of sweetened condensed milk (SCM) before and 7, 14, 21 and 28 days after initial foot-shock exposure. Results showed similar SCM consumption for the pre-test measurement, but cLH rats displayed lower consumption than cNLH rats both when tested 7 and 14 days after the stress exposure and continued to display a trend at 21 days. A second exposure to foot shock stress occurred approximately one month after the initial exposure and SCM intake was assessed 3, 7 and 14 days after this second stress exposure. This reinstated the lower SCM intake in cLH rats compared to cNLH at 3, 7 and 14 days after the second stress exposure. Additionally the impact of stress on hedonic response using the pleasure attenuated startle (PAS) paradigm, which assesses the incentive properties of a conditioned cue by measuring acoustic startle response (ASR), was investigated. Rats were exposed to two foot-shocks and though baseline ASR was shown to be similar between cLH and cNLH rats, after stress exposure the PAS response of cNLH rats was significantly lower which is consistent with previous research. Overall this study

shows that foot-shock stress can be used to induce anhedonia-like behavior in rats bred for learned helplessness and that PAS can be used to assess anhedonic states (Enkel et al., 2010).

One treatment method for depression is repetitive transcranial magnetic stimulation (rTMS) and involves induction of an electric field in the brain over a number of sessions. This procedure has been shown to alter excitability in the brain and is useful as both a therapeutic tool and as a way to study various psychiatric disorders (Rossi et al., 2009). Feng et al. (2012) explored if rTMS could ameliorate anhedonia-like behaviors by first exposing 8 week old rats to a chronic unpredictable mild stress (CUMS) paradigm, which is used to induce depression-like symptoms, for 8 weeks. Rats were acclimated to the procedure of rTMS for a period of 2 weeks, after this acclimation period, 1000 pulses were administered daily for 21 consecutive days. The sucrose preference test (SPT) was used as a behavioral measure of anhedonia. Results showed that after 3 weeks of treatment with rTMS rats exposed to CUMS showed a significant increase in the SPT. Further, after the cessation of treatment the decline in the SPT was gradual, suggesting the reversal of symptoms of anhedonia that remain after the end of treatment. These results further the validity of rTMS as a therapeutic tool by illustrating its ability to ameliorate anhedonic symptoms.

Depression involves both a biological predisposition (Zhang et al., 2006; Zoratto et al., 2011) and an environmental aspect (Macri et al., 2009). To create an animal model with similar features, Zoratto et al. (2011)exposed mouse dams and thus the mice in their litter (postnatal day 0-8) to a tryptophan deficient diet leading to decreases in serotonin levels which is associated with depressive-like abnormalities (Gross et al., 2002). Mimicking the environmental aspect of depression was achieved through a high dose of corticosterone in the water of the dams, which has been used as a model of neonatal stress (Macri et al., 2007). These procedures lead to a

control group (AFR),tryptophan deficient only group (T), corticosterone only group (C) and a tryptophan deficient and corticosterone group (TC).At adulthood mice were tested in an approach-avoidance paradigm to assess anxiety-like behavior and a progressive ratio paradigm to assess reward motivation. In the approach-avoidance paradigm mice were exposed to an environment consisting of two unique compartments, one of which was novel. The progressive ratio schedule required mice to make an increasing number of nose pokes in order to obtain a food reward. The performance of each mouse resulted in a breakpoint value which represented the highest ratio of nose pokes the mouse was able to reach within an 8 min criterion. In the approach-avoidance paradigm, TC mice showed a reduced preference for the unfamiliar compartment whereas no difference was observed between T, C and AFR mice, indicative of a higher level of anxiety in TC mice. Progressive ratio schedule task results showed T, C and TC mice all displayed significantly lower breakpoint values compared to AFR mice, suggesting lower levels of motivation (Zoratto et al., 2012). This paradigm shows that the combination of a biological predisposition and environmental stressors lead to higher levels of anxiety-like behavior. Further, a biological predisposition, environmental stressors or their combination results in diminished reward motivation.

The chick anxiety-depression has illustrated many of the characteristic endophenotypes of anxiety and depression including behavioral patterns of despair (Sufka et al. 2006; Feltenstein & Sufka, 2005; Sufka et al. 2009), stress and depression biomarkers (Sufka et al. 2006, Warnick et al. 2009) pharmacological sensitivity (Feltenstein & Sufka, 2005; Sufka et al. 2009), cognitive biases (Salmeto et al., 2011; Hymel & Sufka, 2012) and the effect of environmental enrichment (Kim & Sufka, 2011). However, it has not demonstrated the expression of anhedonia, one of the cardinal features of depression (Diagnostic and Statistics Manual-IV-TR). By showing the

presence of this phenotype within the model, its validity as a simulation will be increased. Creation of a chick model of anhedonia will involve both utilizing previous research in rodents and developing a model which is more ecologically relevant to chicks.

One strategy that could be adopted for assessments of anhedonia involves measures of social reinstatement with conspecifics. For example, Marin et al. (2001) exposed chicks to an acute crush stressor and 1 hr later assessed runway performance to reinstate contact with conspecifics. Chicks exposed to the stressor displayed quicker exit from the start box and increased time near social companions compared to chicks not exposed to the stressor when tested in an open field apparatus. The current experiment will utilize a straight alley maze to assess social reinstatement. This apparatus has previously demonstrated the ability to detect the display of cognitive biases as a result of the chick anxiety-depression model (Salmeto et al., 2011; Hymel & Sufka, 2011). It is theorized that exposure to the isolation stressor will decrease both start and goal latencies as well as distance traveled in the straight alley maze.

A standard measurement of anhedonia is the sucrose preference task which has been used in rodents to show the impact of stress on consumption of sweetened liquids (Hayase, 2011; Paul et al., 2000; Delgado y Palacios et al., 2011) and could be used in aves. Research by Gentle and Harkin (1979) illustrated avian taste sensitivity by recording the reactions of 6 month old hens to varying concentrations of sucrose, fructose and carboxymethyl cellulose. Results showed an increase in beak and tongue movements for sucrose and fructose, increased head shaking for only sucrose and increased beak wiping for sucrose and carboxymethyl cellulose. These results show the sensitivity of aves for different oral stimuli. Additionally, Gentle (1972) exposed hens to a choice situation to assess preference for a variety of liquids including 1, 2.5, 5, 10, 20 and 30% concentrations of glucose, fructose, and sucrose. All were rejected at 30% concentrations,

glucose above 5%, and fructose at 1% but displayed insignificance at 10 and 20%. Concentrations of 5% sucrose did show a preference but higher concentrations yielded insignificant results. Further, Ganchrow et al. (1990) showed that gustatory distinction is present in recently hatched chicks in an experiment that involved exposure to distilled water, 0.3 and 1.7 M fructose, 0.005 and 0.2 M sodium saccharin, 0.02 M quinine hydrochloride and 0.01 and 0.1 N citric acid. Hedonic reactions were assessed through measurements of pecking contact, drinking contact, beak clapping, gaping, beak wiping, head shaking, walking away or re-approaching the liquid dish, vocalizations and pecking at the floor. Results showed significant unpleasant reactions to the higher quinine and citric acid concentrations compared to water, while the mean hedonic reactions for fructose, saccharin and water were similar. Since chicks do show a reaction to gustatory stimuli, it is posited that they will show a similar behavioral response as rodents within the sucrose test, where exposure to the isolation stressor will lead to decreases in sucrose consumption.

A novel strategy that could be employed in studies of anhedonia is dust bathing which is a naturally occurring behavior of aves (Duncan et al., 1998; Petherick, 1992; Olsson et al., 2002). Research has shown that hens will work for the opportunity to dust bath independent of recent dust bath availability, suggesting that it represents a pleasurable activity (Widowski and Duncan, 2000). It is thus hypothesized that exposure to the isolation stressor will lead to decreases in time spent dust bathing.

The ability to obtain information on anhedonia is enhanced by the use of models of anhedonia based on previous research in rodents and aves as well as a novel model that more closely represents the natural behavior of chicks. Since literature exists on strain differences in stress vulnerability and measures of anhedonia the importance of the strain used in studies of

anhedonia is apparent. For example, Stedenfeld et al. (2011) exposed rats that were breed to express either low (bLR) or high (bHR) locomoter activities in a novel environment to chronic mild stress (CMS). Next, rats were assessed for hedonic preference through weekly sucrose preference test measures. Results showed that bLR rats expressed anhedonia more quickly and to a greater extent than bHR rats. These findings suggest that increased emotional reactivity is important in investigations of stress susceptibility. Recent research (Hymel et al., 2013; Loria et al., 2013; Sufka & White, 2013) in this lab has elucidated two different strains of cockerels, where Black Australorps display stress vulnerability and Production Reds show stress resiliency. Based upon the finding of Stedenfield et al. (2011) the present study will use the Black Australorp strain for investigations of measuring anhedonia in chicks.

Once the best method for assessing anhedonia in the chick anxiety-depression model is determined it will be important to show the impact of antidepressant pharmaceuticals on the display of anhedonia-like behavior. For example, the work of Hayase (2011) showed the ability of fluvoxamine maleate (FL), amitriptyline (AT) and clomipramine hydrochloride (CL) to reverse anhedonia-like behavior in the sucrose preference task. Research has also shown the ability of the novel antidepressant ketamine to attenuate the decrease in sucrose consumption following stressor exposure (Garcia et al., 2009; Li et al., 2011). Using Black Austrolorps in the chick anxiety-depression model, Sufka and White (2013) showed no effect of Imipramine but an alleviation of behavioral despair with Maprotiline and Ketamine. The present research will involve administration of different doses of the tricyclic Imipramine or the novel antidepressant Ketamine. It is posited that Imipramine will neither attenuate behavioral despair or anhedonic behavior but Ketamine will.

MATERIALS AND METHODS

Part 1

Experiment 1

Subjects

Black Australorp cockerels (*Gallus gallus;* Ideal Poultry) were received two days post hatch and housed in 34 x 57 x 40 cm stainless steel cages with 12 chicks per cage. Food (Purina Start and Grow, St. Louis, MO) and water were available *ad libitum* through one quart gravity-fed feeders (Murray MacMurray; Model 4YQW0) and waterers (Murray MacMurray, Model 4YQWO). Room temperature was maintained at 29 +/- 1^{o} C and overhead illumination was maintained on a 12 hr light-dark cycle.

Materials

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 (Radio Shack, Omnidirectional Model 33-3013 (modified for AC current))

mounted on the top of the Plexiglas chamber and routed to a computer equipped with custom designed software for data collection (continuous acquisition with sample rates > 10/sec).

Procedure

Baseline Social Reinstatement

At 5 days post-hatch a baseline measure of social reinstatement was taken for all chicks. Twelve cage mate conspecifics were placed into the holding arena and individually tested in the straight alley maze. Each chick was individually placed in the start box for 10 s after which the guillotine door was raised. Dependent measures included start latency, distance traveled and goal latency. Start latency was defined as the time it took the chick to step completely outside of the start box. Distance traveled was defined as the furthest distance traveled by the chick during the test session. Goal latency was defined as the time to cross a defined mark located 10 cm away from the mirror. A test session was terminated at 5 min if the chick did not reach the goal and a goal latency of 300 sec recorded. After reaching the goal the chick was placed back in the holding arena and the procedure repeated until all were tested.

Chick anxiety-depression model

Testing for the impact of a depressive-like state on social reinstatement occurred on day 6. The Social control group was transported to the isolation test room where each chick was exposed to the isolation apparatus for 90 min, but with 2 conspecifics and mirrors in each chamber. For the Isolated group, 6 chicks were transported to the apparatus test room and each placed into one of the isolation chambers for a period of 90 min during which distress vocalizations were recorded.

Post-stressor Social Reinstatement

The No Test group, not exposed to the isolation apparatus, was assessed for its performance in the straight alley maze following the same procedure as the baseline measure. Following exposure to the isolation stressor, Social chicks were transported to the test room in a 2-quart opaque plastic container and individually tested in the straight-alley maze. Isolated chicks were also transported to the test room in a 2-quart opaque plastic container and tested in the straight alley maze.

Statistical Analysis

One and 2-way ANOVAs were performed where appropriate to look for treatment differences. A MANOVA also analyzed start and goal latencies at baseline compared to the same measures following stressor exposure both within each treatment type (no test, social, isolated) and between treatment types. When applicable, Fischer's LSD was conducted to elucidate group differences.

Experiment 2

Subjects

Black Australorp cockerels (*Gallus gallus;* Ideal Poultry) were received two days post hatch and housed in 34 x 57 x 40 cm stainless steel cages with 6 chicks per cage and paper boards placed between cages to prevent social facilitation of liquid consumption. Further, water availability via one quart gravity-fed feeders (Murray MacMurray; Model 4YQW0) was titrated down to 1 hr a day to ensure accurate measurement of liquid intake. Specifically, chicks had 12 hr access at 2 days post hatch, 6 hr at 3 days post hatch, 3 hr at 4 days post hatch and1 hr at days 5 and 6 post hatch. The time of day for water access roughly corresponded to the time of day at which chicks had access during testing. Food (Purina Start and Grow, St. Louis, MO) weas available *ad libitum* through one quart gravity-fed feeders (Murray MacMurray; Model

4YQW0). Room temperature was maintained at 29 +/ 1^{o} C and overhead illumination was maintained on a 12 hr light-dark cycle.

Materials

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 (Radio Shack, Omnidirectional Model 33-3013 (modified for AC current)) mounted on the top of the Plexiglas chamber and routed to a computer equipped with custom designed software for data collection (continuous acquisition with sample rates > 10/sec).

Procedure

Baseline Sucrose Intake

At 5 days post hatch a baseline measure of liquid intake via one quart gravity-fed feeders (Murray MacMurray; Model 4YQW0) for 1.0 M sucrose compared to water was taken during the first 15 min of the 1 hour liquid access period, in the home cage of 6 chicks at the same time of day that their post-stress measure occurred. This was recorded on video to allow for measurement of the amount of time spent drinking of all 6 chicks in a cage.

Chick anxiety-depression model

Testing for the impact of a depressive-like state on sucrose intake occurred on day 6. The Social group was transported to the isolation test room where each chick was exposed to the isolation apparatus for 90 min, but with 2 conspecifics and mirrors in each chamber. For the Isolated group, 6 chicks were transported to the apparatus test room and each placed into one of the isolation chambers for a period of 90 min during which distress vocalizations were recorded.

Post-stressor Sucrose Intake

The No Test group, not exposed to the isolation apparatus, was assessed in a measure of liquid intake for 1.0 M sucrose compared to water in the first 15 min of the 1 hr liquid access period, in the home cage of the 6 chicks. Immediately following exposure to the isolation stressor, a measure of liquid intake for 1.0 M sucrose compared to water was taken in the first 15 min of the 1 hr liquid access period in the home cage of the 6 chicks. This was recorded on video to allow for measurement of the amount of time spent drinking of all 6 chicks in a cage.

Statistical Analysis

One and 2-way ANOVAs were performed where appropriate to look for treatment differences. Next a MANOVA compared baseline preference for water and sucrose and time spent drinking after stressor exposure both within each treatment type (No Test, Social, Isolated) and between treatment types. When applicable, Fischer's LSD was conducted to determine group differences.

Experiment 3

Subjects

Black Australorp cockerels (*Gallus gallus;* Ideal Poultry) were received two days post hatch and housed in 34 x 57 x 40 cm stainless steel cages with 12 chicks per cage. Food (Purina Start and Grow, St. Louis, MO) and water were available *ad libitum* through one quart gravity-

fed feeders (Murray MacMurray; Model 4YQW0) and waterers (Murray MacMurray, Model 4YQWO). Room temperature was maintained at 29 +/- 1^{o} C and overhead illumination was maintained on a 12 hr light-dark cycle.

Materials

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 (Radio Shack, Omnidirectional Model 33-3013 (modified for AC current)) mounted on the top of the Plexiglas chamber and routed to a computer equipped with custom designed software for data collection (continuous acquisition with sample rates > 10/sec).

Dustbath

Beginning at 3 days post hatch the dust bath was placed in the home cage for a period of 1 hr a day to allow for familiarization and to lessen novelty induced stress. The dust bath consisted of sand and gravel in a closed bottom container that was easily entered by the chicks.

Procedure

Baseline Dust Bathing

At 5 days post-hatch a 20min baseline measure of activity in the dust bath was taken at the same time of day chicks were exposed to it on the isolation test day. This was recorded on video to allow for measurement of the dependent measures of time of first event, time spent

preening, number of foraging events and number of dust bathing events for each chick. The dependent measure of time of first event was defined as the time at which a chick performed any of the other dependent measures. Time spent preening was the total amount of time a chick engaged in wing lifting and pecking towards itself to clean. A foraging event was defined as a chick performing a strong kick at the grit immediately followed by pecking at the grit. A dust bathing event consisted of chicks in a ventral recumbent posture manipulating the grit around them so as to get it on their bodies.

Chick anxiety-depression model

Testing for the impact of a depressive-like state on dust bathing occurred on day 6 post-hatch. The Social group was transported to the isolation test room where each chick was exposed to the isolation apparatus for 90 min, but with 2 conspecifics and mirrors in each chamber. For the Isolated group, 6 chicks were transported to the apparatus test room and each placed into one of the isolation chambers for a period of 90 min during which distress vocalizations were recorded.

Post-stressor Dust Bathing

The No Test group, not exposed to the isolation apparatus, was assessed in a 20 min measure of activity in the dust bath in the home cage. Immediately following exposure to the isolation stressor, a 20 min measure of activity in the dust bath was taken in the home cage. This was recorded by video to allow for measurement of the dependent variables.

Statistical Analysis

One and 2-way ANOVAs were performed where appropriate to look for treatment differences.. A MANOVA also analyzed the time of first event, time spent preening, number of foraging events and number of dust bathing events at baseline to these measures following

stressor exposure both within each treatment type (no test, social, isolated) and between treatment types. When applicable, Fischer's LSD was conducted to determine group differences.

Part 2

Experiment 4

Subjects

Black Australorp cockerels (*Gallus gallus;* Ideal Poultry) were received two days post hatch and housed in 34 x 57 x 40 cm stainless steel cages with 12 chicks per cage. Food (Purina Start and Grow, St. Louis, MO) and water were available *ad libitum* through one quart gravity-fed feeders (Murray MacMurray; Model 4YQW0) and waterers (Murray MacMurray, Model 4YQWO). Room temperature was maintained at 29 +/- 1^{o} C and overhead illumination was maintained on a 12 hr light-dark cycle.

Materials

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 (Radio Shack, Omnidirectional Model 33-3013 (modified for AC current)) mounted on the top of the Plexiglas chamber and routed to a computer equipped with custom designed software for data collection (continuous acquisition with sample rates > 10/sec).

Procedure

Baseline Social Reinstatement

At 5 days post-hatch a baseline measure of social reinstatement was taken for all chicks. Twelve cage mate conspecifics were placed into the holding arena and individually tested in the straight alley maze. Each chick was individually placed in the start box for 10 s after which the guillotine door was raised. Dependent measures included start latency, distance traveled and goal latency. Start latency was defined as the time it took the chick to step completely outside of the start box. Distance traveled was defined as the furthest distance traveled by the chick during the test session. Goal latency was defined as the time it took the chick to cross a defined mark located 10 cm away from the mirror. A test session was terminated at 5 min if the chick did not reach the goal and a goal latency of 300 sec recorded. After reaching the goal the chick was placed back in the holding arena and the procedure repeated until all were tested.

Chick anxiety-depression model

Testing for the impact of separation stress on behavior occurred on days 6-7 post- hatch. The No Test group was injected with 10 mg/kg imipramine and placed back in the home cage 90 min prior to testing for anhedonia-like symptoms. Fifteen minutes prior to exposure to the isolation apparatus, the Social group was intraperitoneally injected with 10 mg/kg Imipramine while Isolated chicks were intraperitoneally injected with vehicle or 10 mg/kg or 15 mg/kg imipramine. The social control group was transported to the isolation test room where each chick was exposed to the isolation apparatus and DVocs recorded for 90 min, but with 2 conspecifics and mirrors in each chamber. For the Isolated group, 6 chicks were transported to the apparatus test room and each placed into one of the isolation chambers for a period of 90 min during which distress vocalizations were recorded.

Anhedonia Measurement

The No Test group, not exposed to the isolation apparatus, was assessed 90 min after injection for its performance in the straight alley maze following the same procedure as the baseline measure. Following exposure to the isolation apparatus, chicks were individually transported to the test room in a 2-quart opaque plastic container and tested in the straight-alley maze.

Statistical Analysis

One and 2-way ANOVAs were performed where appropriate to look for treatment differences. A MANOVA also analyzed start and goal latencies at baseline compared to the same measures following stressor exposure both within each treatment type (no test, social, isolated) and between treatment types. When applicable, Fischer's LSD was conducted to elucidate group differences.

Experiment 5

Subjects

Black Australorp cockerels (*Gallus gallus;* Ideal Poultry) were received two days post hatch and housed in 34 x 57 x 40 cm stainless steel cages with 12 chicks per cage. Food (Purina Start and Grow, St. Louis, MO) and water were available *ad libitum* through one quart gravity-fed feeders (Murray MacMurray; Model 4YQW0) and waterers (Murray MacMurray, Model 4YQWO). Room temperature was maintained at 29 +/- 1^{o} C and overhead illumination was maintained on a 12 hr light-dark cycle.

Materials

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 (Radio Shack, Omnidirectional Model 33-3013 (modified for AC current)) mounted on the top of the Plexiglas chamber and routed to a computer equipped with custom designed software for data collection (continuous acquisition with sample rates > 10/sec).

Procedure

Baseline Social Reinstatement

At 5 days post-hatch a baseline measure of social reinstatement was taken for all chicks. Twelve cage mate conspecifics were placed into the holding arena and individually tested in the straight alley maze. Each chick was individually placed in the start box for 10 s after which the guillotine door was raised. Dependent measures included start latency, distance traveled and goal latency. Start latency was defined as the time it took the chick to step completely outside of the start box. Distance traveled was defined as the furthest distance traveled by the chick during the test session. Goal latency was defined as the time it took the chick to cross a defined mark located 10 cm away from the mirror. A test session was terminated at 5 min if the chick did not reach the goal and a goal latency of 300 sec recorded. After reaching the goal the chick was placed back in the holding arena and the procedure repeated until all were tested.

Chick anxiety-depression model

Testing for the impact of separation stress on behavior occurred on days 6-7 post- hatch. The No Test group was intraperitoneally injected with 10 mg/kg Ketamine and placed back in the home cage 90 min prior to testing for anhedonia-like symptoms. Fifteen minutes prior to exposure to the isolation apparatus, the Social group was intraperitoneally injected with 10 mg/kg Ketamine while the Isolated group was intraperitoneally injected with vehicle or 5 mg/kg or 10 mg/kg mg/kg ketamine. The social control group was transported to the isolation test room where each chick was exposed to the isolation apparatus and DVocs recorded for 90 min, but with 2 conspecifics and mirrors in each chamber. For the isolation group, 6 chicks were transported to the apparatus test room and each placed into one of the isolation chambers for a period of 90 min during which distress vocalizations were recorded.

Anhedonia Measurement

The No Test group, not exposed to the isolation apparatus, was assessed 90 min after injection for its performance in the straight alley maze following the same procedure as the baseline measure. Following exposure to the isolation apparatus, chicks were individually transported to the test room in a 2-quart opaque plastic container and tested in the straight-alley maze.

Statistical Analysis

One and 2-way ANOVAs were performed where appropriate to look for treatment differences. A MANOVA also analyzed start and goal latencies at baseline compared to the same measures following stressor exposure both within each treatment type (no test, social, isolated) and between treatment types. When applicable, Fischer's LSD was conducted to elucidate group differences.

RESULTS

Part 1

Experiment 1

Baseline (i.e., prior to the stress manipulation) social reinstatement behavior in the straight alley maze, as measured by start and goal latency, is presented in Table 1. The distance traveled measure was not included herein as most chicks completed the entire maze within the 300 sec criterion. In general, both start and goal latencies were shorter in the No Test group compared to the Social and Isolated groups. A 1-way ANOVA of start latency data failed to reveal a significant group effect, $F(2, 46)= 1.765$, $p = 0.183$. Planned comparisons approached a longer start latency for the Isolated group compared to the No Test group ($p = 0.073$). A 1-way ANOVA for goal latency failed to detect a significant group effect, $F(2, 46) = 0.909$, $p = 0.41$. No further analyses were conducted on these data.

The effects of isolation stress, as measured by DVocs, over the 90 min test session are presented in Figure 1. In general, Social birds display a low level of DVocs throughout the test period while isolated birds initially display a high level of DVocs which by 30 min declines by about 45% and remains relatively stable thereafter. Consistent with these observations, a 2-way ANOVA on DVoc rates revealed significant main effects of phase, $F(29, 986) = 7.011$, $p < 0.001$, stress condition, $F(1, 34) = 12.874$, $p < 0.001$, and a phase by stress condition interaction $F(29, 986) = 24.92$, $p < 0.001$. Simple effects analysis revealed a significant effect of phase in the Isolated ($p < 0.001$) but not in the Social group (p = n.s.). This pattern of DVocs in the Isolated group illustrates the two phases of the model.

Change scores for social reinstatement behavior in the straight-alley maze, as measured by start/goal latency at test (i.e., following stress manipulation) – start/goal latency at baseline (prior to stress manipulation), is presented in Figures 3 A and B, respectively. In general, both start and goal latencies were delayed in the Social and Isolated groups compared to the No Test group. A 1-way ANOVA of change in start latency yielded a group effect that approached significance, $F(2, 46) = 2.544$, $p = 0.088$. Planned comparisons demonstrated that start latency was longer in the Isolated group compared to the No Test group ($p = 0.029$). A 1-way ANOVA on goal latency failed to detect a significant group effect, $F(2, 46) = 0.909$, $p = 0.41$. No further analyses were conducted on these data.

Experiment 2

Baseline (i.e., prior to the stress manipulation) sucrose preference behavior in the home cage, as measured by change in weight of the container divided by the change in weight for both containers, is presented in Table 2. In general, sucrose preference appears higher in the No Test and Isolated groups compared to the Social group. A 1-way ANOVA of sucrose preference revealed a significant group effect, $F(2, 51) = 53.08$, $p < 0.001$. Planned comparisons demonstrated lower sucrose consumption for the Social group compared to the Isolated and No Test groups ($p < 0.009$).

Baseline (i.e., prior to the stress manipulation) water and sucrose preference behavior in the home cage, as measured by mean number of drinks, is presented in Table 3. In general the baseline number of water drinking events appears higher in the Social group compared to the No Test and Isolated groups. Additionally, the baseline number of sucrose drinking events appears lower in the Social group compared to the No Test and Isolated groups. A 1-way ANOVA of baseline number of water drinks illustrated a significant group effect, $F(2,39) = 3.932$, $p = 0.028$.

Planned comparisons showed the Social group to engage in more water drinking events than both the No Test ($p = 0.019$) and Isolated ($p = 0.002$) groups. A 1-way ANOVA of baseline number of sucrose drinks also revealed a significant effect of group, $F(2, 39) = 18.63$, $p < 0.001$. Planned comparisons showed significantly less sucrose drinking events in the Social ($p < 0.001$) and Isolated ($p = 0.037$) groups compared to the No Test group. Additionally, less sucrose drinking events were displayed by the Social compared to the Isolated group ($p = 0.003$).

The effects of isolation stress, as measured by DVocs, over the 90 min test session are presented in Figure 3. In general, Social birds display a low level of DVocs throughout the test period while Isolated birds initially display a high level of DVocs which by 30 min declines by about 45% and remains relatively stable thereafter. A 2-way ANOVA on DVoc rates revealed significant main effects of phase, $F(29, 986) = 11.252$, $p < 0.001$, stress condition, $F(1, 34) = 7.204$, $p = 0.011$, and a phase by stress condition interaction, $F(29, 986) = 6.72$, $p = 0.002$. Simple effect analyses revealed a significant effect of phase in the Isolated group ($p < 0.001$) and in the Social group ($p = 0.016$). This pattern of DVocs in the Isolated group reveals the two phases of the model.

Sucrose preference change scores, measured by preference at test (i.e., following stress manipulation) - preference at baseline (prior to stress manipulation), are presented in Figure 4. In general, sucrose preference increases at test for all groups. A 1-way ANOVA of change in sucrose preference revealed a significant treatment effect, $F(2, 45) = 3.45$, $p = 0.04$. Planned comparisons demonstrate significantly less sucrose preference in the Isolated group compared to the No Test group ($p = 0.017$) and approaches a significant decrease compared to the Social group ($p = 0.065$).

Number of water and sucrose drinking events change scores, as measured by test number of drinking events – baseline number of drinking events, for water and sucrose are presented in Figure 5. In general, the total number of water drinking events between baseline and test appeared to decrease in the Social and increase in the Isolated group compared to the No Test group. Additionally, the total number of sucrose drinking events between baseline and test appeared to increase in both the Social and Isolated groups compared to the No Test group. A 1-way ANOVA of change in number of water drinking events revealed a significant effect of group, $F(2, 45) = 15.604$, $p < 0.000$. Planned comparisons demonstrated a significant decrease in the Social group and a significant increase in the Isolated group in the number of water drinking events compared to the No Test group ($p = 0.004$). A 1-way ANOVA of change in number of sucrose drinking events also revealed a significant effect of group, $F(2, 42)= 4.992$, $p= 0.012$. Planned comparisons demonstrated an increased number of sucrose drinking events in the Social group ($p=0.003$) and approached a significant increase in the Isolated group ($p= 0.087$), compared to the No test group.

Experiment 3

Baseline (prior to stress manipulation) behavioral measures in the dust bath apparatus, as measured by time of first event, time spent preening, number of foraging events and number of dust bath events, are presented in Table 4. In general, time of first event appears delayed in the Isolated and Social groups compared to the No Test group. A 1-way ANOVA on these data shows a statistically significant difference between the groups, $F(2, 93) = 5.853$, $p = 0.004$. Planned comparisons showed a significantly longer latency in the Isolated group compared to the Social (0.009) and No Test groups ($p = 0.002$). In general, time spent preening appears longer in the Social and shorter in the Isolated group compared to the No Test group. A 1-way ANOVA

shows a significant difference between the groups, $F(2, 93) = 10.409$, $p < 0.0001$. Planned comparisons showed a marginally significant increase in time spent preening by the Social group ($p = 0.05$) and a significant decrease by the Isolated group ($p = 0.002$) compared to the No Test group. Planned comparisons also discovered significantly more preening by the Social group compared to the Isolated group ($p < 0.001$). In general, the number of foraging events appears lower in both the Social and Isolated groups compared to the No Test group. A 1-way ANOVA of the number of foraging events yielded a group effect which approached significance, $F(2, 93) = 2.668$, $p = 0.075$. Planned comparisons showed significantly less foraging in the Isolated group compared to the No Test group ($p = 0.023$). Planned comparisons failed to detect significant differences between the Social and No Test group. In general, the number of dust baths appears similar between the groups. A 1-way ANOVA on number of dust bath events failed to detect significant group differences, $F(2, 93) = 0.689$, $p = 0.505$. No further analyses were conducted on these data.

The effects of isolation stress, as measured by DVocs, over the 90 min test session are presented in Figure 6. In general, Social birds display a lower level of DVocs compared to Isolated birds throughout the test period while Isolated birds initially display a high level of DVocs which by 30 min declines by about 50% and remains relatively stable thereafter. Consistent with these observations, a 2-way ANOVA on DVoc rates revealed significant main effects of phase, $F(29, 1682) = 13.709$, $p < 0.001$, stress condition, $F(1, 58) = 274.97$, $p < 0.001$, and a phase by stress condition interaction, $F(29, 1682) = 4.95$, $p < 0.001$. Simple effects analysis revealed a significant effect of phase in the Isolated group ($p < 0.001$) and in the Social group ($p < 0.001$). This pattern of DVocs in the Isolated group reveals the two phases of the model.

Change scores for time of first event, time spent preening, number of foraging events and number of dust bath events, as measured by the test measure – baseline measure, are presented in Figures 7 A, B, C and D, respectively. In general, a longer latency for first event was displayed by chicks in the Social group compared to the Isolated and No Test groups. A 1-way ANOVA of change in time of first event revealed a significant treatment effect, $F(2, 93) = 11.458$, $p < 0.001$. Planned comparisons demonstrated an increased latency for the Social group compared to both the Isolated and No Test groups ($p < 0.001$). In general, time spent preening appears decreased for the Social group compared to the No Test group. A 1-way ANOVA of change in time spent preening revealed significant treatment effects, $F(2, 93) = 16.571$, $p < 0.0001$. Planned comparisons show a significant decrease in time spent preening for the Social group compared to both the Isolated and No Test groups ($p < 0.001$). In general, no difference in the number of foraging events was observed between the treatment conditions. A 1-wayANOVA of change in foraging events failed to reveal significant treatment effects, $F(2, 93) = 0.187$, $p = 0.83$. No further analyses were conducted on these data. In general, the number of dust bathing events appears decreased in the Social group compared to the No Test group. A 1-way ANOVA of change in number of dust bath events failed to reveal significant treatment differences, $F(2, 93) = 1.624$, $p = 0.2$. Planned comparisons approached a significant decrease in the number of dust bath events for the Social group compared to the No Test group ($p = 0.088$). No further analyses were conducted on these data.

Part 2

Experiment 4

Baseline social reinstatement (i.e., prior to stress manipulation), as measured by start and goal latency, is presented in Table 5. Distance traveled was not included herein as most chicks

completed the maze within the 300 sec criterion. In general, baseline start and goal latencies appear longer in the Social and 15 mg/kg Imipramine groups compared to the No Test group. A 1-way ANOVA did not reveal significant group differences, $F(4, 85) = 1.525$, $p = 0.202$. Planned comparisons approached significance for delayed start latency of Social chicks compared to the No Test group ($p = 0.077$) and the Vehicle group ($p = 0.095$). A 1-way ANOVA did not reveal significant group differences in goal latency, $F(4, 85) = 1.5$, $p = 0.209$. Planned comparisons detected a significantly longer goal latency for the Social group ($p = 0.037$) compared to the No Test group and approached significance compared to the Vehicle group ($p = 0.065$).

The effects of Imipramine on separation induced DVocs over the 90 min test session are presented in Figure 8 A. In general, Social chicks display a lower level of DVocs compared to Vehicle chicks throughout the test period while Vehicle chicks initially display a high level of DVocs which by 30 min declines by about 50% and remains relatively stable thereafter, indicative of a depression-like state. Both Imipramine doses displayed DVoc rates that were attenuated compared to the Vehicle group. Consistent with these observations, a 2-way ANOVA on DVoc rates revealed significant main effects of phase, $F(29, 1972) = 22.77$, $p < 0.001$, drug treatment condition, $F(3, 68) = 29.0$, $p < 0.001$, and a phase by drug treatment condition interaction, $F(29, 1972) = 1.57$, $p = 0.001$. Simple effects analyses revealed a significant effect of phase in the Isolated group ($p < 0.001$) and in the Social group ($p = 0.033$). This pattern of DVocs in the Isolated group reveals the two phases of the model.

Drug treatment effects on the display of behavioral despair, as measured by DVoc rates for min 30-90, are presented in Figure 8 B. In general, DVocs are higher for both Imipramine doses compared to the Vehicle treatment. A 1-way ANOVA of DVocs, during the depression-like phase, revealed significant drug treatment effects, $F(2, 51) = 12.65$, $p < 0.001$. Planned

comparisons detected significantly higher DVoc levels in the 10 and 15 mg/kg Imipramine treatments compared to the Vehicle treatment (ps < 0.001). This suggests that both doses of Imipramine were able to attenuate the display of behavioral despair.

Change scores for social reinstatement behavior in the straight-alley maze, as measured by start and goal latency is presented in Figures 9 A and B, respectively. In general, start and goal latencies were delayed following stress manipulation compared to the No Test group. A 1-way ANOVA for change in start latency detected a significant treatment effect, $F(4,85) = 3.788$, $p= 0.007$. Planned comparisons demonstrated a significant increase in the Vehicle group ($p < 0.001$) and a marginally significant increase in the 10 mg/kg Imipramine group ($p = 0.049$) compared to the No Test group. Additionally, planned comparisons showed a significant decrease in start latency in the 15 ($p = 0.014$) and approached significance with the 10 ($p = 0.077$) mg/kg Imipramine groups compared to the Vehicle group. A 1-way ANOVA for change in goal latency detected a significant treatment effect, $F(4,85) = 2.72$, $p = 0.035$. Planned comparisons showed a significant increase for the Vehicle group compared to the No Test group ($p = 0.004$). Additionally, goal latency is decreased in the 15 mg/kg Imipramine group compared to the Vehicle group ($p = 0.032$).

Experiment 5

Baseline social reinstatement (i.e., prior to stress manipulation), as measured by start and goal latency, is presented in Table 6. Distance traveled was not included herein as most chicks completed the maze within 300 sec. In general, baseline start and goal latencies appear similar between the groups of chicks. A 1-way ANOVA of baseline start latency failed to reveal a significant group effect, $F(4, 85) =0.744$, $p=0.565$. A 1-way ANOVA of baseline goal latency also failed to reveal a significant group effect, $F(4, 85) = 0.546$, $p=0.703$.

The effects of Ketamine on separation induced DVocs over the 90 min test session are presented in Figure 10 A. In general, Social chicks display a lower level of DVocs compared to Isolated chicks throughout the test period while Isolated chicks initially display a high level of DVocs which by 30 min declines by about 50% and remains relatively stable thereafter, indicative of a depression-like state. Both Ketamine groups initially display a high level of DVocs which by 30 min declines by about 40% and remains relatively stable thereafter. Consistent with these observations, a 2-way ANOVA on DVoc rates revealed significant main effects of phase, $F(29, 1972) = 24.19$, $p < 0.001$, drug treatment condition, $F(3, 68) = 31.05$, $p < 0.001$, and a phase by drug treatment condition interaction, $F(29, 1972) = 2.093$, $p < 0.001$. Simple effects analyses revealed a significant effect of phase in the Isolated group ($p < 0.001$) and in the Social group ($p = 0.033$). This pattern of DVocs in the Isolated group reveals the two phases of the model.

Drug treatment effects on the display of behavioral despair, as measured by DVoc rates for min 30-90, are presented in Figure 10 B. In general, similar DVoc rates are displayed by Vehicle and the two Ketamine treatment groups. A 1-way ANOVA failed to reveal a significant treatment effect, $F(2, 51) = 0.164$, $p = 0.849$. No further analyses were conducted on these data.

Test social reinstatement (i.e., following stress manipulation), as measured by start and goal latency, is presented in Figures 11 A and B, respectively. In general, both start and goal latency appears increased in all treatment conditions compared to the No Test condition. A 1-way ANOVA for start latency detected a significant treatment effect, $F(4, 85) = 3.095$, $p = 0.02$. Planned comparisons demonstrated a significant delay in start latency for the Social ($p < 0.001$), Vehicle ($p = 0.038$), 5 ($p = 0.04$) and 10 mg/kg Ketamine conditions ($p = 0.044$) compared to the No Test condition. A 1-wayANOVA for goal latency detected a significant treatment effect,

$F(4,85) = 3.091$, $p = 0.02$. Planned comparisons demonstrated a significant delay in goal latency in the Social ($p = 0.002$), Vehicle ($p = 0.038$), 5 ($p = 0.006$) and 10 mg/kg Ketamine groups ($p = 0.016$) compared to the No Test group.

DISCUSSION

Depression is characterized by a general feeling of sadness, hopelessness, loss of motivation, loss of pleasure in previously pleasurable activities, and problems concerned with sleep, attention, and eating habits (Diagnostic and Statistics Manual-IV-TR) and is the most common mental disorder in the U.S. (NIMH, 2014). Current treatment strategies include behavioral therapy (Wuthrich & Rapee, 2013) and pharmaceutical treatment (Dombrovskiet al., 2007; Quitkin et al. 2002). However, 28-55% of patients do not respond or experience delayed effects and/or residual symptoms from pharmaceutical treatment (Nutt et al., 2007). As such, researchers continue to explore the etiology, biology and symptomology of depression as well as assessments of new treatment strategies.

Investigations of depression often employ animal model simulations. Ideally these are isomorphs of the human clinical syndrome, where both show similar but species typical responses in behavioral, physiological, biochemical, endocrinological and/or neuroanatomical characteristics of the disease state (van der Staay, 2006). The chick anxiety-depression simulation has illustrated many of the characteristic endophenotypes of anxiety and depression (Sufka et al. 2006; Feltenstein & Sufka, 2005; Sufka et al. 2009; Warnick et al. 2009; Salmeto et al., 2011; Kim & Sufka, 2011). However, it has not demonstrated the expression of anhedonia, one of the cardinal features of depression (Diagnostic and Statistics Manual-IV-TR). As such, the current series of experiments were aimed at quantifying anhedonia by utilizing social reinstatement latency, which has previously been used in chicks (Salmeto et al. 2009, Hymel

Sufka, 2011), sucrose preference, which has been used as a measure of anhedonia in rodents (Hayase, 2011; Paul et al., 2000), and the novel measure of behavior in a dust bath apparatus.

Part 1

The first goal of Experiment 1 was the induction of behavioral despair using the chick anxiety-depression model simulation. Results suggest chicks show a similar pattern in DVocs as that seen in previous studies (Suka et al., 2006; Salmeto et al., 2009; Hymel & Sufka, 2011; Loria et al., 2013). Specifically, Social chicks display relatively low DVoc levels throughout the test session whereas Isolated chicks initially display high levels of DVocs which by 30 min declines by about 50% and remains relatively stable thereafter. This pattern of DVocs in the Isolated group represents behavioral despair (Feltenstein & Sufka, 2005; Sufka et al., 2006; Sufka et al., 2009; Salmeto et al., 2011; Kim & Sufka, 2011; Hymel & Sufka 2012; Sufka & White, 2013).

The second goal of Experiment 1 was to quantify anhedonia in the straight alley maze apparatus after the induction of behavioral despair. Since baseline measures in the straight alley maze (i.e., prior to stress manipulation), revealed group differences in start and goal latency, change scores were computed to assess the impact of isolation stress on behavior in the straight alley maze. Change scores suggest that stress exposure leads to increased start latency, as shown by the Isolated group compared to the No Test group. Previous research has also demonstrated delays in start latency, toward a mirror stimulus cue, following exposure to an isolation stressor (Salmeto et al., 2011; Hymel & Sufka, 2012). The delayed start latencies of the Isolated group are considered indicative of anhedonia-like behavior since decreased motivation to pursue pleasurable activities is a defining feature of anhedonia (Der-Avakian & Markou, 2012). This interpretation is consistent with previous research demonstrating runway tests as accepted

methods for measurements of motivation to be near conspecifics (Mills et al., 1995; Clarke & Jones, 2001). For example, Clarke and Jones (2001) demonstrated that chicks are attracted to video images of feeding chicks in a goal box, as shown by quicker approach responses compared to an image of a food dish.

Change scores for goal latency failed to reveal group differences. This is proposed to be related to the lack of variability in goal latency, which is similar to previous studies using the straight alley maze (Salmeto et al., 2011; Hymel & Sufka, 2012). For example, Salmeto et al. (2011) was unable to show significant differences in goal latency for a mirror stimulus cue in non-isolated chicks. These equivocal effects suggest that similar to the measure of distance traveled, goal latency is not a sensitive measure for the quantification of anhedonia-like behavior.

The issues and limitations of Experiment 1 included a computer malfunction and a lack of homogeneity of variance in start latency. A computer malfunction during min 15 and 18 for an Isolated group resulted in abnormally low DVocs. This artificially impacted the ability to detect the effects of isolation, so these data points were removed from analyses. Once these were removed, the typical pattern of behavioral despair became apparent. Another issue was the lack of homogeneity of variance in start latency for No Test birds, likely resulting from social reinstatement being a powerful motivator. Change scores were calculated to help address this limitation. These allowed for the accurate quantification of the impact of stressor exposure on runway performance as well as showing the predicted pattern of runway results following isolation exposure, where Isolated chicks showed increased start latency relative to the No Test chicks. .

Experiment 2 also aimed to induce behavioral despair in the chick anxiety-depression model and measure anhedonia through sucrose preference. Results suggest chicks show a similar

pattern of DVocs as that demonstrated in previous studies (Sufka et al., 2006; Salmeto et al., 2009; Hymel & Sufka, 2011; Loria et al., 2013). As in Experiment 1, DVocs were low in Social chicks whereas the high DVocs which decline by 50 % in Isolated birds show the induction of behavioral despair indicative of a depression-like phase (Feltenstein & Sufka, 2005; Sufka et al., 2006; Sufka et al., 2009).

Measurement of anhedonia-like behavior used an adapted version of the rodent sucrose preference task using the dependent measures of preference and number of drinking events for water and sucrose. Since preference scores at baseline (i.e., prior to stress manipulation) were found to differ between the groups change scores were computed. Change scores suggest that though sucrose preference generally increases in all groups, it is not equivalent across groups. Specifically, sucrose preference in the Isolated group approached being significantly lower than the Social group and was significantly lower than the No Test group. The lower sucrose preference following stress exposure is interpreted as anhedonia-like behavior. This interpretation is consistent with previous research that suggests decreased consumption of a sucrose solution to be indicative of an anhedonia-like state (Delgado y Palacios et al., 2011; Hayase, 2011; Enkel et al., 2010).

The mean number of water drinks at test remained stable in the No Test group, decreased in the Social group and increased in the Isolation group. It is proposed that Isolated birds satiated their need for liquid but did not put forth the effort to have access to the sucrose solution. This is similar to the results of Nowend et al. (2001). Their study showed that interference with the dopamine (DA) system led to decreased lever pressing for a preferred food reward but did not alter concurrently available chow consumption. They suggest rats were not altered in their food motivation but in their ability to overcome restraints to obtain food.

Explanations for the change in the number of sucrose drinks at test was not as clear as it was shown to decrease in the No Test group, increase in the Social group and somewhat increase in the Isolated group. These behavioral patterns may result from No Test birds being better able to recall the unforeseen negative gastrointestinal impact (i.e. vomiting, limp posture) of excessive liquid consumption during the baseline test. This explanation is consistent with research by Abidin et al. (2004) showing chronic restraint stress to negatively impact memory. Another factor that may have influenced the obtained results is the lack of specificity in the measure of number of sucrose drinks. Though specific behaviors were required for quantification as a drinking event, the amount of liquid in a particular drink could not be measured. As such, the accuracy of the drinking events measure does not provide a quantifiable way to assess anhedonia-like behavior.

Limiting factors of Experiment 2 included a power outage during testing, camera picture quality, liquid deprivation effects and gastrointestinal difficulties. A power outage during testing resulted in the loss of behavioral data which led to a lower number of subjects. Specifically, the Social group became n = 12 (2 cages) whereas the No Test and Isolated groups were n = 18 (3 cages). Additionally, the camera picture quality only allowed for the assessment of a cage as a whole, preventing the intended measure of individual chick consumption scores. Moreover, the liquid deprivation led to chicks getting into both the water and sugar containers thus altering volume but not due to consumption. Further, as previously mentioned excessive liquid consumption was observed to lead to gastrointestinal difficulties which may have impacted results.

Another factor that may have influenced the current results is shown in the work of Barbato et al. (1982) who demonstrated the existence of heritable components involved in the

gustation preferences of fowl. Specifically, preference for quinine sulfate and dextrose was assessed in two different lines of chicken, one expressing low and the other high juvenile body weight. Results showed lower hedonic thresholds in low juvenile body weight chickens compared to the high juvenile body weight chickens, suggesting a heritable component in taste preference. This may help explain the ambiguity of the current results as the strain may not have a natural preference for the concentration of sucrose used since it was chosen based on research with a different chick strain.

The first goal of Experiment 3 was to induce behavioral despair with the chick anxiety-depression model. Chicks demonstrated a similar pattern of DVocs to that shown in previous studies (Suka et al., 2006; Salmeto et al., 2009; Hymel & Sufka, 2011; Loria et al., 2013). As in Experiments 1 & 2, DVocs were low in Social chicks whereas the high DVocs which declined by 50 % in Isolated birds show the induction of behavioral despair indicative of a depression-like phase (Feltenstein & Sufka, 2005; Sufka et al., 2006; Sufka et al., 2009).

Anhedonia was quantified by assessing behaviors in a dust bath apparatus that have been shown to indicate relaxation and pleasure (Delius et al., 1988, Widowski and Duncan, 2000). In general, a low number of foraging and dust bath events were observed. As such, behavioral data could not be meaningfully tested statistically for group differences. Since baseline measures (i.e., prior to stress manipulation) of time of first event and time spent preening were found to differ between the groups of chicks, change scores were computed.

Change scores for the time of first event and time spent preening do show significant group differences, but the outcome was in contrast to predictions. It was predicted that Isolated chicks would display a longer latency for time of first event and less time preening, reflecting anhedonia. However, Social chicks displayed a longer time of first event and less preening

relative to both the Isolated and No Test chicks. One potential explanation is that during isolation testing, Isolated chicks were attempting to reinstate social contact which prevented engaging in grooming behavior. In contrast, Social birds were able to engage in grooming/preening behavior, such that upon return to their home cage they engaged in less because they were not engaged in a competing behavior during test. However, this explanation is an unlikely given that the No Test chicks did not show the same decrease in preening behavior as the Social chicks. No existing literature could be found to account for this unusual difference in behavior. The notion that preening and dust bathing behaviors are social comfort behaviors may be true as related to general animal welfare. However, they are not measures sensitive to an isolation stressor that is intended to produce a state of behavioral despair and induce anhedonia. It is recommended that dust bath apparatus exposure not be considered as a measure of anhedonia.

Part 1 results revealed significant group differences in both the straight alley maze and the sucrose measure. However, accuracy concerns of the sucrose measure decrease the validity of it in quantifying anhedonia-like behavior. Therefore, the straight alley maze was chosen to measure the potential of pharmaceuticals to reverse the display of behavioral despair and anhedonia-like behavior. Specifically, the tricyclic Imipramine or the novel antidepressant Ketamine was administered prior to exposure to the isolation apparatus.

Part 2

Experiment 4 assessed the ability of Imipramine, a gold standard tricyclic antidepressant (Sufka et al., 2006), to alleviate behavioral despair in the chick anxiety-depression model and the display of anhedonia-like behavior in the straight-alley maze. The ability of a pharmaceutical to alleviate the display of anhedonia-like behaviors in the straight alley maze would further its validation as a measure of anhedonia. Chicks were injected with vehicle, 10, or 15 mg/kg

Imipramine prior to exposure to the isolation apparatus or for No Test chicks, return to the home cage. Distress vocalizations served as the dependent measure in the chick anxiety-depression model. As in Experiments 1, 2, & 3 DVocs were low in Social chicks, whereas the high DVocs which decline by 50 % in Isolated chicks show the induction of behavioral despair indicative of a depression-like phase (Feltenstein & Sufka, 2005; Sufka et al., 2006; Sufka et al., 2009). However, inconsistent with previous research in the Black Australorp strain, where Imipramine did not alleviate behavioral despair (Sufka & White, 2013), current results show both doses of Imipramine to alleviate behavioral despair.

The differing results of the present study are proposed to be related to the different experimental procedures used in each study. Specifically, the present study involved behavioral testing prior to the isolation stressor, whereas Sufka & White (2013) isolated chicks without any previous behavioral testing. This difference may be important as exposure to a previous test procedure has been shown to alter drug responsivity (File et al., 1990; File et al., 1992; Hymel, 2013). For example, File et al. (1990) showed that a single prior exposure to a plus maze eliminates the anxiolytic effects of chlordiazepoxide upon a second exposure to the plus maze. Further, prior exposure to the plus maze impacts the effects of chlordiazepoxide on neurochemical measures. Specifically, basal release of 5-HT from both the cortex and hippocampus were decreased in rats with prior exposure to the plus maze compared to rats that did not have previous plus maze exposure. These studies support the idea that prior testing can influence behavioral reactions to pharmaceuticals.

Ninety minutes after injection, chicks were tested in the straight alley maze apparatus, where the dependent measures of start and goal latency were assessed. An increase in mean start latency was observed in all groups relative to the No Test group. The increased latency for

Vehicle chicks indicates a stress effect from exposure to the isolation apparatus and replicates the findings from Experiment 1 as well as previous research (Salmeto et al., Hymel & Sufka, 2012). Additionally, results show decreased start latency in the 15 mg/kg Imipramine group compared to the Vehicle group, indicative of an alleviation of anhedonia-like behavior. This is consistent with the work of Hayase (2011) which showed drugs that inhibit the reuptake of monoamines administered prior to immobilization stress, reverse the display of anhedonia as measured by the sucrose preference test.

Surprisingly, all groups displayed increased goal latency relative to the No Test group, showing a stress effect from isolation apparatus test procedures on the display of anhedonia-like behaviors. Though no effect of goal latency was demonstrated in Experiment 1, current results are consistent with the results of Salmeto et al. (2009) that showed goal latency to increase in White Leghorn chicks following exposure to the isolation apparatus. Additionally, a significant increase in goal latency was shown by the Vehicle group compared to the 10 and 15 mg/kg Imipramine groups. This suggests the ability of 10 and 15 mg/kg Imipramine to alleviate the display of anhedonia-like behavior, and is also consistent with the work of Hayase (2011). Combined DVoc and straight-alley maze results suggest that 15 mg/kg Imipramine administered prior to stress exposure can prevent behavioral despair and the display of anhedonia-like behavior. These results strengthen the validity of the straight alley maze in assessments of anhedonia.

Experiment 5 involved the administration of vehicle, 5 or 10 mg/kg Ketamine prior to isolation apparatus exposure. Ketamine is a novel antidepressant that has previously been shown to be effective in alleviating behavioral despair in Black Australorps tested in the chick anxiety-depression model (Sufka & White, 2013). It was predicted that Ketamine would alleviate

behavioral despair in the isolation apparatus and reverse the display of anhedonia-like behavior as assessed in the straight alley maze. Distress vocalizations served as the dependent measure in the chick anxiety-depression model. As in Experiments 1, 2, 3 & 4 DVocs were low in Social chicks, whereas the high DVocs which decline by 50 % in Isolated chicks show the induction of behavioral despair, indicative of a depression-like phase (Feltenstein & Sufka, 2005; Sufka et al., 2006; Sufka et al., 2009). Contrary to the results of Sufka and White (2013) which showed the alleviation of behavioral despair with 10 mg/kg Ketamine, neither dose of Ketamine prevented the onset of behavioral despair in the current study. As previously discussed, exposure to a previous test procedure has been shown to alter drug responsivity (File et al., 1990; File et al., 1992; Hymel, 2013) and may explain the present results. Additionally, the inability of 10 mg/kg Ketamine to reverse behavioral despair is consistent with Hymel (2013) that similarly involved testing procedures which occurred prior to isolation apparatus exposure.

Ninety minutes after injection, chicks were tested in the straight alley maze apparatus. Results revealed increased start latency for all groups compared to the No Test group. The increased start latency for Vehicle chicks indicates a stress effect from exposure to the isolation apparatus and replicates the findings from Experiments 1 and 4 as well as previous research (Salmeto et al., Hymel & Sufka, 2012). Surprisingly, goal latency was increased in all groups relative to the No Test group. This pattern of behavior in Vehicle chicks is consistent with Experiment 4 as well as previous research (Salmeto et al., 2009). These results suggest the administration of Ketamine prior to isolation apparatus exposure did not alleviate behavioral despair or anhedonia-like behaviors, as assessed in the straight alley maze. Therefore, it is suggested that Ketamine may not be effective in the alleviation of behavioral despair and anhedonia, as assessed in the straight alley maze.

General Conclusions

One unexpected finding was the simple effects analyses in Experiments 2, 4 and 5 showing a decline in DVocs over time in the Social group. However, this is not the pattern that typifies the two phases of the chick anxiety-depression model. Isolated chicks do show a pattern indicative of behavioral despair, which is characterized by initially high DVoc levels which declines by about 50% after 30 min and remains stable thereafter. One reason for the increased DVocs of Social chicks may be testing in behavioral measures prior to exposure to the isolation apparatus. Similar DVoc patterns in Social chicks have been shown in studies which involved exposure to behavioral tests prior to isolation apparatus exposure (Hymel, 2011).

Another issue encountered in all of the studies was large error for behavioral data. It was assumed all chicks would show near identical behavioral response because of identical genetics and rearing environments. However, this may have been an oversight as studies assessing personality in non-human animals illustrate that similar to humans, animals show a range of expression in a given personality trait. Further, research has identified natural personality differences in a wide range of animals. For example, extraversion and neuroticism have been identified in species ranging from chimpanzees to guppies (Gosling & John, 1999). Additionally, the work of Cusseen & Mench (2014) explored the relationship between personality differences and cognition in parrots. Results demonstrate that different baseline levels of neuroticism impact performance in a cognitive task. Specifically, higher levels of neuroticism led to poorer performance when an additional observer was present during testing. This study is similar to studies in humans which have shown a correlation between higher levels of anxiety and altered cognitive processing. Therefore, it is suggested that the baseline level of neuroticism for

individual chicks could be a confounding factor in their responses to testing procedures leading to the large observed error.

Surprisingly, the results obtained in Part 2 stand in stark contrast to those of Sufka and White (2013). Specifically, Sufka and White (2013) demonstrated that Imipramine failed to alleviate behavioral despair while Ketamine did, within the Black Australorp strain. In contrast, present results in the Black Australorp strain, showed Imipramine was able to alleviate behavioral despair while Ketamine was not. As such, it will be necessary that future studies be conducted to elucidate the drug responsiveness of the Black Australorp strain.

Since females are more likely to suffer from depression (NIMH, 2014), another area of exploration that should be assessed involves testing females in the chick anxiety-depression and straight alley maze paradigms. An example of research that has shown sex differences is the work of Vallortigara et al. (1990) which showed different patterns of social reinstatement response between male and female chicks towards social reinforcement (cage mates) and non-social reinforcement (food). It would be interesting to see what the impact of the chick anxiety-depression model would be on social reinstatement behavior in female chicks.

Another area that should be explored is analysis of the characteristics of chick vocalizations during the isolation and straight alley maze tests. Specifically, assessment of the acoustic frequency (in Hz) signals within a distress call in the isolation apparatus and vocalization in the maze could be measured. Recording the vocalizations of chicks during the straight-alley maze task may help to better quantify behavior. Similar measurements were conducted by Mateus-Pinheiro et al. (2014) who showed different levels of pleasurable ultrasonic vocalizations (USVs) in rats that were positively correlated with sugar pellet preference.

Future studies could also explore physiological measures suggested to be related to anhedonia, such as the catecholamines (Nutt et al., 2007). Further, Paul et al. (2005) suggests the importance of assessing emotional processes through the use of cognitive measures. For example, the work of Henningsen et al. (2009) explored the relationship between cognitive deficits and anhedonic-like responses, by assessing sucrose preference and performance in the spontaneous alternation test.

The current research demonstrates the utility of the straight alley maze as a potential tool for the assessment of anhedonia in chicks. Additionally, 15 mg/kg Imipramine was shown to alleviate behavioral despair and the display of anhedonia. This result shows that anhedonia-like behavior as assessed in the straight-alley maze can be reversed with pharmaceutical manipulations, which strengthen its validity as a measure of anhedonia-like behavior. Further, the current series of studies advance the chick anxiety-depression model by demonstrating another homology between clinical populations and the model.

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LIST OF APPENDICES

APPENDIX A: FIGURE CAPTIONS

Figure 1. The effects of social separation stress across a 90 min isolation test session. Values represent mean DVoc rates (± SEM) in 3 min blocks. The square dots represent social chicks tested in the presence of two social companions and mirrors. The round dot represents chicks tested in isolation. Sample sizes were n = 18.

Figure 2 A. Change scores for start latency in sec across stress conditions. Values represent mean ± SEM. * indicates significantly longer start latency compared to No Test chicks. Sample sizes were n = 16-17.

Figure 2 B. Change scores for goal latency in sec across stress conditions. Values represent mean ± SEM. Sample seizes were n = 16-17.

Figure 3. The effects of social separation stress across a 90 min isolation test session. Values represent mean DVoc rates (± SEM) in 3 min blocks. The square dots represent Social chicks tested in the presence of two social companions and mirrors. The round dot represents Isolated chicks. Sample sizes were n = 12-18.

Figure 4. Preference change scores in % across stress conditions. Values represent mean ± SEM. Bars represent sucrose preference change scores. * indicates significant difference compared to No Test. + indicates approached significance compared to the Social group. Sample sizes were n = 12-18.

Figure 5. The mean change in the number of drinking events across stress conditions for water and sucrose. Values represent mean ± SEM. Blue bars represent the change scores for the number of water drinking events. Red bars represent the change scores for the number of sucrose drinking events. * indicates significant difference compared to No Test chicks. + indicates results approach significance compared to No Test chicks. Sample sizes were n = 12-18.

Figure 6. The effects of social separation stress across a 90 min isolation test session. Values represent mean DVoc rates (± SEM) in 3 min blocks. The dashed line represents Social chicks tested in the presence of two social companions and mirrors. The round dot represents Isolated chicks. Sample sizes were n = 30.

Figure 7 A. Mean change scores across stress conditions for time of first event. Values represent mean ± SEM. * indicates significant difference compared to No Test and Isolated chicks. Sample sizes were n = 30-36.

Figure 7 B. Mean change score across stress conditions for time spent preening. Values represent mean ± SEM. * indicates significant difference compared to No Test and Isolated chicks. Sample sizes were n = 30-36.

Figure 7 C. Mean change scores across stress conditions for number of foraging events. Values represent mean ± SEM. Sample sizes were n = 30-36.

Figure 7 D. Mean change scores across stress conditions for number of dust bath events. + indicates results approach significance compared to the No Test chicks. Values represent mean ± SEM. Sample sizes were n = 30-36.

Figure 8 A. The effects of social separation stress across a 90 min isolation test session. Values represent mean DVoc rates (± SEM) in 3 min blocks. The square dot represents Social chicks tested in the presence of two social companions and mirrors. The round dot represents Vehicle chicks. The dot and dash line represents 10 mg/kg Imipramine chicks. The solid line represents 15 mg/kg Imipramine chicks. Sample sizes were n = 18.

Figure 8 B. The effects of Imipramine dose on DVocs during the depression-like phase (30-90 min). * indicates significant differences from Vehicle. Sample sizes were n = 18.

Figure 9 A. Change scores for start latency in sec across treatment conditions. Values represent mean ± SEM. * indicates significantly longer start latency compared to Vehicle chicks. + indicates approaches significant increase compared to No Test chicks. Sample seizes were n = 18.

Figure 9 B. Change scores for goal latency in sec across treatment conditions. Values represent mean ± SEM. * indicates significantly shorter goal latency compared to the Vehicle chicks. Sample seizes were n = 18.

Figure 10 A The effects of social separation stress across a 90 min isolation test session. Values represent mean DVoc rates (± SEM) in 3 min blocks. The dashed line represents Social chicks tested in the presence of two social companions and mirrors. The round dot represents Isolated chicks. The dot and dash line represents 5 mg/kg Ketamine chicks. The solid line represents 10 mg/kg Ketamine chicks. Sample sizes were n = 18.

Figure 10 B. The effects of Ketamine dose on DVocs during the depression-like phase (30-90 min). Sample sizes were n = 18.

Figure 11 A. Start latency in sec across treatment conditions. Values represent mean ± SEM. * indicates significantly increased start latency compared to the No test chicks. + indicates results approach significance compared to the No Test group. Sample seizes were n = 18.

Figure 11 B. Goal latency in sec across treatment conditions. Values represent mean ± SEM. * indicates significant increase in goal latency compared to No Test chicks. Sample seizes were n = 18.

APPENDIX B: TABLES

Table 1

Means and standard deviations for start and goal latency at baseline across the different groups

Baseline Measure	No Test	Social	Isolated
Mean Start Latency (SD)	1.875 (1.09)	16.13 (33.5)	21.82 (41.91)*
Mean goal Latency (SD)	7.44 (9.32)	30.13 (73.09)	44.29 (82.68)

Note. * indicates significant difference

Table 2

Means and standard deviations for sucrose preference at baseline across the different groups

Baseline Measure	No Test	Social	Isolated
Sucrose Preference % (SD)	37.99 (4.83)	29.45(5.86)*	49.18(2.88)

Note. * indicates significance difference from No Test and Isolated

Table 3

Means and standard deviations for number of water and sucrose drinks at baseline across the different groups

Baseline Measure	No Test	Social	Isolated
Mean # of Water Drinks (SD)	189.33 (29.51)	211.50 (32.90)*	183.5(1.57)
Mean # of Sucrose Drinks (SD)	161.0 (28.71)	92.0 (42.82)*	112.0 (22.98)*

Note. * indicates significant difference with the No Test group

Table 4

Means and standard deviations for baseline measures in the dust bath apparatus

Baseline Measure	No Test	Social	Isolated
Mean Time (s) First Event (SD)	282.69 (209.57)	313.07 (219.09)	498.03 (364.15)*
Mean Time (s) Preening (SD)	47.89 (36.76)	64.8 (44.2)	24.43 (14.76)
Mean # Foraging Events (SD)	2.92 (6.51)	1.67 (3.02)	0.4 (1.48) *
Mean # Dust Bath Events (SD)	1.67 (2.33)	2.37 (3.32)	1.57 (3.07)

Note. * indicates significance difference from No Test

Table 5

Means and standard deviations for start and goal latency at baseline across treatment groups

Baseline Measure	No Test	Social	Vehicle	10 mg Imip.	15 mg Imip
Mean Start Latency (SD)	2.44 (2.15)	15.44 (30.73)+	3.17(3.31)	3.72 (3.83)	13.78 (37.4)
Mean goal Latency (SD)	5.33 (4.59)	48.67(93.95)*	10.39(15.35)+	24.39(58.32)	34.72(79.61)

Note. * indicates significant difference to No Test. + indicates approaches significance compared to No Test

Table 6

Means and standard deviations for start and goal latency at baseline across treatment groups

Baseline Measure	No Test	Social	Vehicle	5 mg Ket.	10 mg Ket.
Mean Start Latency (SD)	3.28(3.88)	3.22 (3.52)	13.61(48.2)	7.17 (16.07)	2.44(3.38)
Mean goal Latency (SD)	19.72(51.62)	10.5(20.15)	22.94(69.52)	14.22(23.02)	5.0(4.09)

APPENDIX C: FIGURES

Figure 1

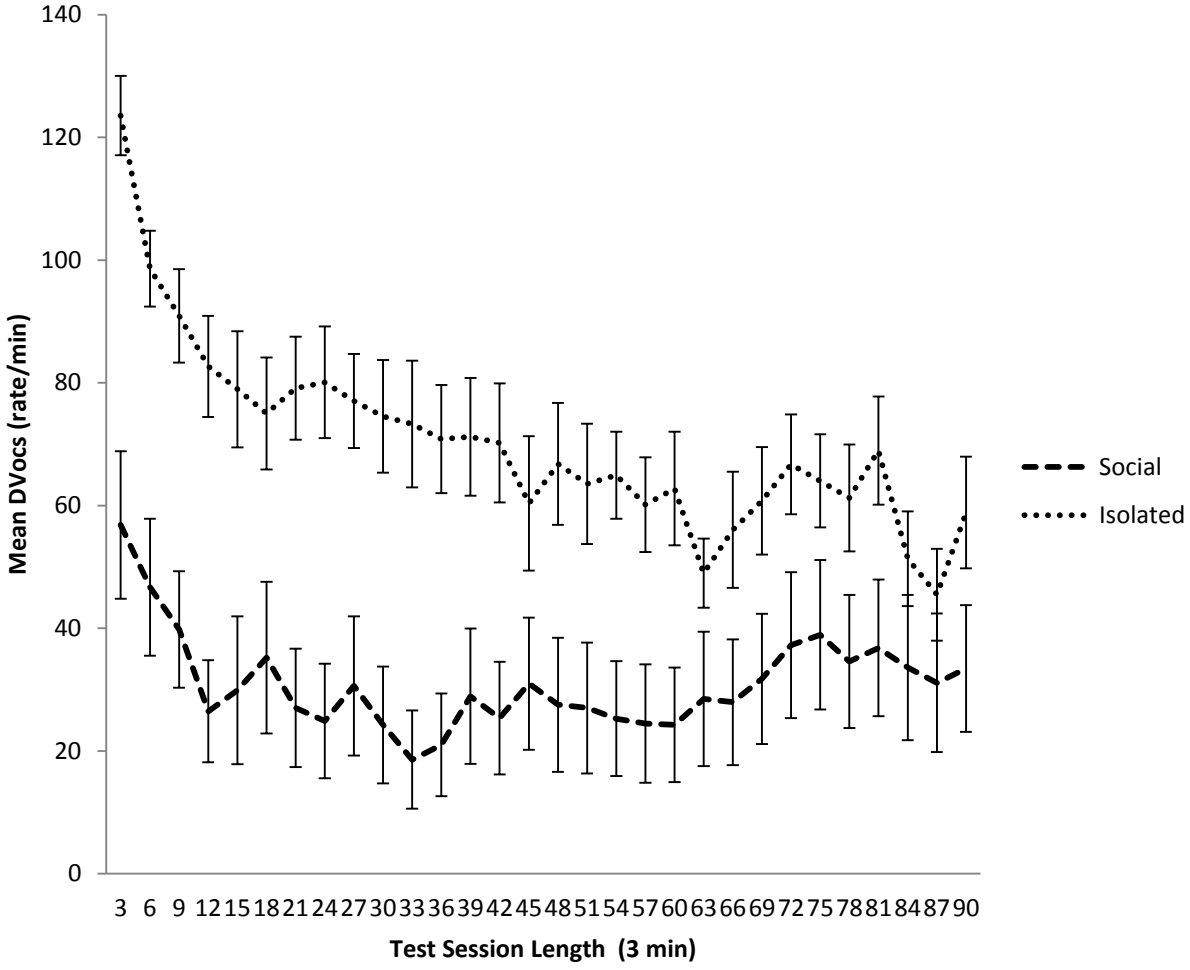

140
120
100
80
60
40
20
0
Mean DVocs (rate/min)
3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90
Test Session Length (3 min)
Social
Isolated

Figure 2A

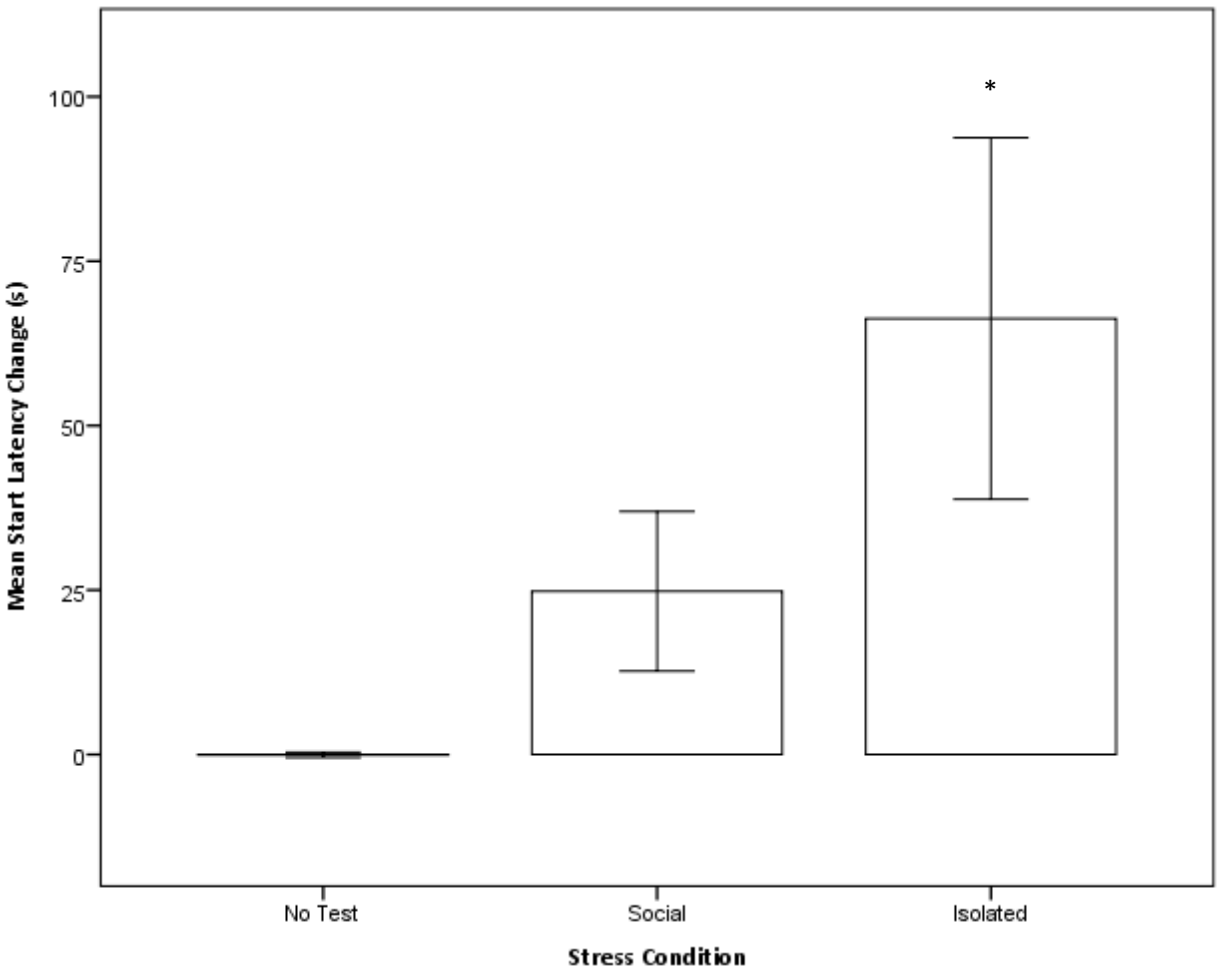

100
75
50
25
0
*
Mean Start Latency Change (s)
No Test
Social
Isolated
Stress Condition

Figure 2 B

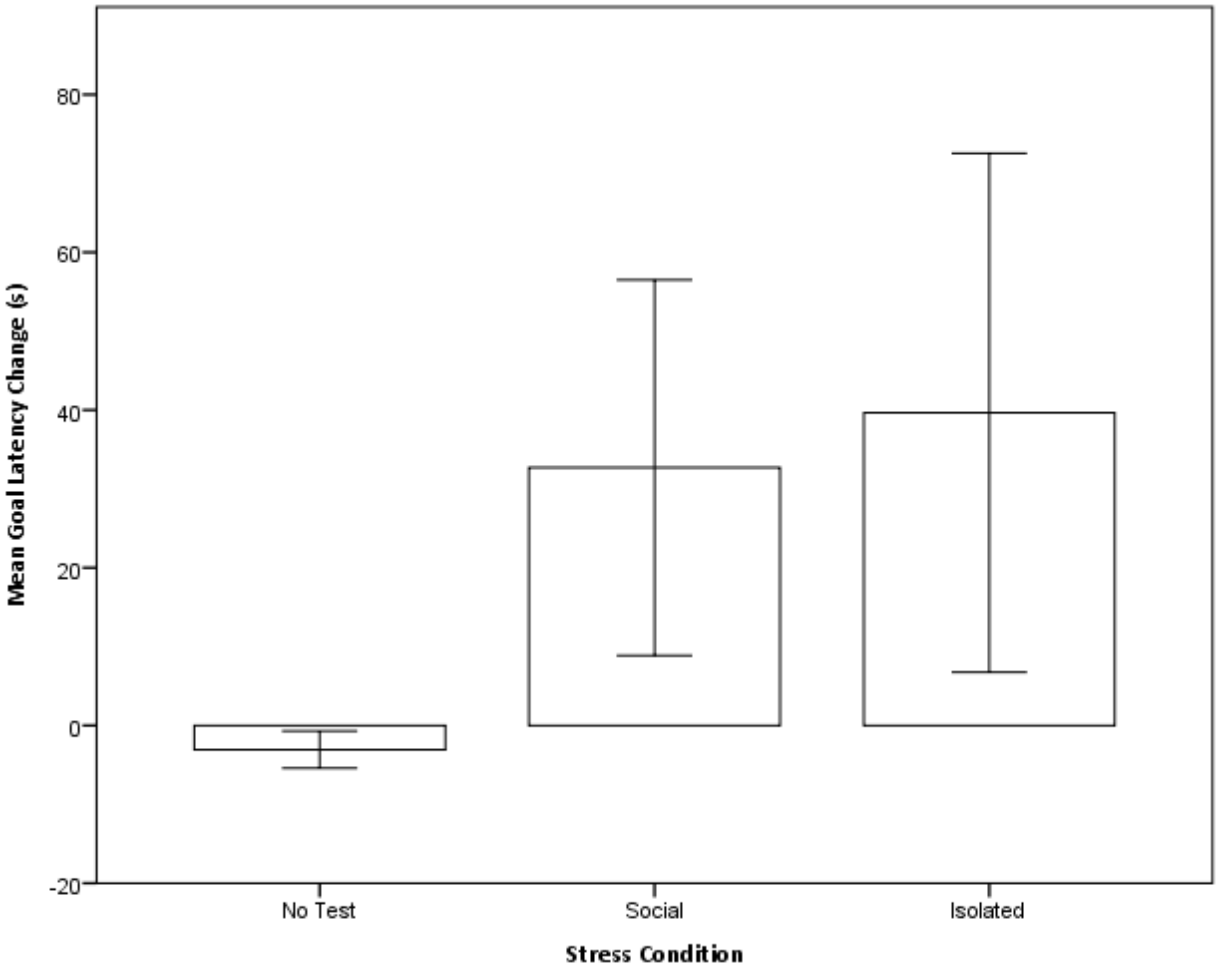
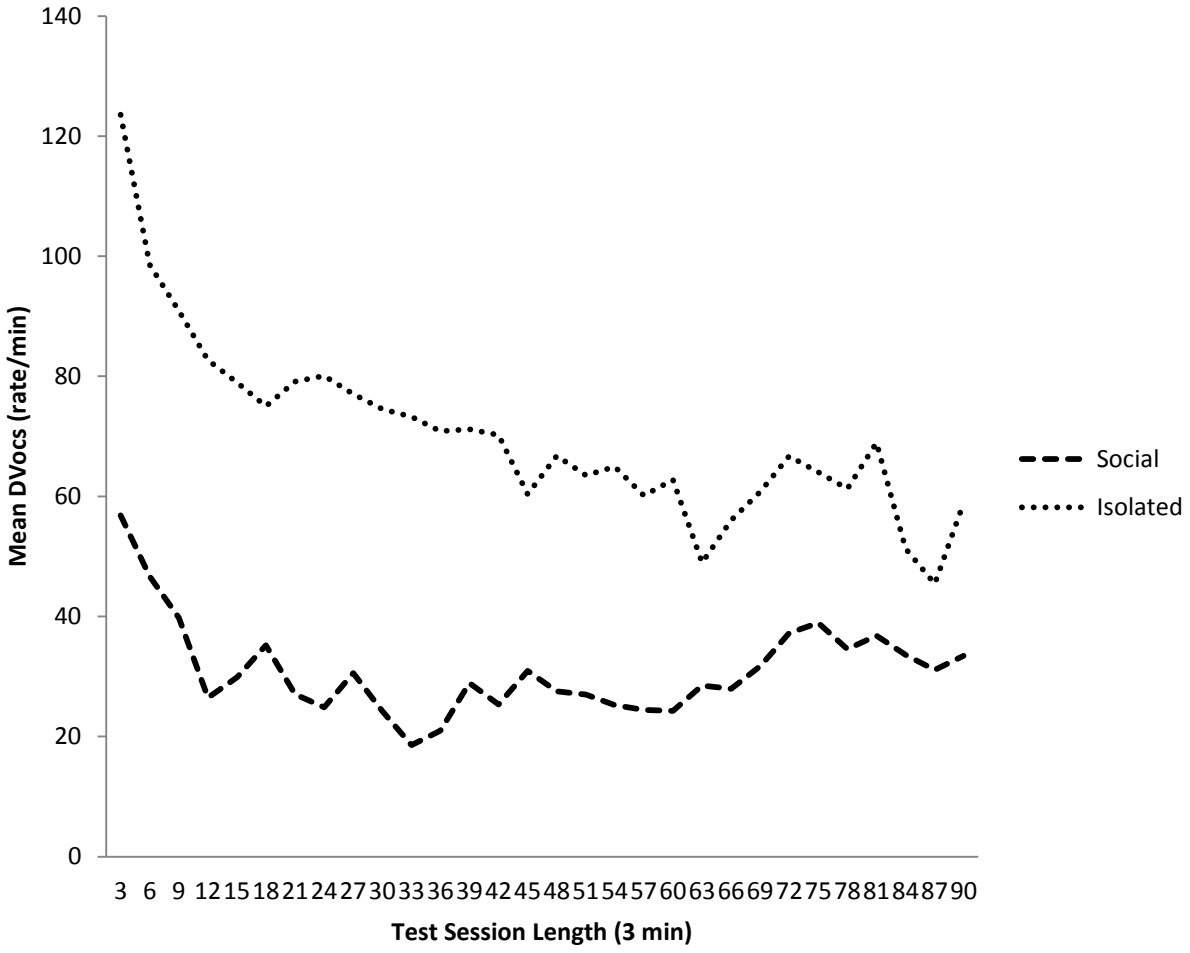

80
60
40
20
0
-20
Mean Goal Latency Change (s)
No Test
Social
Isolated
Stress Condition

Figure 3


140
120
100
80
60
40
20
0
Mean DVocs (rate/min)
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Test Session Length (3 min)
Social
Isolated

Figure 4

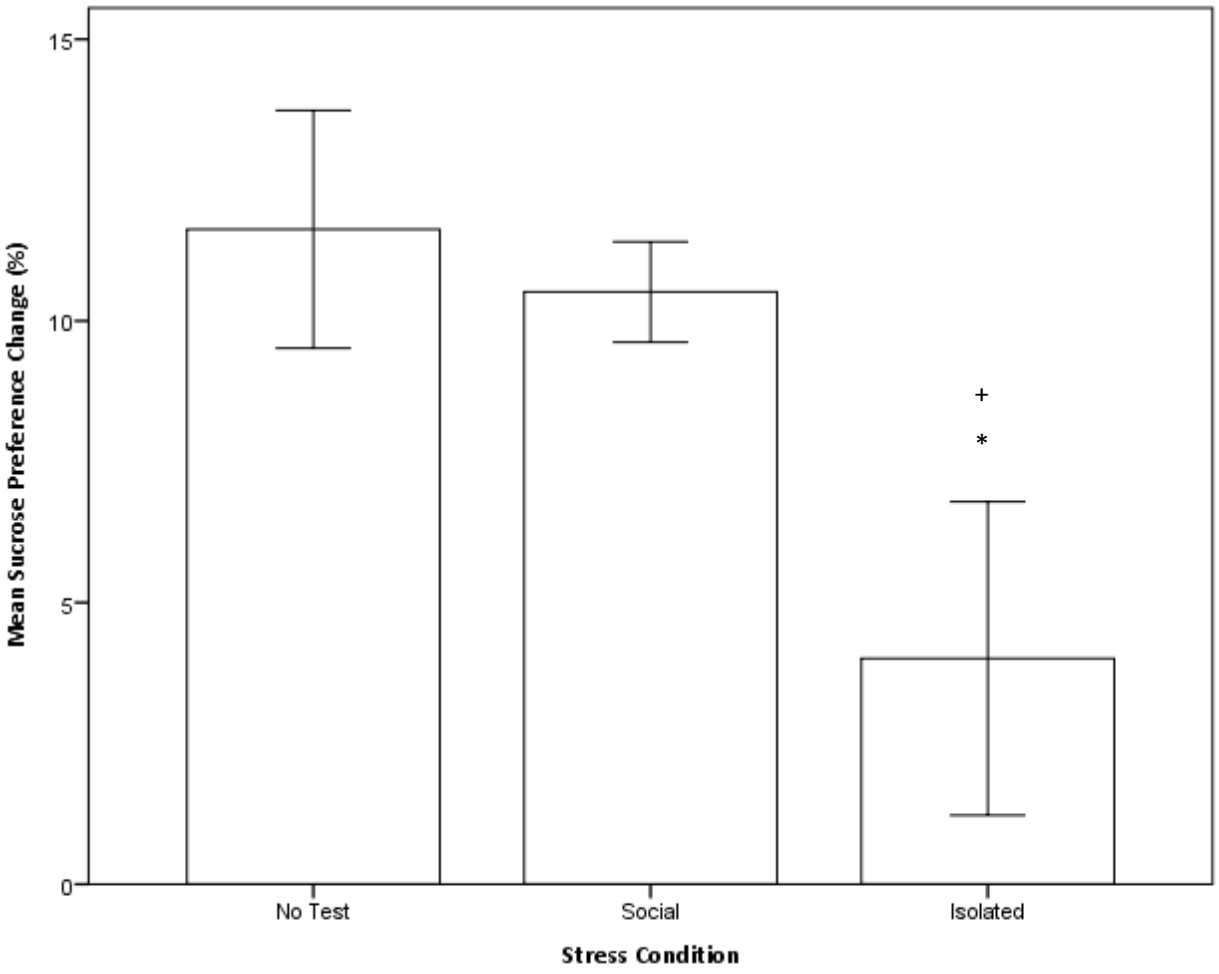

15
10
5
0
Mean Sucrose Preference Change (%)
No Test
Social
Isolated
Stress Condition

Figure 5

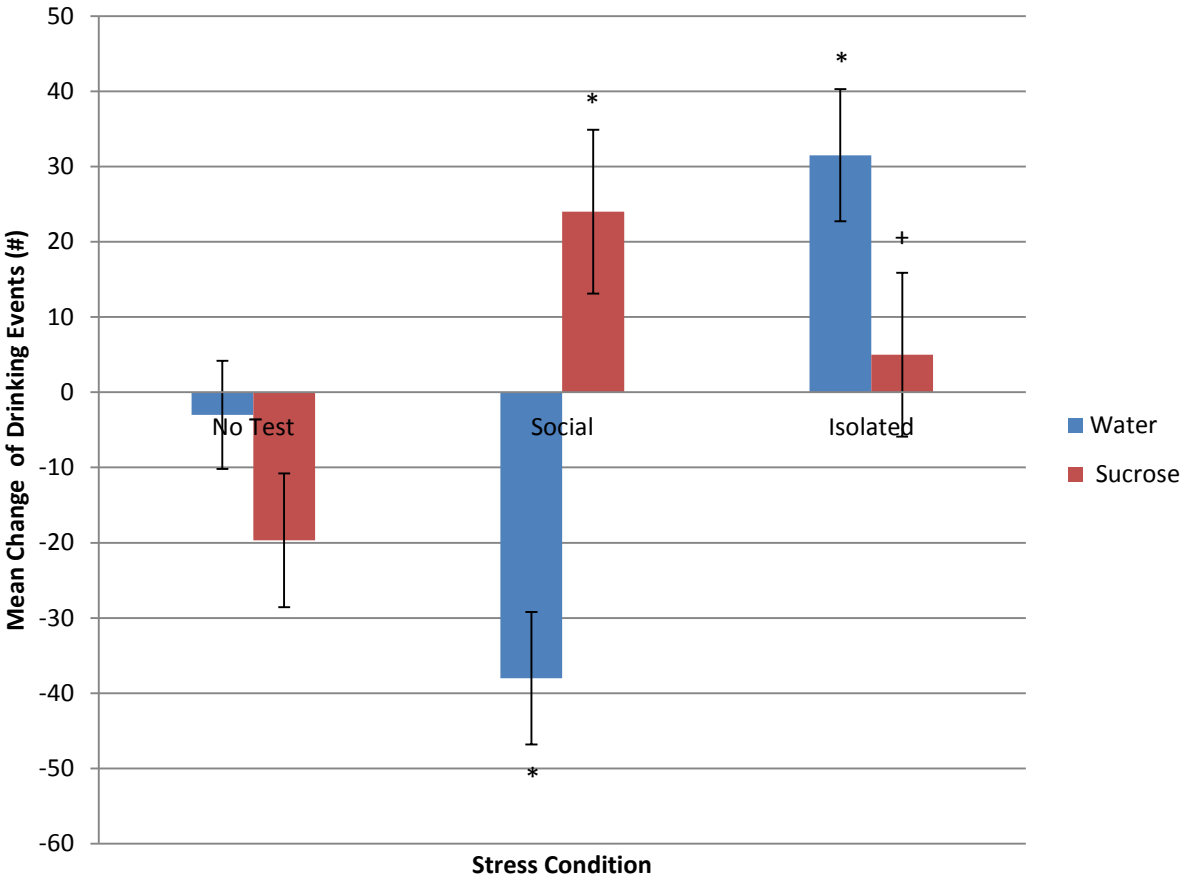
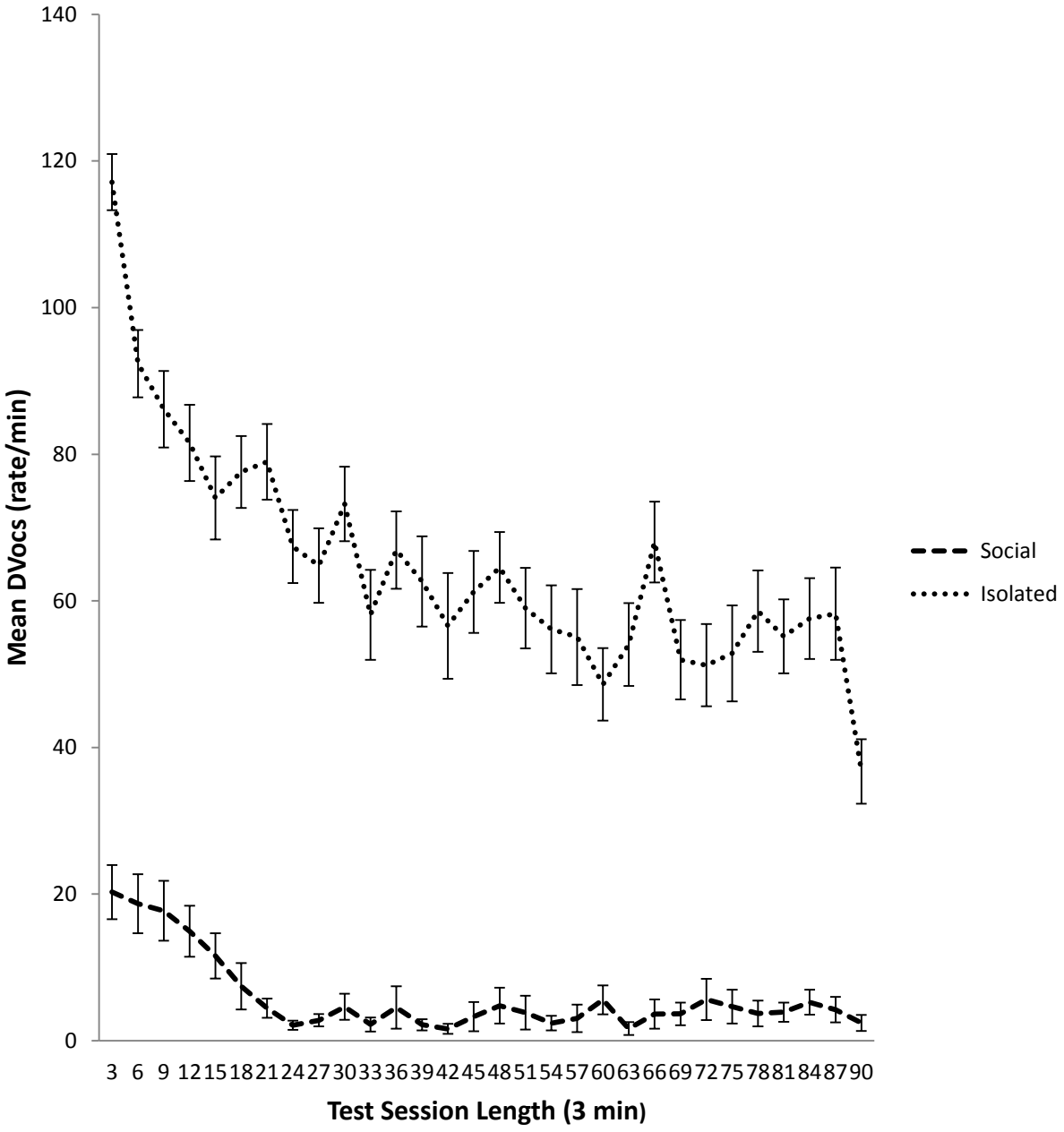

50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
Mean Change of Drinking Events (#)
No Test
Social
Isolated
Stress Condition
Water
Sucrose
*
*
*
+

Figure 6


140
120
100
80
60
40
20
0
Mean DVocs (rate/min)
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Test Session Length (3 min)
Social
Isolated

Figure 7 A

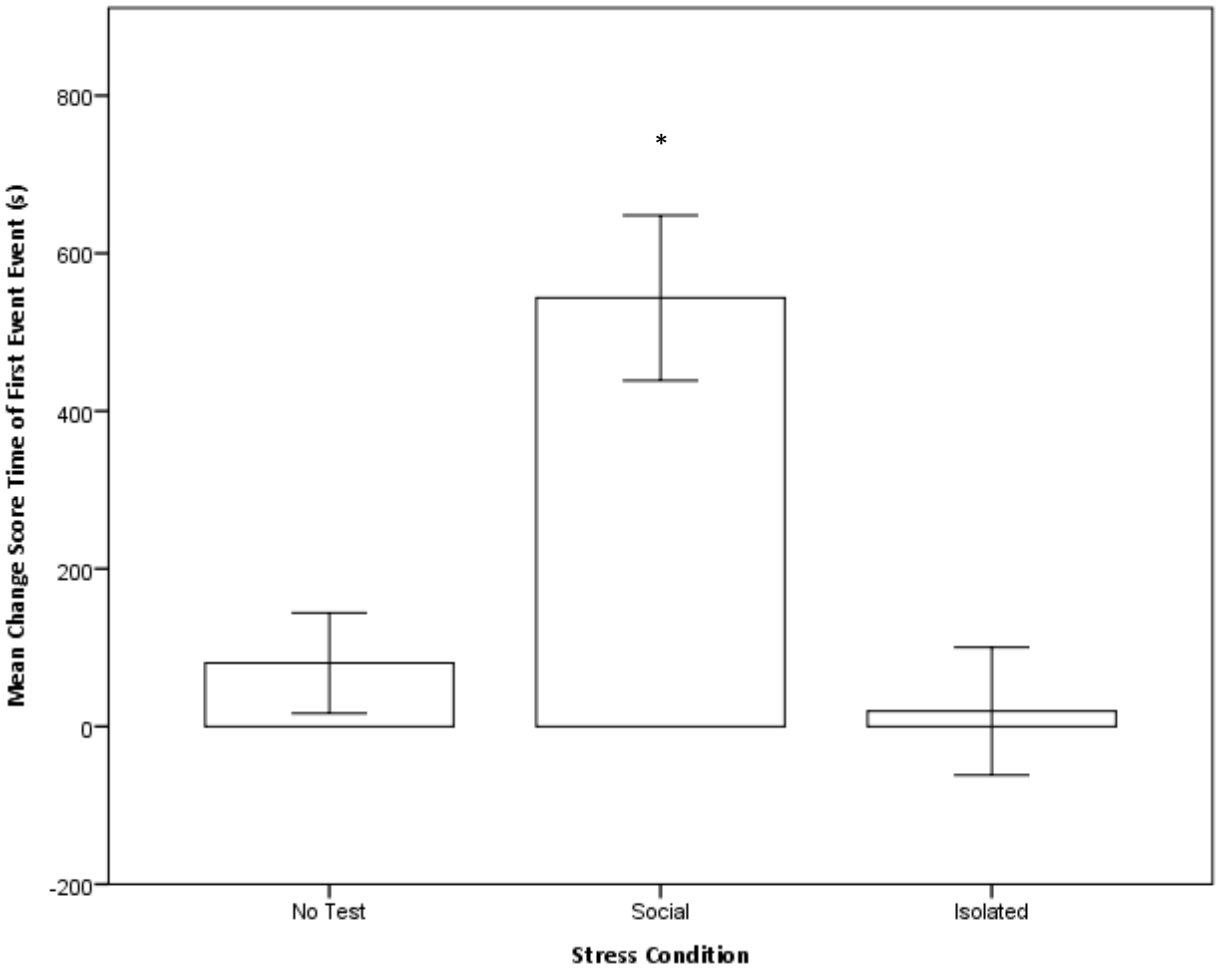

800
600
400
200
0
-200
*
Mean Change Score Time of First Event Event (s)
No Test
Social
Isolated
Stress Condition

Figure 7 B

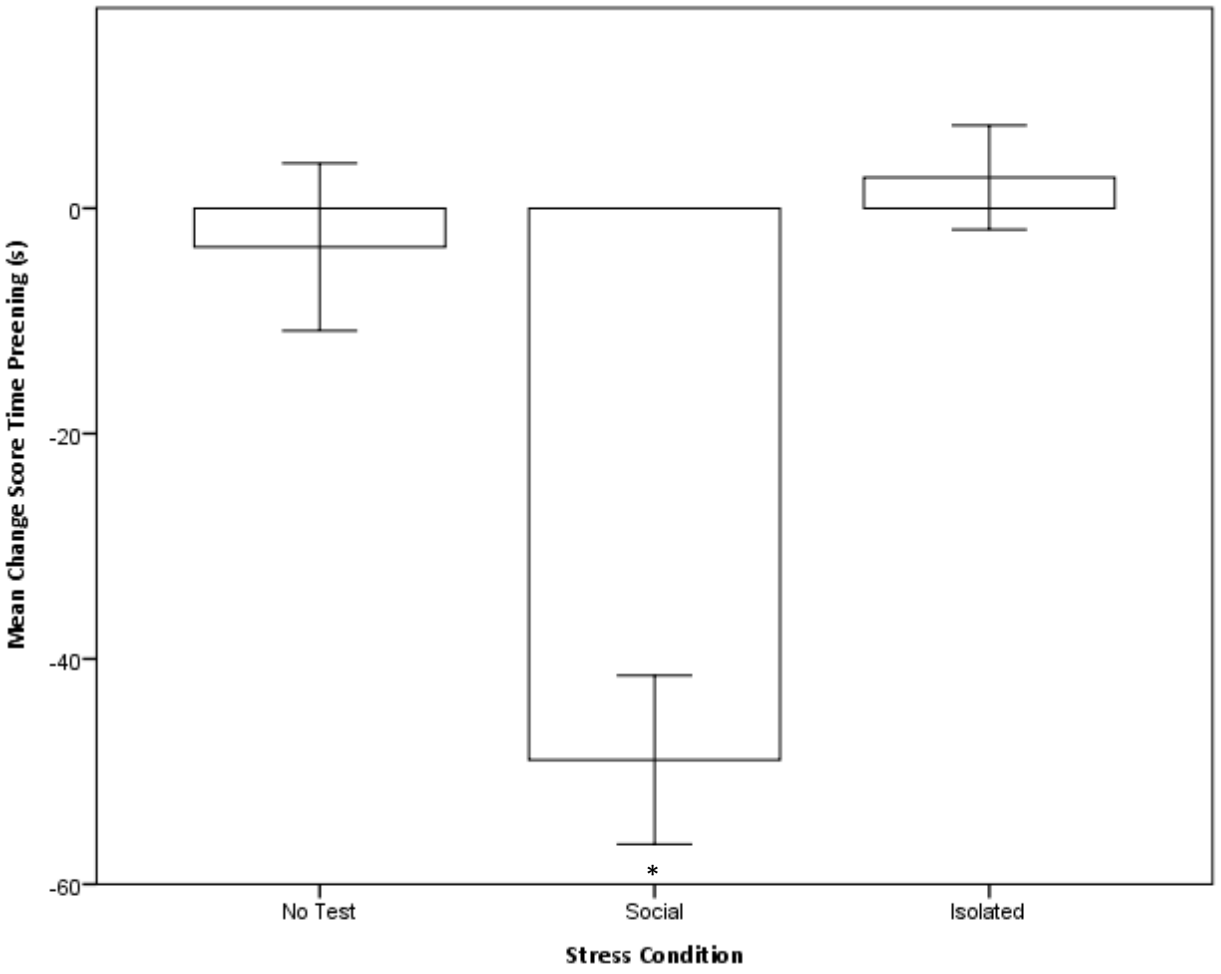

Mean Change Score Time Preening (s)
0
-20
-40
-60
*
No Test
Social
Isolated
Stress Condition

Figure 7 C

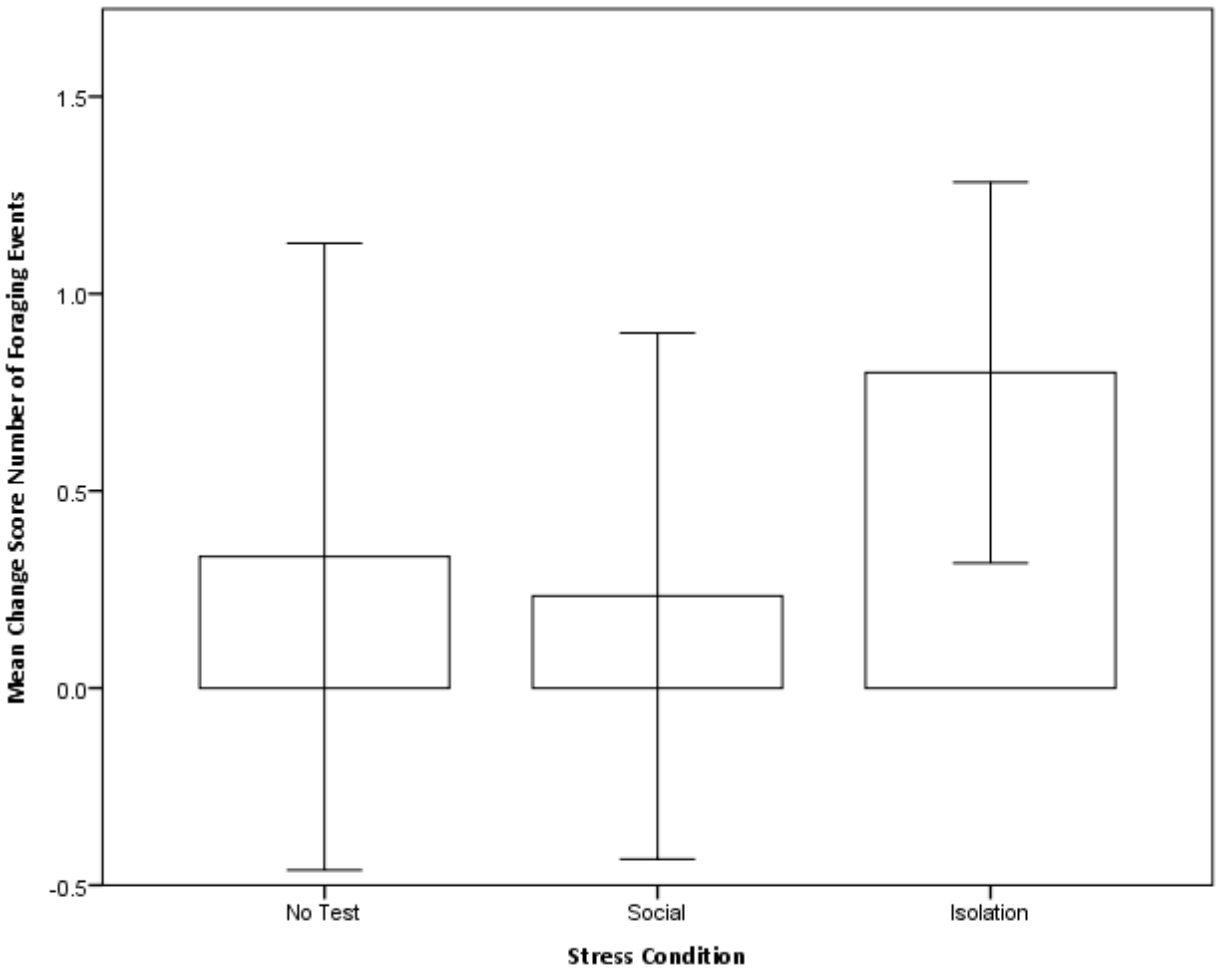

Mean Change Score Number of Foraging Events
1.5
1.0
0.5
0.0
-0.5
No Test
Social
Isolation
Stress Condition

Figure 7 D

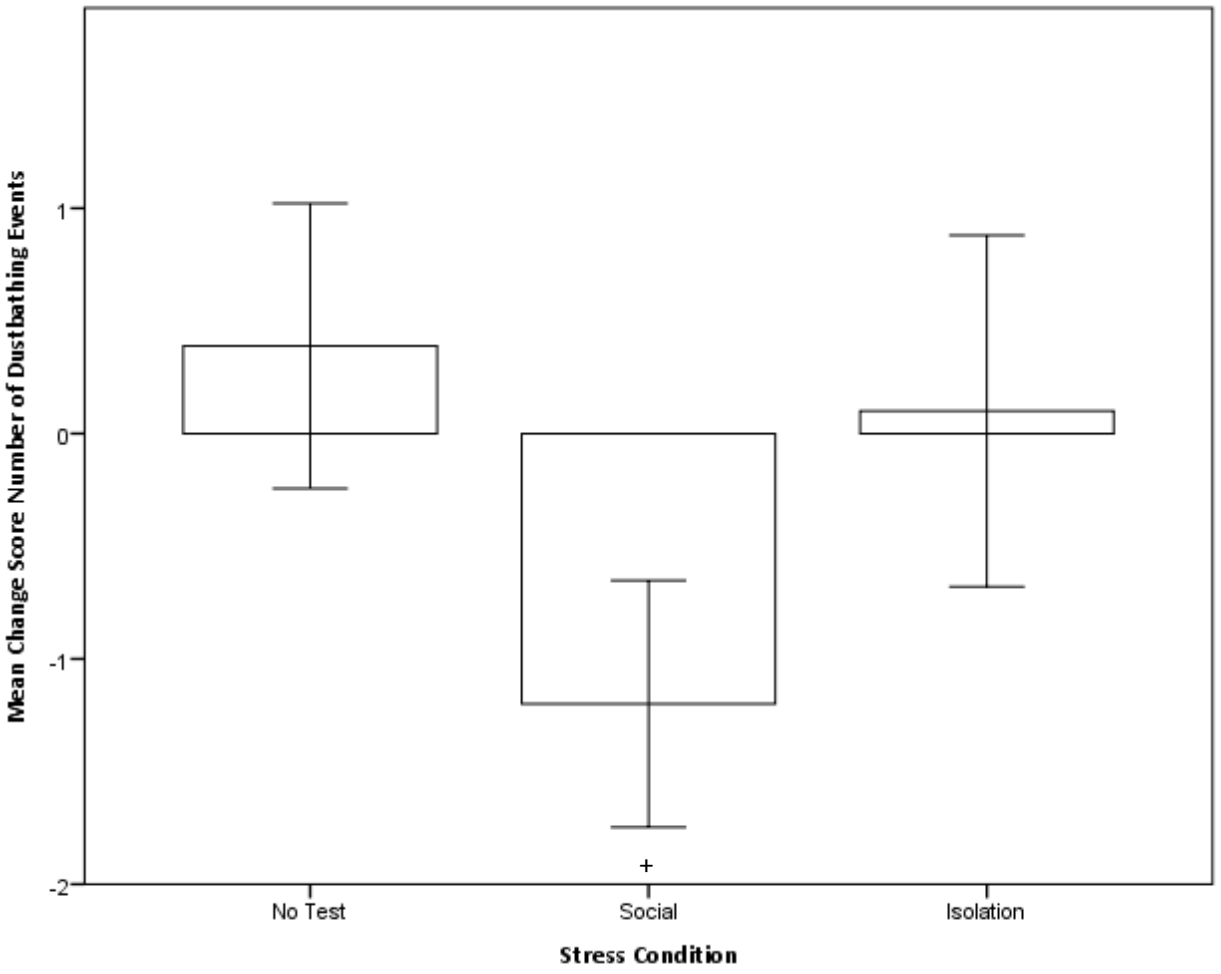

Mean Change Score Number of Dustbathing Events
1
0
-1
-2
No Test
Social
Isolation
Stress Condition

Figure 8 A

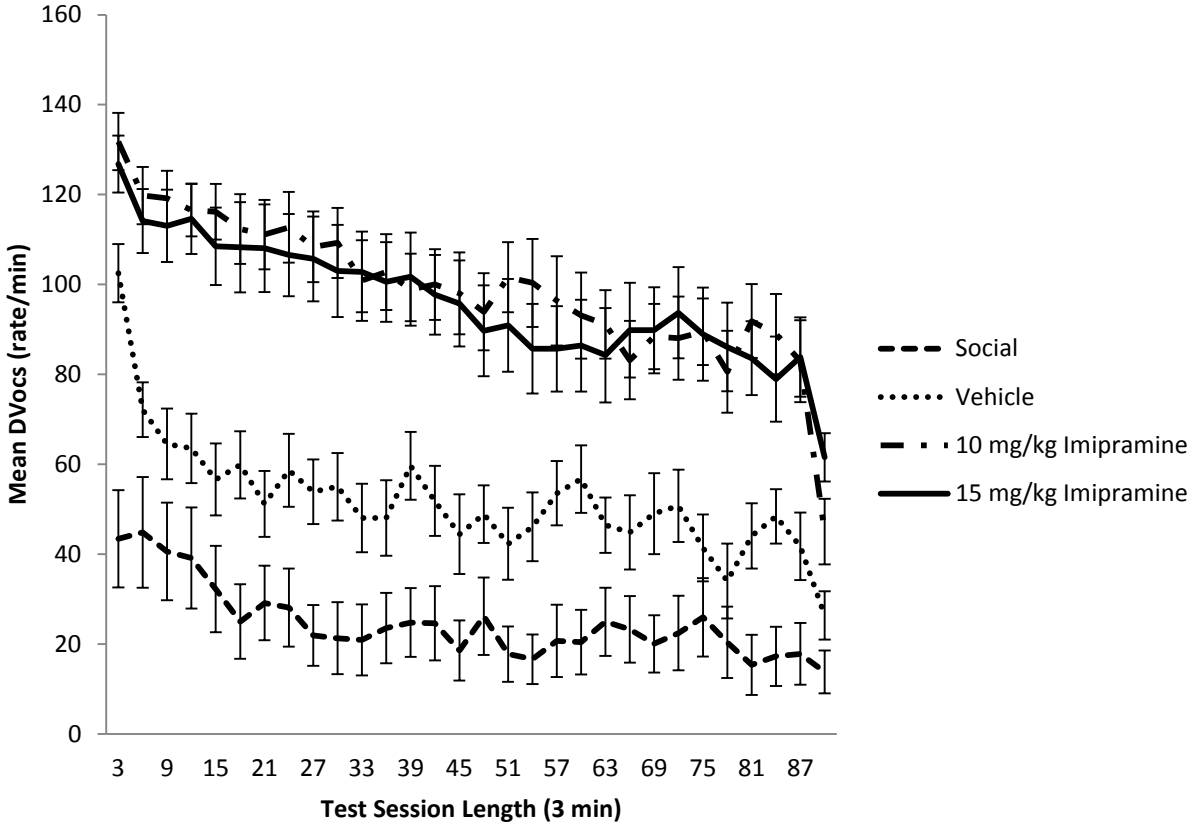

160
140
120
100
80
60
40
20
0
Mean DVocs (rate/min)
3
9
15
21
27
33
39
45
51
57
63
69
75
81
87
Test Session Length (3 min)
Social
Vehicle
10 mg/kg Imipramine
15 mg/kg Imipramine

Figure 8 B

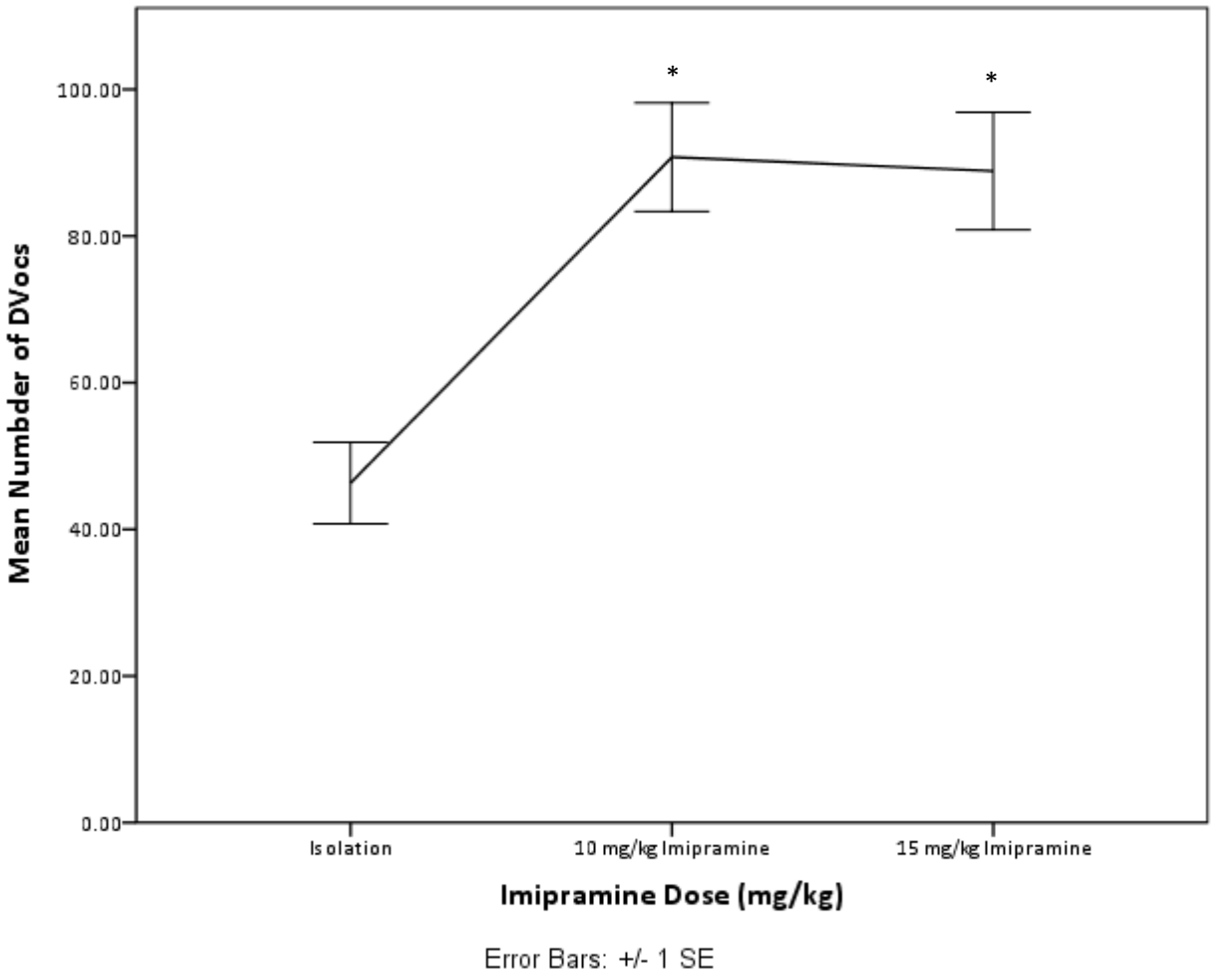

Mean Numbder of DVocs
100.00
80.00
60.00
40.00
20.00
0.00
*
*
Isolation
10 mg/kg Imipramine
15 mg/kg Imipramine
Imipramine Dose (mg/kg)
Error Bars: +/- 1 SE

Figure 9 A

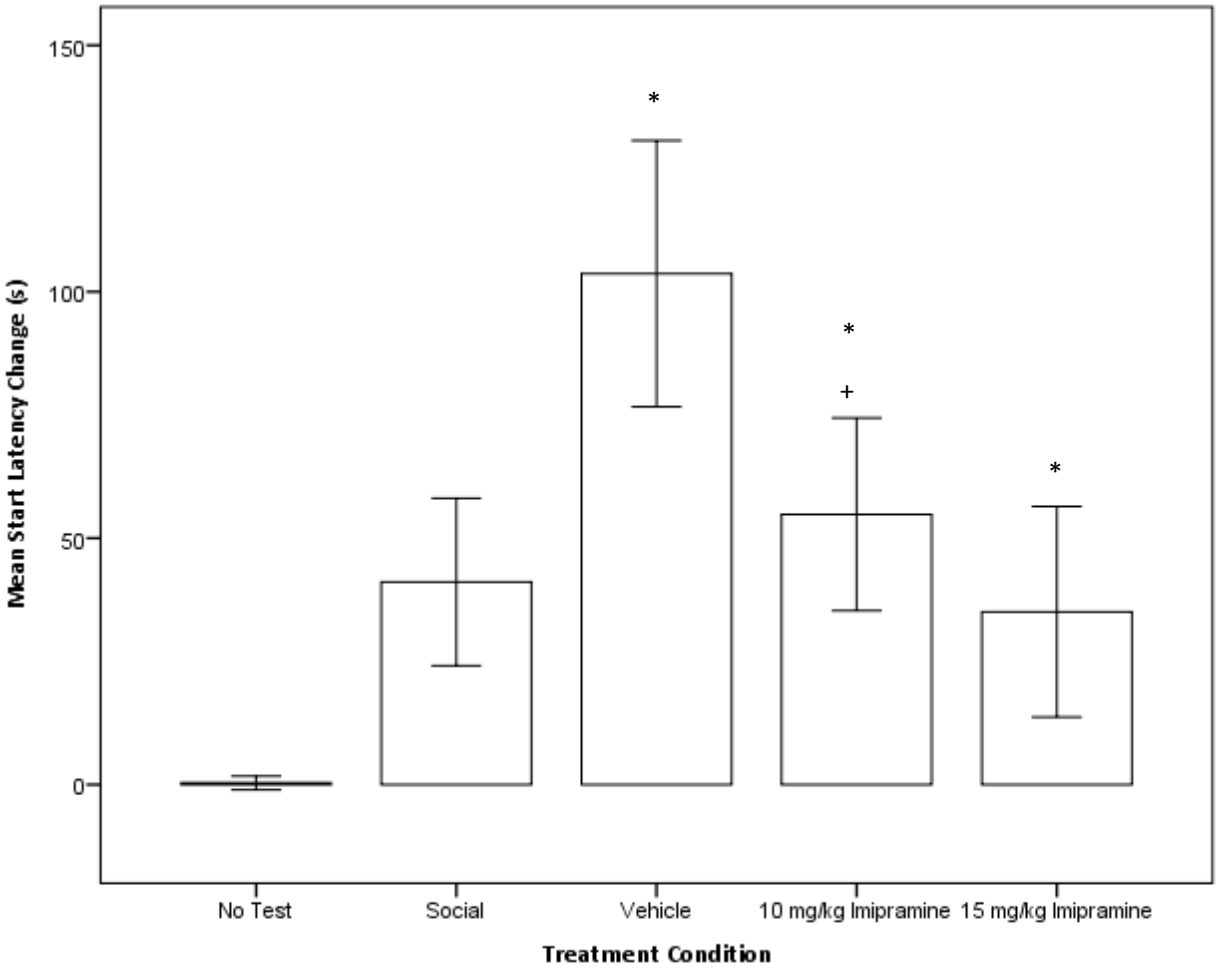


Figure 9 B

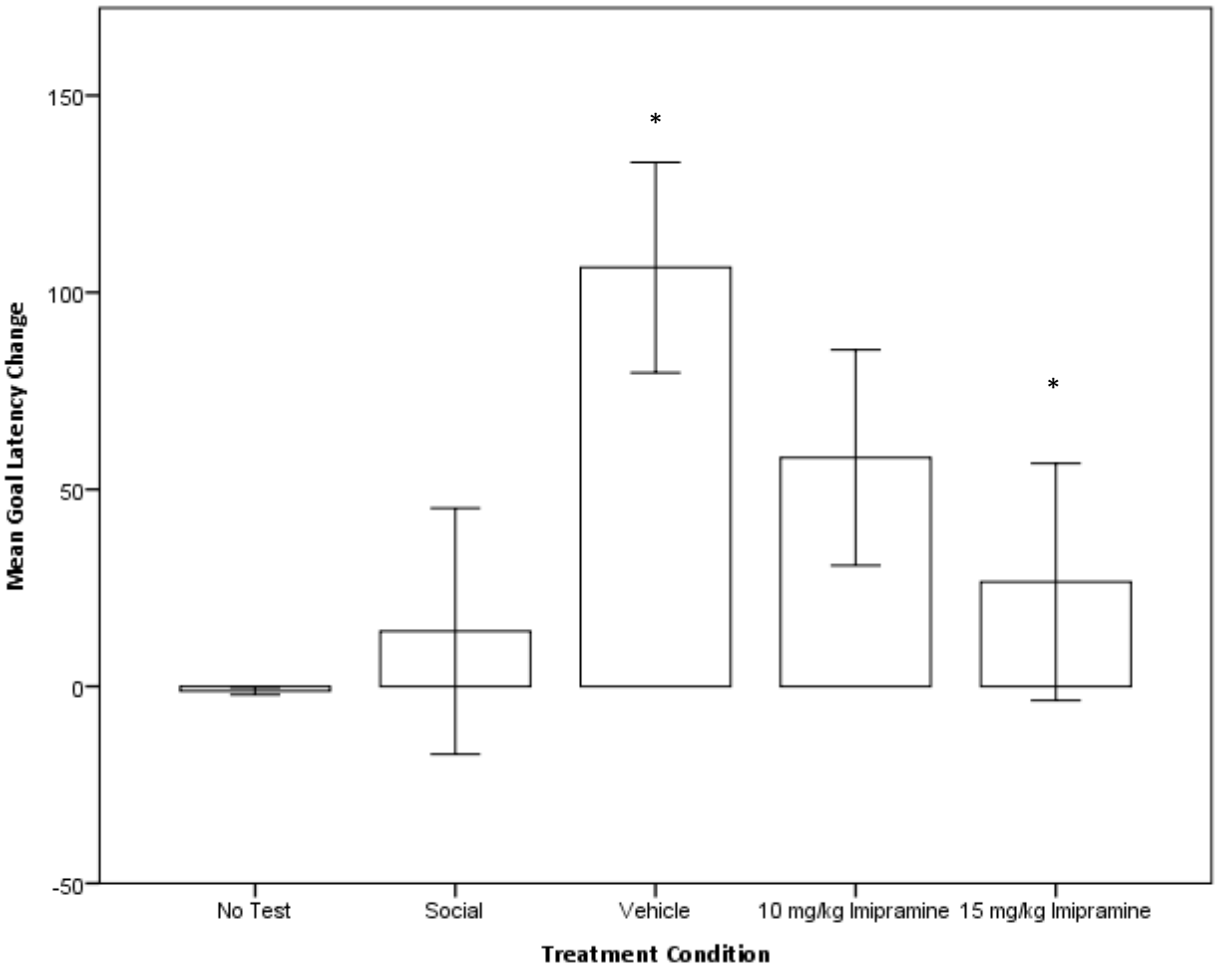

150
100
50
0
-50
*
*
Mean Goal Latency Change
No Test
Social
Vehicle
10 mg/kg Imipramine
15 mg/kg Imipramine
Treatment Condition

Figure 10 A

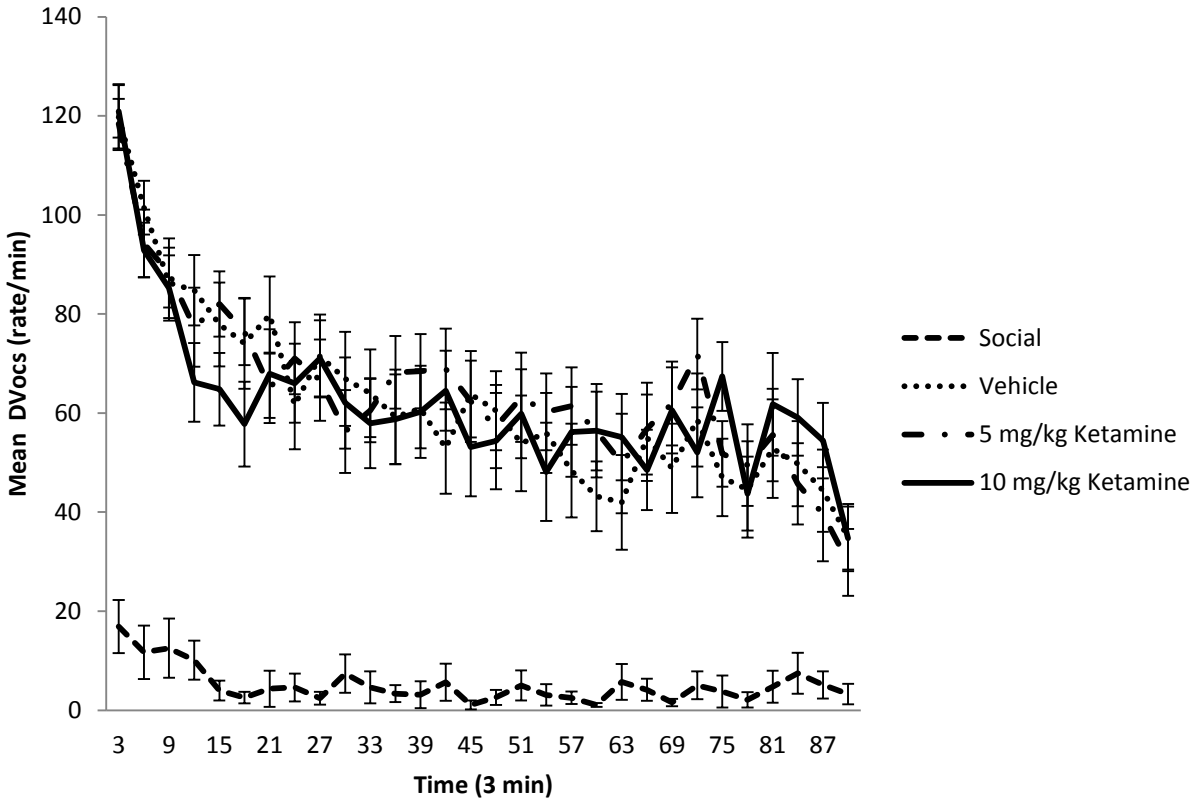

140
120
100
80
60
40
20
0
Mean DVocs (rate/min)
3
9
15
21
27
33
39
45
51
57
63
69
75
81
87
Time (3 min)
Social
Vehicle
5 mg/kg Ketamine
10 mg/kg Ketamine

Figure 10 B

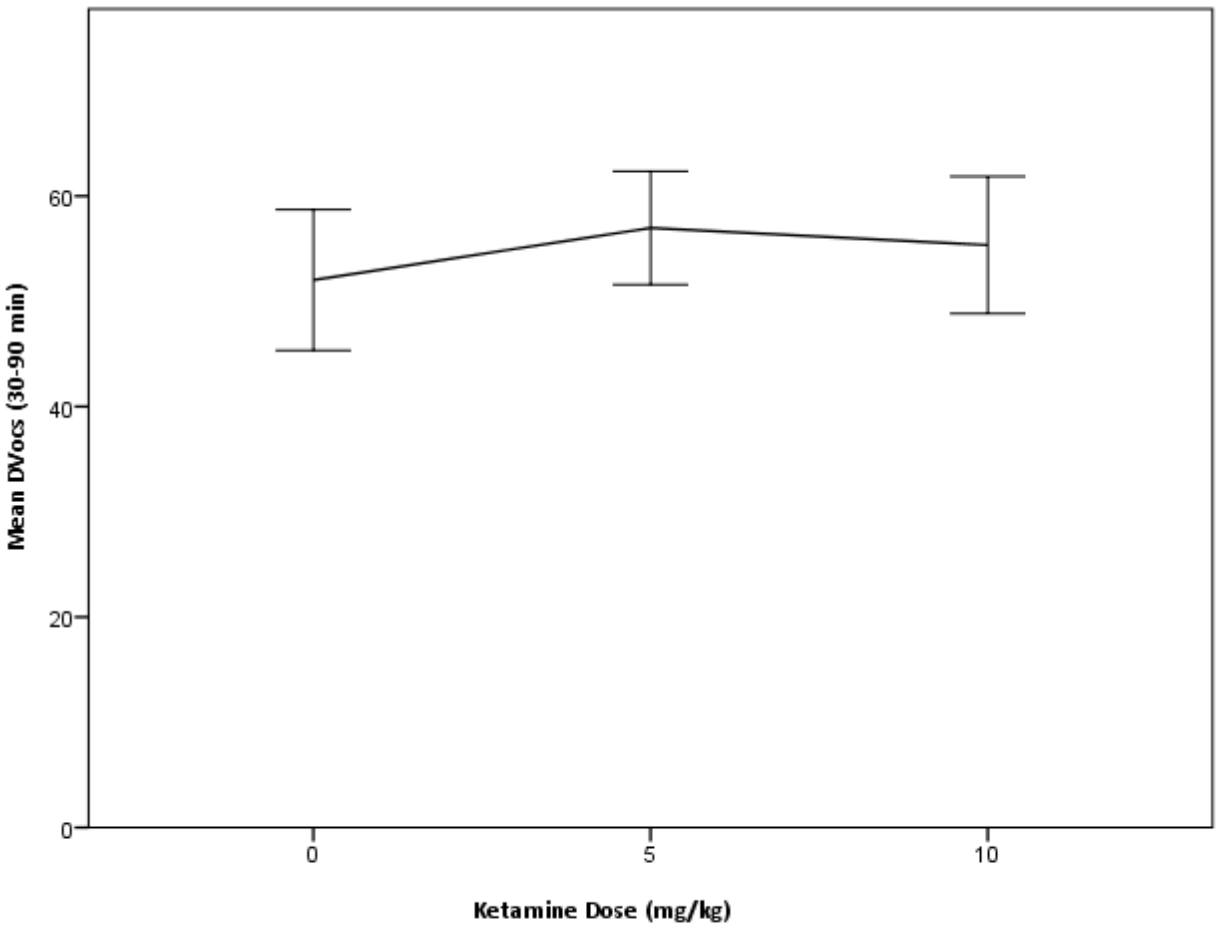


Figure 11A

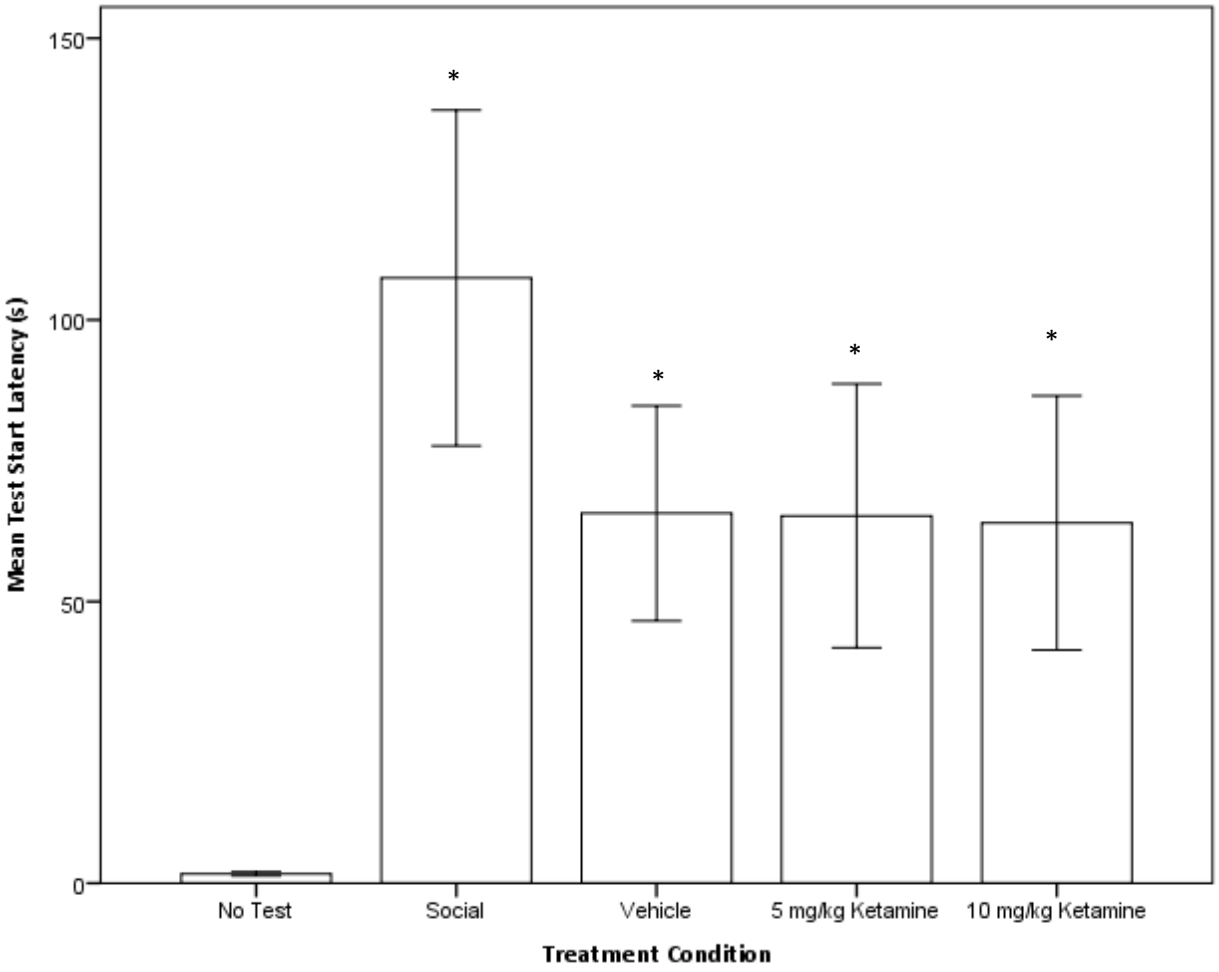
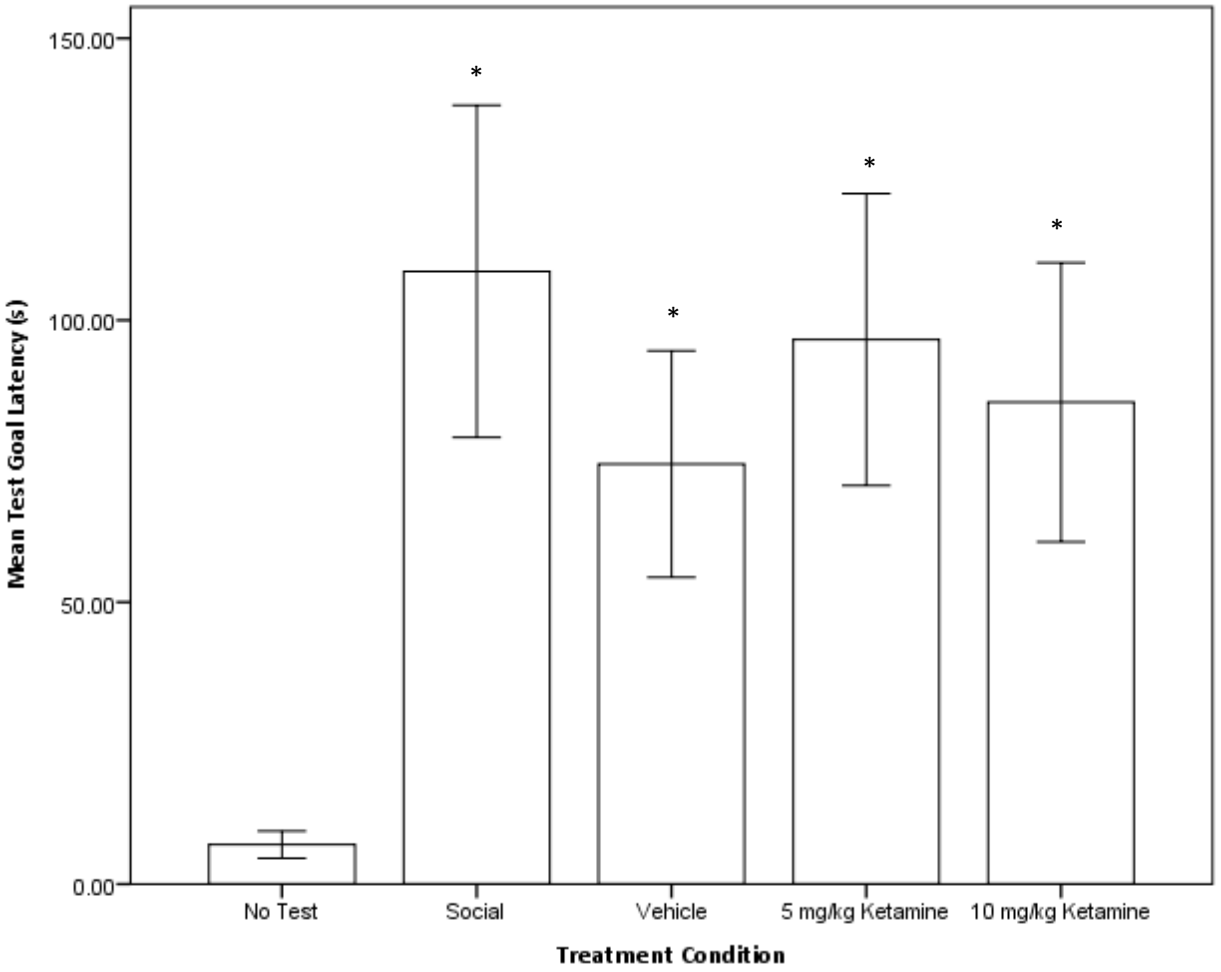

150
100
50
0
Mean Test Start Latency (s)
*
*
*
*
No Test
Social
Vehicle
5 mg/kg Ketamine
10 mg/kg Ketamine
Treatment Condition

Figure 11 B


150.00
100.00
50.00
0.00
Mean Test Goal Latency (s)
*
*
*
*
No Test
Social
Vehicle
5 mg/kg Ketamine
10 mg/kg Ketamine
Treatment Condition

VITA

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University of Mississippi, Oxford, MS (Fall 2008 to present). Kenneth J. Sufka laboratory. Conducted studies involving investigations of anxiety and depression model building, statistical analysis of experimental results through use of Excel programs designed and manipulated to evaluate data and other statistics software, personnel IACAUC compliance management including help understanding online modules and personnel training.

Graduate Research Assistant
University of Mississippi, Oxford, MS (Fall 2009 to Spring 2010). Michael T. Allen laboratory. Conducted studies investigating the impact of life stressors on physiological reactions to an acute stressor.

Undergraduate Research Assisstant
Albion College, Albion, MI (Fall 2006- Spring 2008). W. Jeffrey Wilson laboratory. Studies involving assessments of learning in rats. Data collection and analysis involved use of computer programs designed to capture data in real-time and use of statistical software to analyze data.

Undergraduate Research Fellow
Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA (Summer, 2007).Etienne Sibille laboratory. Conducted studies involving anatomical investigations of the impact of a depressive-like state in mice. This included use of a computer program allowing visualization of specific brain images that could then be highlighted and the new image captured.

TEACHING EXPERIENCE

Graduate Instructor, General Psychology
University of Mississippi, Oxford, MS (Fall 2010, Spring 2011, Fall 2011, Spring 2012, Fall 2013)

Graduate Instructor, Brain and Behavior
University of Mississippi, Desoto, MS (Fall 2011)
University of Mississippi, Tupelo, MS (Spring 2012)
Graduate Instructor, Cognitive Psychology
University of Mississippi, Tupelo, MS (July 2011, Fall 2012, Summer 2013)
University of Mississippi, Desoto, MS (June 2012, Spring 2013)
Teaching Assistant, Research, Design and Analysis
Albion College, Albion, MI (Fall, 2006, Spring, 2007)

INTELLECTUAL CONTRIBUTIONS

PUBLICATIONS

Hymel KA, Loria MJ, Salmeto AL, White SW, Sufka KJ (2013). Strain vulnerability and resiliency in the chick anxiety-depression model. Physiology and Behavior, 120, 124-129.

Loria MS, White SW, Robbins SA, Salmeto AL, Hymel KA, Murthy SN, Manda, P, Sufka KJ (2013). Brain-derived neurotrophic factor response in vulnerable and resilient genetic lines in the chick anxiety-depression model. Behavioural Brain Research, 245, 29-33.

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.

CONFERENCE PRESENTATIONS

Hymel KA, Salmeto AL, Sufka KJ (November 2013). Strain mediated stress vulnerability and resiliency on cognitive bias in the chick anxiety-depression model. Society for Neuroscience. San Diego, CA.

Salmeto, A.L.Hymel, K.A., Loria, M.J., White. S.W., Sufka, K.J. (June 2013) Strain vulnerability and resiliency in the chick anxiety-depression model. 20th International "Stress and Behavior" North America Conference June 22-24, 2013 (New Orleans, USA)

Loria M.J., White S.W., Robbins S.A., Salmeto, A.L., Hymel, K.A., Murthy, S.N., Manda, P.,Sufka, K.J. (October 2012). Brains, strains and neurotrophic gains: Strain differences in BDNF response in the chick anxiety-depression model.

Hymel K.A., Loria M.J., Salmeto A.L., White S.W., Sufka K.J. (October 2012). The sky is falling: Strain vulnerability and resiliency in the chick anxiety-depression model. Society for Neuroscience. New Orleans, LA.

Salmeto, A.L., Hymel, K.A., Carpenter, E.C., Sufka, K.J. (2009, October). *Cognitive Bias in the Chick Anxiety-Depression Model*. Poster presented at the annual meeting of the Society for Neuroscience, Chicago, IL.

Bowling, J. J.; Salmeto, A. L.; Hymel, K. A.; Sufka, K. J.; Hamann, M. T. (2008, December). *In Vivo Functional Studies of the Anxiolytic Marine Natural Product Aaptamine in Domestic Fowl Chicks.* Neuroscience and Behavior Research Day 2008, The University of Mississippi Medical Center, Jackson, Mississippi.

Salmeto, A.L.,Wilson, W.J. (May 2008). *Impact of a Depression-like State on Spatial Learning and Memory*. Poster presented at annual meeting of the American Psychological Society, Chicago, IL.

PROFESSIONAL AND HONOR ORGANIZATIONAL MEMBERSHIP

Society for Neuroscience, 2007 – present.

Faculty for Undergraduate Neuroscience, 2007- present.

Psi Chi National Honor Society in Psychology, 2006 – present.

American Psychological Society, 2008 – 2009.

Sigma Zi National Honor Society, 2008-2009.

HONORS, AWARDS, AND RECOGNITION

Virginia Delores Cantu Fellowship Endowment, University of Mississippi, 2013-2014

Magna Cumae Laude, Albion College, 2008

President, Psi Chi Honor Society, Albion College, 2007-2008

Treasurer, Psi Chi Honor Society, Albion College, 2006-2007