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ANTIDEPRESSANT EFFICACY AND BEHAVIORAL COMPARISONS

OF TWO ANIMAL MODELS OF DEPRESSION

An Honors Thesis

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Antidepressant Efficacy and Behavioral Comparisons of Two Animal Models of Depression

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Abstract

The current study examined the validity of the chronic mild stress and learned helplessness models of depression in addition to testing the efficacy of the tricyclic antidepressant imipramine. Thirty-six male albino rats were randomly assigned to three experimental conditions: control, chronic mild stress (CMS), and learned helplessness (LH). Half of the animals in each condition received IP injections of imipramine at 10 mg/kg/day, and the other half received equal volumes of isotonic saline. All animals were weighed daily throughout experimentation. The CMS animals were chronically stressed for a period of five weeks after which learned helplessness was induced in the LH group through one two hour shock session. All groups were tested weekly for sucrose consumption and following the initiation of learned helplessness behavioral tests were conducted. A Morris Water Maze and open field test were used to evaluate the spatial memory and anxiety behavior respectively of the rats. The results of this study indicate that the CMS model of depression is not very reliable in that sucrose consumption did not decrease even after 6 weeks of testing. The animals given imipramine showed significant growth delays which is indicated by their delayed weight gain in comparison to saline animals. LH rats showed more hyperactivity in the open field test on days 2, 3, and 7 of testing and deficits in spatial memory on the first day of Morris Water Maze testing.
Antidepressant Efficacy and Behavioral Comparisons of Two Animal Models of Depression

Although every person will experience some sadness and grief throughout his or her life, many individuals find that these symptoms significantly impair their daily functioning, persist for long periods of time, and are accompanied by other unpleasant symptoms such as loss of pleasure (anhedonia), hopelessness, and possibly suicidal thoughts. These individuals are suffering from major depressive disorder, a mood disorder that many estimate will affect 15 percent of Americans throughout their lifetime (Keen, p. xi). According to the Surgeon General's Report on Mental Health in 1999 (hereafter cited as SGRMH), depression is ranked number one as a cause of worldwide disability and if untreated will last an average of 9 months. While 90 percent of individuals will feel substantially better within two years, at least 50 percent will have recurring episodes of depression. For every additional episode of depression a person experiences, the odds greatly increase that the disorder will be chronic and perhaps lifelong (SGRMH, 1999). Therefore, depression is not only a disorder that affects a great proportion of Americans, but it is a disorder that is not easily overcome. Because of this and many other factors, it is very important to continue searching for new and ongoing treatments for depression and also attempt to discover what causes this disorder so that it may be prevented in the future.

For many years the biomedical community has recognized the importance and utility of animal models of depression. These models are invaluable in screening a wide variety of antidepressants to determine their efficacy and ability to properly treat the symptoms of depression without producing a great number of unpleasant side effects. Though these models have served a great purpose in discovering new and improved antidepressants, there are always questions of their clinical relevance (Bourin, Fiocco, & Clenat, 2001). In order for an animal model of depression to be clinically useful, it must measure up to certain standards of validity.
Namely, the animal model must show similar responses to antidepressants as in a clinic (predictive validity), similar phenomenon or symptoms as in clinical settings (face validity), and the model must have a strong theoretical rationale (construct validity). According to Bourin et al. (2001), there is no animal model in existence that meets all of these requirements at all times. However, there certainly is still merit in using animal models to investigate depression. Holmes (2003) suggests that the problem with animal models of depression is that they claim to actually induce depression in the animals. These models rely on the assumption that the behaviors observed in the animals are caused by certain underlying psychological states which cannot be measured. Depression is one of a number of disorders of cognition in humans and it remains possible that these disorders are unique to humans and that animals themselves cannot experience them. Given that depression is considered to manifest itself in psychological, behavioral, and physiological ways, it may also be true that I will never be able to fully model this disorder since I lack the ability to communicate with animals about their psychological state (Cryan & Mombereau, 2004). However, it could be greatly beneficial to study depression in animals and look at the symptoms for what they are. Instead of inferring a psychological state from the behaviors observed, it would be useful to instead study the symptoms that are similar to those in depression and the biological or environmental influences that caused them (Holmes, 2003). Successfully creating an animal model of depression is of great interest to the scientific community. Finding a reliable animal model of depression could lead to valuable information about humans and how they react to stress.

Over the past twenty years, the Chronic Mild Stress (CMS) model has become increasingly established as a valid model for depression in animals. While this model was initially designed by Katz and colleagues around 1982, their chronic stress model involved
severe stressors that caused significant trauma to the rats that were tested. Katz et al. used stressors such as mild uncontrollable foot shock, cold swims, changes in housing conditions, reversal of the light/dark cycle, and food and water deprivation (Murison & Hansen, 2001; Willner, Muscat, & Papp, 1992). Therefore, the chronic stress model was modified by Paul Willner (1992) to create the chronic mild stress paradigm. This model focuses mainly on the symptom anhedonia, or loss of pleasure in normally pleasurable activities. This symptom, according to the DSM-IV-TR, is one of the main characteristics of depression (American Psychiatric Association, 2000). Therefore, the CMS model focuses on anhedonia measured by sucrose consumption or sucrose preference in rats or mice. The consumption of sucrose water is a pleasurable event for rats and they often prefer it to water when given the choice. Previous research by Willner has shown that there are not significant differences between testing sucrose preference or sucrose consumption in rats therefore the more simplistic of the two methods, sucrose consumption, is preferred (Willner, 1997). Many laboratories have published studies indicating that they have successfully found decreases in sucrose consumption following exposure to the CMS procedure (D’Aquila, Brain, & Willner, 1994; Papp, Klimek, & Willner, 1994; Matthews, Forbes, & Reid, 1995; Monleon, D’Aquila, Parra, Simon, Brain, & Willner, 1995; Forbes, Stewart, Matthews, & Reid, 1996; D’Aquila, Newton, & Willner, 1997; Azpiroz, Fano, Garmendia, Arregi, Cacho, Beitia, & Brain, 1999; Zurita, Martijena, Cuadra, Brandão, & Molina, 2000; Dunčko, Kiss, Škultétyová, Rusnák, & Ježová, 2001; Grippo, Beltz, & Johnson, 2003).

Several characteristics establish the CMS model as a valid model of depression in rats. First, CMS has a relatively large amount of construct validity. CMS simulates anhedonia, or loss of pleasure, which is one of the key symptoms of depression in humans. In Willner’s 1997
review of the CMS procedure he notes the large amount of construct validity that it possesses. The sucrose consumption test is truly believed to be a measure of loss of pleasure as CMS does not cause decreases in consumption of regular water, the caloric value of the sucrose solution appears irrelevant, and decreases in sucrose consumption occur regardless of whether food deprivation is utilized. However, the anhedonia modeled by CMS is certainly not limited to sucrose consumption. Numerous studies have found a decline in response pleasurable stimuli using place preference conditioning and brain stimulation reward threshold studies as well (Papp, Lappas, Muscat, & Willner, 1992; Willner, 1997c). CMS also has a large amount of face validity as is indicated by its production of certain behavioral changes that mirror the symptoms of major depressive disorder in humans. For example, CMS has produced loss of body weight, decreases in sexual and aggressive behavior, decreases in locomotor activity, and the symptoms tend to be worse at the beginning of the rat's active cycle which also mirrors depression in humans (Willner, 1997c; Willner, 1997b). These symptoms have persevered for up to three months which is a good estimate of the duration of symptoms untreated in humans as well (Overstreet & Steiner, 1998). Lastly, because of the positive response of rats to antidepressant drugs, this model also has a considerable amount of predictive validity. After being treated with antidepressants the animals typically return to normal consumption of sucrose solution in three to four weeks which corresponds very highly to the recovery times reported in most clinics. In addition, these antidepressants do not affect the behavior of control animals that have not been exposed to the CMS procedure (Willner, 1997c). According to a review done by Overstreet and Steiner (1998), CMS has been effectively reversed by nearly all of the antidepressants that are used clinically but has not been affected by other psychoactive drugs such as anxiety or schizophrenia medications.
However, while some laboratories appear to have no trouble producing this decrease in sucrose consumption associated with anhedonia, there have been mounting concerns about the reliability of the CMS model. An increasing number of laboratories are having difficulties with the sucrose consumption tests. For example, Konkle, Baker, Kentner, Barbagallo, Merali, and Bielajew (2003) found no significant changes in sucrose consumption after implementing the CMS regimen for six weeks. Murison and Hansen (2001) actually found significant increases in sucrose consumption after approximately three weeks of the stress regimen. Our own laboratory, while previously able to produce decreases in sucrose consumption, has not been able to successfully complete a CMS experiment with consumption deficits for the past year and a half. Another laboratory has published several experiments in which they report obtaining significant decreases in sucrose consumption in CMS rats, however they believe these decreases are due to the extreme food deprivation and weight losses that the rats sustained (Matthews, Forbes, & Reid, 1995; Forbes, Stewart, Matthews, & Reid, 1996). In a 1997 review of the CMS model of depression, Paul Willner admits that even his own laboratory has had some difficulties with the CMS procedures after moving from London to Swansea. In addition, D’Aquila, Newton, and Willner (1997) found that their Wistar rats only decreased consumption of sucrose when tested during the dark cycle, and not during the light cycle. All of these studies give rise to doubt about the reliability of the CMS model of depression.

While the CMS model of depression has only been in existence for approximately 20 years, the second model of depression chosen for the current experiment, Learned Helplessness (LH), has been utilized for almost 40 years. This model was inadvertently discovered in the early 1960s by graduate students at the University of Pennsylvania who were studying avoidance learning. They discovered that after the animals were exposed to an uncontrollable and traumatic
event, they showed distinct and unexpected behavioral changes (Overmier, 2002). This discovery was followed up by J. Bruce Overmier and Martin Seligman (1967). In their experimentation they exposed one group of dogs to inescapable shock and another group to escapable shock. When these animals were tested later in an escapable shock situation, the animals exposed to escapable shock were indistinguishable from control animals who had never been shocked. However, of those animals who were previously exposed to inescapable shock, two thirds passively accepted the shock and did not make any attempt to escape (Seligman & Maier, 1967; Seligman, 1968; Willner, 1986). These studies have gradually evolved to use rats as subjects instead of dogs, and a number of behavioral symptoms have been noted to appear after a session of inescapable shock such as sleeping and eating problems, decreased immune functioning, ulcers, impairments in responding for brain reward stimulations, decreased locomotor activity, weight loss, and decreases in aggressive behavior (Shors, 2004; Willner, Muscat, & Papp, 1992; Willner, 1986). Seligman asserts that the cause of depression is that an individual believes that he or she is helpless and has no control over events and consequences. This belief affects motivation, emotions, and cognition (Willner, 1986). The learned helplessness model of depression is widely used as a stress model of depression, although appears much more severe in comparison to the mild stressors used in chronic mild stress.

Learned helplessness, while a well-established model of depression, has often been criticized for its lack of reliability across laboratories similar to CMS (Bourin et al., 2001; Vollmayr & Henn, 2001). Across the various studies conducted using learned helplessness there appears to be some variation in the criteria used to classify the animals. In some studies the delay of an escape attempt for greater than 20 seconds constituted helpless behavior. Other studies have used 45 seconds as the criteria for helplessness (Musty, Jordan, & Lenox, 1990). These
discrepancies make it difficult to compare different studies and the effectiveness of the learned helplessness procedure as a whole. In addition, in any given experiment it is reported that anywhere from 10 to 80 percent of the animals may develop certain helpless behaviors such as an escape deficit (Cryan & Mombereau, 2004). The variability in the number of helpless animals could be the result of several factors. When animals are tested multiple times in the escape task it is possible that they will improve solely based on learning (Vollmayr & Henn, 2001). In addition, some studies have found that the escape tasks are too simple. An animal could accidentally complete the escape action without making any effort. Therefore Musty, Jordan, and Lenox (1990) suggest the use of a hurdle in the middle of the box that separates the animal from the lever necessary to escape the shock. This hurdle reduces random escape behaviors by the animal and significantly increases reliability. This problem could also be circumvented by using a more difficult escape task (Cryan & Mombereau, 2004). While some efforts have been made to increase the reliability of learned helplessness, it is clear that additional steps still need to be taken.

Just as complaints have been made about the reliability of learned helplessness, there are also some concerns with regards to validity. According to Willner (1986), there are several components to the construct validity of learned helplessness. First, it must be shown that the animals in the study who are exposed to uncontrollable shock really become helpless. Second, it is important to make sure that humans in similar positions of uncontrollability experience a helpless state. Lastly, Willner believes it is important to establish that helplessness is truly a main symptom of depression in humans. Although numerous studies have been conducted to address these questions and issues, the exact answers remain unclear as contradictory evidence often emerges. Therefore, the point of view presented by Willner is that we cannot definitely establish
helplessness in animals, humans show even less of a connection between uncontrollability and helplessness, and evidence still is not clear about whether helplessness is a solid part of depression so the construct validity of learned helplessness remains unknown.

Learned helplessness appears to have a great amount of face validity. It produces a wide variety of symptoms that are listed under the DSM-IV-TR criteria for depression such as psychomotor retardation, passivity, and loss of weight and appetite (Willner, 1986). However, a large problem with learned helplessness is the duration of the symptoms. Studies have found that the symptoms of depression do not linger for much longer than two to three days which certainly does not mirror the duration of these symptoms in humans (Cryan & Mombereau, 2004). According to J. Bruce Overmier (personal communication), one of the principal investigators of learned helplessness when it was first discovered, the escape deficit can be observed for approximately 24 hours, avoidance behavior will continue for 48 hours, open field deficits can be observed for about a month, and gastric ulcerations are still present after three months. The more primary an action is to survival, such as escape, the sooner this symptom will be reversed after the uncontrollable shock session (Overmier, personal communication).

The predictive validity of learned helplessness appears to be somewhat debatable. Willner (1986) maintains that learned helplessness has relatively good predictive validity because it responds well to sub-chronic treatments of various tricyclics, atypical antidepressants, and electroconvulsive therapy. In addition, Overstreet and Steiner (1998) report that learned helplessness shows a positive response to selective serotonin reuptake inhibitors (SSRIs), the newest class of antidepressants. However, Willner warns that there are some potential drugs in existence that will appear to reverse the symptoms of learned helplessness but are not antidepressants. Bourin et al. (2001) and Holmes (2004) report that anxiolytics have been known
to reverse the behavioral effects of depression which would be considered a false positive response. Therefore, it would appear from the previous analysis that learned helplessness possesses some validity, although like most animal models of depression it is certainly not perfect.

In order to evaluate and compare the two chosen animal models of depression in the current experiment, several behavioral tests were used. The first behavioral measure employed was a weekly sucrose consumption test as described with regards to chronic mild stress. Sucrose consumption tests are thought to provide a method of assessing the hedonic changes that occur in depression. Willner (1997c) points out that sucrose consumption mirrors closely some tests of anhedonia that are used in humans such as taste reactivity tests. Because of its possible applicability to anhedonia, this measure has been endlessly assessed for validity and reliability as an indicator of depression in CMS. However, very little research has examined the effects of learned helplessness on sucrose consumption. A study conducted by Vollmayr, Bachteler, Vengeliene, Gass, Spanagel, and Henn (2004) investigated rats bred for congenital learned helplessness. These rats were the 48th and 50th generation descendents of rats who showed escape deficits after a learned helplessness shock session. These rats, after undergoing a shock session, were tested on sucrose consumption. Vollmayr et al. found significant decreases in responding for sucrose in congenital learned helplessness rats. This indicates that learned helplessness could possibly produce anhedonia in rats.

Depression has many characteristic symptoms besides anhedonia including weight changes. Previous studies of chronic mild stress have not found completely consistent results with regards to weight changes. Several studies have found that the chronic mild stress procedure resulted in significant reductions in weight gain over the course of the procedure (Matthews,
Forbes, & Reid, 1995; Forbes, Stewart, Matthews, & Reid, 1996; Dunčko, Kiss, Škultétyová, Rusnák, & Ježová, 2001; Murison & Hansen, 2001). Though some maintain that this is because of the strict food deprivation procedure that is employed in the CMS procedure, Dunčko, Kiss, Škultétyová, Rusnák, and Ježová (2001) found this reduction in weight gain despite their decision to omit the food deprivation regimen. However, while a number of labs have consistently found this reduction in weight gain among CMS animals, other studies, such as that conducted by Grippo, Beltz, and Johnson (2003), found that CMS animals did not differ significantly in weight from control animals. In fact, Grippo et al. (2003) found that CMS animals actually weighed slightly more on average than control animals although this difference was not significant. These discrepancies regarding the effects of CMS on weight are generally not mirrored in the effects of learned helplessness on weight. Several studies of learned helplessness have found weight loss following the shock session and reductions in weight gain (Dess, Raizer, Chapman, & Garcia, 1988; Westenbroek, Ter Horst, Roos, Kuipers, Trentani, & den Boer; 2003). In addition, a study conducted by Park, Campbell, and Diamond (2001) used a traumatic stressor comparable to a shock session which led to a reduction in weight gain. Given the discrepancies that exist in the weight changes resulting from chronic mild stress, weight will be monitored daily throughout the study to accurately gauge the animals’ responses to CMS, and to compare these results to those of learned helplessness.

Previous research has attempted to assess the effects of stress and depression on spatial memory. While there is not a great deal of research on this topic, there are several studies that are comparable. Hölscher (1999) experimented with the stress of handling or not handling animals. This study manipulated whether or not the animals were held until comfortable before assessing the results in a spatial memory task. Hölscher found significant impairments in spatial learning
and memory using a radial arm water maze. While the previous study tested normal rats’ reactivity to stress, Grauer and Kapon (1993) chose to use two different breeds of rats. Wistar-Kyoto (WKY) rats are known to be hyper-reactive to stress while Sprague-Dawley (SD) rats served as the control group. WKY rats showed significant impairments with regards to spatial memory when compared to SD rats. In addition, several studies have modeled the relationship between spatial memory and depression using more severe stressors such as predator or shock exposure. Park, Campbell, and Diamond (2001) exposed rats to a predator and measured its impact on their ability to perform in a radial arm water maze. They found that spatial learning and memory were significantly impacted by the stress regimen in the same day trials in the radial arm water maze. However the impact noted was transient and lasted only for a short amount of time. Multiple studies have attempted to investigate the impact of learned helplessness on spatial memory. Vollmayr et al. (2004) found no significant impacts of learned helplessness on Morris water maze performance in congenital learned helplessness rats. Similarly, Warren, Castro, Rudy, & Maier (1991) found that when rats were exposed to inescapable shock they showed no deficits in Morris water maze performance. However, a study conducted by Shors (cited in her 2004 review) found that the exposure of rats to inescapable shock resulted in performance deficits in an eight arm radial maze. While some of the previous studies have chosen to investigate spatial memory using a radial arm water maze, the current study employed a Morris Water Maze. According to D’Hooge and De Deyn (2001), the Morris Water Maze is an effective way to test spatial learning with significant flexibility with regards to the type of spatial cue provided. While other valuable spatial learning tests are available, the Morris Water Maze is more simplistic and easier to use than some alternatives. Because of this, the Morris Water Maze has become one of the most commonly utilized research tools (D’Hooge & De Deyn, 2001). This
maze, originally developed by Richard Morris (1984), was designed to be a test of spatial memory in rats so that the neural basis of spatial memory could be investigated. Therefore, this study will use the Morris Water Maze as a valid test of spatial learning and memory in rats.

Few research studies in the past have attempted to examine the effects of chronic mild stress and learned helplessness on anxiety behavior. Several experiments have found that stress can result in animals showing more anxiety in an elevated plus maze (Shors, 2004; Zurita et al., 2000). However, D’Aquila et al. (1994) attempted to examine the effects of chronic mild stress on anxiety as evaluated in an elevated plus maze and found that the stress regimen did not result in any behavioral changes indicating anxiety. Therefore, it is not clear the impact that stress as a whole or learned helplessness and chronic mild stress specifically have on anxiety behaviors.
The current study seeks to further explore the impact of stress on anxiety behavior using the chronic mild stress and learned helplessness models of depression. Previous studies have made use of the elevated plus maze which certainly is useful in exploring anxiety, however the current study chooses to use the open field test. This model was created by Calvin Hall (1934) who was studying emotionality in rats and has since been endlessly tested and validated. Ramos and Mormède (1998) cite numerous behaviors that can be investigated using the open field test such as urination, defecation, locomotion, and time spent in the center among other behaviors. This study will make use of the ability of the open field test to detect anxiety in the animals measured by whether or not they venture into the middle of the open field where the squares are not adjacent to a wall. The open field test has been used in a large number of studies to investigate anxiety and will provide a solid measure of anxious behavior in the animals of this study (Carola, D’Olimpio, Brunamonti, Mangia, & Renzi, 2002; Romeo, Mueller, Sisti, Ogawa, McEwen, & Brake, 2003; Kanari, Kikusui, Takeuchi, & Mori, 2005).
The current study also chooses to investigate the effects of imipramine administration on the developing of symptoms associated with depression. Imipramine is a tricyclic antidepressant that has been used for many years in the treatment of depression. Its use with chronic mild stress and learned helplessness are well-established through a variety of studies. Bourin et al. (2001) go to great lengths to describe the various animal models of depression and all of the treatments that have been used to attempt to reverse the effects. Imipramine has certainly been tested with chronic mild stress and been found to successfully reverse the behavioral effects in studies such as Muscat et al. (1992), Willner et al. (1987), Muscat et al. (1990), and Papp et al. (1996) (as cited in Bourin et al., 2001). In addition, Papp, Klimek, and Willner (1994) used chronic mild stress on rats for three weeks monitoring their sucrose consumption, and then began injecting half with imipramine in a volume of 10 mg/kg per day and the other half with a similar volume of saline for another four weeks. They found that the imipramine reversed the decreases in sucrose consumption that had occurred as a result of the chronic mild stress regimen (Papp, Klimek, & Willner, 1994). Similarly, Monleon, D’Aquila, Parra, Simon, Brain, and Willner (1995) examined chronic mild stress in mice and observed their decreases in sucrose consumption that took place over the course of four weeks. After four weeks they began imipramine administration in a volume of 20 mg/kg per day and found that over the course of the next three weeks the sucrose consumption of the stressed animals returned to normal (Monleon, D’Aquila, Parra, Simon, Brain, & Willner, 1995). Learned helplessness has been similarly studied to investigate not only whether imipramine can reverse the behavioral effects of inescapable shock, but also whether imipramine can prevent these behavioral effects. A review conducted by Bourin et al. (2001) reports that imipramine has indeed been successful in reversing the effects of learned helplessness in at least two published studies by Sherman et al.
(1979) and Leshner et al. (1979). Additionally, Gambarana, Scheggi, Tagliamonte, Tolu, and De Montis (2001) found that long term treatments with imipramine could prevent the deficits that were associated with the learned helplessness paradigm after an inescapable shock session.

While many previous studies have examined different parts of the current experiment, the current study has several new aims. First, this study attempts to reestablish the reliability of the Chronic Mild Stress model of depression using sucrose consumption as a measure of anhedonia. This study will use extra care to ensure that all aspects of the sucrose test are carefully and accurately conducted. Second, the current study will compare the Chronic Mild Stress and Learned Helplessness models of depression on sucrose consumption, weight changes, spatial memory, and anxiety behaviors in hopes of expanding on the previous research that has been conducted. Lastly, the current study seeks to investigate the efficacy of imipramine at preventing symptoms of depression from developing. I hypothesize that the rats undergoing the CMS regimen and receiving saline will show signs of anhedonia as represented by a decrease in sucrose consumption but that the learned helplessness procedure will not cause a decrease in sucrose consumption. I believe that CMS will lead to only slight weight loss if any because food deprivation is not used in this study. In addition, I think that the animals in the CMS group will not show deficits in spatial memory but that learned helplessness animals will because of the more severe nature of the stressor. I believe that learned helplessness animals will also show more anxious behavior in the open field test. Lastly, I hypothesize that for both models those animals injected with imipramine will not develop the same behavioral symptoms as those animals would receive saline injections and so imipramine will prevent the development of depression.
Method

Subjects

Thirty five male albino Sprague-Dawley rats (Charles River, Wilmington, Mass., USA) approximately seven weeks old and weighing between 235 and 315 grams at the start of drug administration were used in this experiment (one rat was dropped from the study due to health problems). The animals were individually housed under conditions of controlled temperature and humidity. Food and water were freely available except when otherwise specified. The animals were kept on a 12:12 hour light:dark cycle with the lights on from 7:30am – 7:30pm. Behavioral experiments and shock sessions were conducted during the light cycle while the chronic mild stressors were implemented during the dark cycle.

Apparatus

Weight measures. Animals were weighed daily throughout experimentation at approximately 11:00 am. They were weighed individually on a Scout Pro 2000 gram scale (purchased from Ohaus). This weight was recorded in grams and used to calculate the proper amount of drug or saline to administer to each animal.

Shock apparatus. The LH animals were exposed to a single shock session in a Lafayette Instrument Co. Operant Chamber (11"x 8"x 8") with electrified bars. This chamber was attached to a C.H. Stoelting Co. Electric Shock Stimulator and to a computer which randomly distributed the shocks across the shock session. Each animal had a two hour shock session during which it was given 80 shocks or one shock approximately every 90 seconds. Each shock had a duration of 5 seconds and an intensity of 1 milliamp (at the recommendation of J. Bruce Overmier).

Morris water maze. The animals were tested in a Morris Water Maze. It was a blue circular plastic pool approximately six feet in diameter, three feet tall, and colored blue. The
maze was filled to a depth of a little over two and a half feet with lukewarm tap water and made opaque using powdered milk. A plastic pitcher was turned upside down and weighted with rocks for the platform. This clear platform was exactly five inches in diameter, two and a half feet tall, and located just below the surface of the water. This platform was located at a different place in the maze each testing session. The room containing the water maze was well lit and contained several visual clues including colored paper taped to the walls that were kept constant in the experimentation. Rat performance in the maze was recorded on a stopwatch that was started immediately after the rat entered the water and stopped as soon as the rat reached the platform.

*Open field test.* The animals were also tested in an open field test for signs of anxiety and hyperactivity. The open field test was a large square wooden box with dimensions of about 3.2' x 3.2', a wooden wall approximately 10" high, and with clear plexiglass walls extending above the wooden walls approximately 21". The floor of the box was painted white and subdivided into 16 equally sized compartments using masking tape (4 across and 4 tall). Twelve of the compartments were adjacent to a wall while four compartments in the center were not. Time in the open field test was recorded using a stopwatch and the number of squares entered was counted along with whether or not the rat entered one of the four middle squares.

*Procedure*

The animals were delivered to the laboratory at approximately three weeks of age. For the first week they were group housed with six per cage and given free access to both food and water. At the end of this week the animals were separated into individual cages with free access to food and water. To habituate the animals to the sucrose testing procedure, they were given free access to a 1% sucrose solution for two days continuously. At the beginning of the following week, the sucrose was taken away and after a period of 24 hours, the first sucrose test was given.
In the following three weeks periodic sucrose tests were given to accurately assess the average amount consumed by each animal. In addition, daily weighings began at 11:00 am and continued for the remainder of the experiment. After three weeks, the animals were divided into six groups based on sucrose consumption: Chronic Mild Stress-Imipramine, Chronic Mild Stress-Saline, Learned Helplessness-Imipramine, Learned Helplessness-Saline, Control-Imipramine, and Control-Saline.

At this point the CMS procedure and drug administration began. Animals in the two CMS groups were stressed every night from approximately 6:00 pm to 8:00 am. All stressors were implemented in the animal colony except for having the lights on or a strobe light on during the dark cycle at which time the CMS animals were moved to an adjacent room so as to not disturb the other animals. Drug administration took place every day at approximately 4:00 pm, and sucrose tests were conducted every Tuesday from 11:30 am to 12:30 pm.

After approximately five weeks of the chronic mild stress procedure, the twelve LH animals were subjected to a two hour shock session. On the day following the shock sessions, the LH animals were weighed at 11:00 am, tested for sucrose consumption at 11:30 am, began open field testing at 2:00 pm, injected with either imipramine or saline at 4:00 pm, and run in the Morris Water Maze at 5:00 pm. This behavioral testing took place on Days 1, 2, 3, and 7 following the completion of the learned helplessness shock session. The CMS and control animals were tested similarly on separate days in which they were first weighed and given the sucrose test, and then run through the behavioral tests in the same manner and for the same number of days as the LH animals. Throughout all of this testing the CMS procedure and drug administration continued. Following the completion of all days of behavioral testing, the data were entered into a spreadsheet for analysis.
Drugs. The three experimental stress conditions of animals were randomly divided in half with the first group receiving daily IP injections of imipramine in a dose of 10 mg/kg/day and the second group receiving an equal dose of isotonic saline. Imipramine hydrochloride (purchased from Sigma-Aldrich) was dissolved in isotonic saline solution. Animals were injected daily at approximately 4:00 pm in a volume of 1.0 mL/kg in a 26-gauge, $\frac{3}{8}$ inch long needle (purchased from Fisher Scientific) for approximately 50 days during the course of experimentation.

Stress regimen. A modified version of Willner’s (1997) chronic mild stress model of depression was used in this experimentation. According to a previous Willner study, implementing the chronic mild stressors at night during the beginning of the rat’s active cycle was found to be just as if not more effective than their similar implementation during the light cycle (D’Aquila, Newton, & Willner, 1997). Therefore, chronic mild stressors were selected from a published list by Willner and these stressors were put into effect around 6:00 pm every day and removed approximately 14 hours later. The selected stressors in this experiment included: Monday – strobe light during dark cycle, Tuesday – foreign object in cage, Wednesday – cage tilted 45°, Thursday – animals switched cages, Friday – lights on during dark cycle, Saturday – wet bedding (water), and Sunday – pair housing (new partner each time) (D’Aquila, Newton, & Willner, 1997).

Sucrose consumption testing. To evaluate anhedonia, all animals were given a weekly sucrose consumption test. The sucrose consumption test entailed the rats having free access to a 1% concentration of sucrose water every Tuesday for one hour from 11:30 am – 12:30 pm. During this test the animals did not have access to normal tap water. The bottles were weighed prior to placement in the cage and weighed again after the hour was completed. The difference
between these weights was the amount of sucrose water consumed measured in grams. Prior to sucrose testing, the animals were water deprived for 24 hours.

*Morris water maze.* Subjects were tested for spatial learning ability and possible concentration deficits in the Morris Water Maze on four separate occasions (Days 1, 2, 3, and 7 following completion of stress treatment). Each animal was placed on top of the submerged platform for approximately one minute prior to beginning the swimming task. Then, each rat was released into the water maze facing the wall from a random starting point. The stopwatch began timing as soon as the rat was in the water and stopped when the rat reached the platform. The rat was allowed a maximum of 60 s to reach the platform before it was removed from the water and placed on the platform. In this case the animal would have a time of 60 seconds recorded. Whenever the rat reached the platform, the animal was allowed to remain on the platform for approximately 10 seconds before the next trial was started. Each animal completed ten trials per day and each time it was once again released into the water from the same point as before and timed as to how long it took the animal to reach the platform. After the tenth trial the animal was thoroughly rinsed off, returned to its cage, and put back in the room with the rest of the animals. For the purposes of statistical testing, data were used from all four days of testing and all 10 trials completed.

*Open field test.* The animals were tested for anxiety and hyperactive behavior in the open field test on four occasions: Days 1, 2, 3, and 7 after completion of either the learned helplessness shock session or CMS stress regimen. Animals were placed in a corner square of the open field test and given three minutes to explore the open field test. During this time, the experimenter kept track of how many squares the animal entered (squares re-entered were counted again) and also if the animal entered one of the four center squares which were not
adjacent to a wall. At the end of three minutes the animals were removed from the open field
test, returned to their cages, and brought back to the animal colony.

Results

Weight

To examine the differences in weight between the different conditions in this experiment,
the animals were weighed daily. For the purpose of statistical testing, the weights of the animals
every Monday were used to obtain a weekly weight measure. The data were collated and a mixed
factorial analysis of variance (ANOVA) was calculated. Statistical analysis revealed a significant
main effect of drug group, $F(1, 29) = 13.70, p < .01$ but no main effect of condition ($p > .05$).
Analysis also revealed significant interactions of week of testing and condition, $F(12, 174) =
1.96, p < .05$, and week of testing and drug, $F(6, 174) = 45.08, p < .01$, and also a significant
repeated measures effect of week, $F(6, 174) = 454.89, p < .01$. However, there was not a
significant interaction between week, condition, and drug ($p > .05$). Subsequent analysis revealed
that Saline animals had significantly greater weights than animals treated with imipramine every
week after the first week (all $p$'s < .05).
Figure 1. Mean weights for Antidepressant and Saline animals for the duration of experimentation. * Antidepressant is significantly different from Saline ($p < .05$)

Sucrose Consumption

To examine the differences in sucrose consumption across stress and drug conditions, a mixed factorial ANOVA was calculated using sucrose data from one week before the CMS procedure was started (Baseline) and seven sucrose tests during experimentation with Week 0 being the first week of drug administration. Statistical analysis revealed a significant repeated measures effect of week of test, $F(7, 203) = 34.00, p < .001$, and significant interactions between week and condition, $F(14, 203) = 2.76, p < .001$, week and drug, $F(7, 203) = 2.72, p < .01$, and week by condition by drug, $F(14, 203) = 2.01, p < .05$. However, there were not significant main effects of condition, drug, or condition by drug (all $p$'s $>.05$). Given the significant triple interaction of week, condition, and drug, further analysis was conducted to investigate whether learned helplessness, which occurred between weeks 4 and 5, had a significant impact on the learned helplessness animals. However, no significant results were found ($p > .05$).
Figure 2. Mean sucrose consumption across experimentation by condition.

Figure 3. Mean sucrose consumption across experimentation by drug.

Morris Water Maze

In order to examine the differences in spatial memory between the stress and drug conditions, a mixed factorial ANOVA was calculated on the average times to reach the platform.
per day. Statistical analysis revealed a significant repeated measures effect of day of testing, $F(3, 87) = 34.85, p < .001$, and a significant interaction of day of testing and stress condition, $F(6, 87) = 2.33, p < .05$. In addition, this analysis showed a marginally significant main effect interaction of condition and drug, $F(2, 29) = 3.29, p = .052$. No significant interactions were found between day of testing and drug or day of testing, condition, and drug, nor were there main effects of drug or condition (all $p$'s $>.05$). Subsequent analysis revealed that learned helplessness rats were significantly slower at finding the platform on day 1 than the control animals, however this difference disappeared by day 2.

![Graph](image)

* Figure 4. Mean time taken to find platform in Morris Water Maze separated by stress condition.

* LH is significantly different from control ($p < .05$).

**Open Field Test**

In order to investigate hyperactivity and anxious behavior, a mixed factorial ANOVA was calculated comparing the number of squares entered in the open field test, and in other
calculations comparing what percentage of rats entered the middle of the open field test.

Statistical analysis on the number of squares entered revealed a significant repeated measures effect of day of testing, $F(3, 87) = 4.73, p < .01$, and a significant main effect of condition, $F(2, 29) = 4.10, p < .05$. Analysis did not reveal any significant interactions between condition and drug, day of testing and condition, day of testing and drug, or day of testing by condition by drug, and also did not reveal a significant main effect of drug (all $p$'s > .05). Subsequent analysis on the effect of condition on the number of squares entered revealed that LH animals entered a significantly greater number of squares than control animals on day 2 ($p < .05$), a marginal effect on day 3 ($p = .073$), and day 7 ($p < .01$). In addition, the CMS animals also entered a significantly greater number of squares on day 7 than control animals ($p < .01$).

![Graph](image)

* Figure 5. Mean number of squares entered in the open field test separated by stress condition.

* LH is significantly different from control ($p < .05$). # LH is marginally significantly different from control ($p = .07$). † CMS is significantly different from control ($p < .05$).
Discussion

The purpose of the current study was to first replicate the results of many previous studies in which the chronic mild stress animals decrease their sucrose consumption thereby reestablishing the reliability of the chronic mild stress model of depression in our laboratory. Second, this study aimed to compare chronic mild stress and learned helplessness on various behavioral measures such as sucrose consumption, weight changes, spatial memory, and anxiety. I hypothesized that the CMS animals would decrease sucrose consumption, but that the shock session would have no impact on the sucrose consumption of the LH animals. I also predicted that the CMS animals would only show slight reductions in weight gain and no impairments in spatial memory or anxious behaviors. The LH animals were predicted to show deficits in spatial working memory and anxious behaviors in the open field test. The last hypothesis was that imipramine would be able to prevent the behavioral symptoms typically characteristic of both of these animal models of depression. The results of the current study show many complicated relationships some of which support the hypotheses and some of which do not.

Unfortunately, the CMS animals injected with saline did not show any significant decreases in sucrose consumption over the course of the six weeks of experimentation. There was no significant main effect of condition on the sucrose data collected in this study. There are several reasons as to why this could have possibly occurred. A study conducted by D’Aquila et al. (1997) yielded interesting results with regards to the effects of time of day on sucrose consumption. They found that those animals tested for sucrose consumption during their light cycle showed no significant differences in consumption from control animals while those animals tested during their dark cycle did show significant decreases (D’Aquila et al., 1997). Therefore, it is entirely possible that the current study did not find any significant differences in
sucrose consumption because the consumption tests were conducted during the light. However, previous research does not necessarily support the claim that sucrose testing is effective during the dark cycle and not during the light cycle. While some studies have found significant decreases in sucrose consumption during the dark cycle (Monleon et al., 1995; D’Aquila et al., 1997; Azpiroz et al., 1999), a study conducted by Murison and Hansen (2001) actually found significant increases in the sucrose consumption of CMS animals when they were tested during the dark cycle. In addition, many other studies have found significant decreases in sucrose consumption despite testing during the light cycle (Matthews et al., 1995; Forbes et al., 1996; Zurita et al., 2000; Duncko et al., 2001; Grippi et al., 2003). Therefore, the inability of the current study to reproduce decreases in sucrose in CMS animals is more likely not caused solely by sucrose testing conducted in the light cycle.

Another possible explanation for our inability to replicate previous studies in which CMS caused decreases in sucrose consumption is that the current study did not use food deprivation in the CMS regimen. Two studies conducted by Matthews et al. (1995) and Forbes et al. (1996) claim that decreases in sucrose consumption are the result of the food deprivation procedures which cause substantial weight loss in the CMS animals. The current study did not find any decreases in sucrose consumption nor was there any weight loss in CMS animals and the two previously mentioned studies would claim that this is because the food deprivation procedures were not employed. Many of the studies that were successful in finding decreases in sucrose consumption following the CMS procedure did in fact utilize food deprivation for varying amounts of time from 3 to 23 hours prior to testing (D’Aquila et al., 1994; Papp et al., 1994; Matthews et al., 1995; Monleon et al., 1995; Forbes et al., 1996; D’Aquila et al., 1997; Azpiroz et al., 1999). However, several studies that utilized the food deprivation procedures for periods of
time well within the above range failed to find significant decreases in sucrose consumption (Murison & Hansen, 2001; Konkle et al., 2003). CMS studies have also been conducted successfully with a robust decrease in sucrose consumption observed without the use of food deprivation (Zurita et al., 2000; Dunčko et al., 2001; Grippo et al., 2003). In addition, our own laboratory was previously successful in using the CMS procedure without food deprivation. Therefore, it would appear that food deprivation is also not the sole reason for the current study’s non-significant sucrose data.

Given the difficulties that the our laboratory has had in reproducing the anhedonic decrease in sucrose consumption, it seems appropriate to consider that the chronic mild stress model of depression, though seemingly valid, lacks substantial reliability as an animal model of depression. While it is difficult to accurately gauge the number of laboratories that have had difficulties in producing significant decreases in sucrose consumption because few of these studies are ever published, it lends great support to the unreliability of CMS that its creator, Willner, admits in his 1997 review that his own laboratory began having difficulties with sucrose consumption after moving from London to Swansea. He also cites several other laboratories such as those of J. Hagan, C.K. Nielsen, and M.-H. Thiebot who have also had significant difficulties with the procedure (Willner, 1997c). Despite Willner’s best attempts he has failed to understand the variability in sucrose data. There are several hypotheses presented such as it could be the result of the strain of rats used, the animal supplier, the preferences of certain strains of rats for concentrations of sucrose solutions, the duration that the rats are housed singly before the beginning of experimentation, and several other conjectures (Willner, 1997c). While any number of these hypotheses may be the reason for the unreliability of the CMS model, it supports the view that small details are largely important for this particular model of depression and any
number of elements could cause this model to not produce accurate results. Though our laboratory has tried to control all extraneous variables, we have still not been able to discover why the procedure stopped working after 2002. One of my hypotheses as to why CMS did not work in our lab is possibly that the age of the animals makes a large difference. The animals in the current study were 7 weeks old at the beginning of experimentation while the animals used in our last successful CMS study were approximately 45 weeks old. This difference suggests that possibly young rats react differently to the CMS procedure than adult animals. Unfortunately, the studies that utilize that CMS procedure have not reported the ages of the animals at the beginning of experimentation. Thus, at the present time this hypothesis is unable to be verified.

Perhaps one of the most clear and surprising findings in the current study was the impact of imipramine on weight gain. When the average weekly weights of the imipramine animals and saline animals are compared, imipramine had no effect on the weight differences between conditions, but a significant main effect on weight and the differences in weight between the two groups was significant beginning the first week after drug administration began. This result came as a great surprise given that much research with humans in the past has revealed that imipramine causes quite a bit of weight gain. Berken, Weinstein, and Stern (1984) studied the effects of imipramine and other tricyclics on body weight and appetite in 40 depressed individuals who were 42 years of age on average. They found that 40% of those patients taking imipramine gained at least 5 pounds over the six month period that was studied (Berken et al., 1984). Further evidence is provided from a study conducted by Sussman, Ginsberg, and Bikoff (2001) which compared the effects of nefazodone and imipramine on weight. This study revealed that imipramine caused a significant increase in weight gain (more than 7% of original body weight) compared to nefazodone in both the short term and long term (Sussman et al., 2001).
Given these two studies, it is surprising that our study revealed such a robust effect of imipramine reducing the amount of weight gain. However, a careful review of past research reveals another study that is applicable to the current experiment. A study conducted by Quinn and Rapoport (1975) examined hyperactive boys who were being treated with imipramine and looked at their heights and weights before and after a one year treatment. They converted the height and weight measures into percentiles and found that the children taking imipramine lost approximately 7.6 percentile points in weight and 2.2 percentile points in height indicating that imipramine did not necessarily cause weight loss, but rather interfered with the natural growth of the children (Quinn & Rapoport, 1975). The animals used in the current study were relatively young and certainly still growing toward their adult size and weight. Therefore, given this previous study, it is very likely that imipramine caused the reduction in growth during the six weeks that they were being injected. Additional evidence is provided by Broitman & Donoso (1978) who investigated the effects of imipramine on mothers and their developing litter. In this study they gave imipramine to virgin female rats before they were impregnated and while they were nursing their litters, and then observed the effects that it later had on their developing litters. They found that the litters of mothers who had been exposed to imipramine had suppressed developmental increases in weight (Broitman & Donoso, 1978). This study provides further evidence of the harmful effects of imipramine on developing rats and children in that it can significantly reduce weight gain during critical phases of development.

The current study predicted that CMS animals would not show any significant deficits in the Morris water maze while the LH animals would show impairments in spatial learning. Our results revealed that CMS animals did not perform significantly differently from control animals in the Morris water maze on any of the four days of behavioral testing. One possible explanation
for this lack of effect is that spatial memory deficits are not known to be associated with depression. Thus, it would be a good sign that CMS did not cause spatial memory deficits. However, it is difficult to make any connections between depression and spatial memory in this particular case since I failed to obtain significant sucrose consumption data which is thought to indicate the presence of depression. It is possible that the reason I did not find any significant differences between CMS animals and control animals is because the CMS animals were not really depressed. Other studies using mild stress such as Hölscher (1999) who used handling stress found that it impaired spatial working memory in the Morris water maze. Similarly, Grauer and Kapon (1993) who used a breed of rats genetically predispositioned to stress and depression found that they had difficulties in the acquisition of the Morris water maze task as well. Therefore, it would seem that mild stress can cause impairments in spatial working memory even though it failed to here with the chronic mild stress model.

I also predicted that the LH animals would show some deficits in the Morris water maze due to the more severe nature of the stressor. After post hoc tests, it appears that the LH animals performed significantly slower in the Morris water test than control animals on day 1 of testing but this difference disappeared by day 2. This pattern is interesting especially in the context of some previous research. Warren et al. (1991) used inescapable shock and a Morris water maze in order to investigate the effects of shock on spatial memory. They found that after a session of 100 tail shocks the animals did not show any deficits in spatial working memory regardless of whether they were tested 30 minutes after the shock session or 6 hours after the shock session (Warren et al., 1991). Vollmayr et al. (2004) examined the impact of congenital learned helplessness on the learning ability of rats in a Morris water maze and found that the helpless animals and control animals showed similar acquisition of the task indicating that shock did not
cause a deficit in spatial learning. Some evidence for the impairment of spatial memory is found in a study conducted by Frisone, Frye, and Zimmerberg (2002). This study investigated the relationship between stress, spatial memory, and age of the rats. They chose two different groups of rats, adults (12.5-14 weeks old) and juveniles (3 weeks). The juveniles were subjected to social isolation stress for seven days and the adults were subjected to restraint stress for seven days before both groups were tested in the Morris Water Maze for spatial learning. Frisone et al. (2002) found that seven days of stress was enough to produce impairment in spatial learning in the juvenile rats, but not in the adult rats. Since the current study did result in some impairments in spatial memory for the adult learned helplessness animals, it indicates that inescapable shock should possibly be considered a more severe stressor than restraint and social isolation. In contrast, chronic mild stress was not a severe enough stress regimen to cause spatial working memory deficits. This draws a clear division between chronic mild stress and learned helplessness. Both claim to have mild stressors that induce depression, however learned helplessness causes impairments in spatial memory while chronic mild stress does not. Perhaps most relevant to the current experiment is a study conducted by Park, Campbell, and Diamond (2001) who used the stressor of predator exposure which could be considered to be as harmful to the animal as inescapable shock. This study found that predator exposure had a transient effect on spatial learning which was recovered later on in training (Park et al., 2001). This seems very similar to the deficits found in the current study. The learned helplessness animals were found to have impairments in spatial memory, but only on the first day of testing. This provides evidence to the assertion that learned helplessness, though it produces symptoms similar to those seen in depression, does not show many effects that last longer than approximately 24 hours (Personal Communication: J. Bruce Overmier). This has been one of the problems with the learned
helplessness paradigm as it produces symptoms that mirror depression in humans but do not last the proper length of time to actually be diagnosable by the DSM-IV-TR (Willner et al., 1992).

Another rather prominent finding from the current experiment is that the learned helplessness animals entered significantly more squares in the open field test on days 2, 3, and 7 than control animals. All of the animals entered a comparable number of squares on day 1 of behavioral testing. However, after day 1 the control animals appeared to habituate to the open field apparatus and entered significantly less squares per day. On the other hand, learned helplessness animals entered approximately the same number of squares each day of testing indicating that they were responding each day as if the environment were novel. This effect is supported by the research of Park, Campbell, and Diamond (2001). Although they used the severe stressor of predator exposure instead of inescapable shock, they found that the stressed animals failed to acclimate to the open field test and exhibited the same amount of motor activity on both periods of testing (Park et al., 2001). Therefore, one possible explanation for the increased activity among the learned helplessness animals is that they fail to acclimate to the new surroundings and therefore continue to act as if each day in the open field test is their first exposure. Further evidence for hyperactivity resulting from inescapable shock is provided by Westenbroek, Ter Horst, Roos, Kuipers, Trentani, and den Boer (2003) who found that animals exposed to chronic shock exhibited significant increases in open field activity. Similarly, Vollmayr et al. (2004) found that congenital learned helpless animals were significantly more hyperactive in the first five minutes of the open field test, but began responding comparable to control animals during the last ten minutes of observation. Since the current experiment only observed the animals for three minutes in the open field, this certainly falls within the hyperactive range of time found by Vollmayr et al. It is possible that had the open field test been
run for longer than five minutes, the learned helplessness animals may have habituated and come
to respond in a similar manner to the control animals. The results of the current study along with
those of previous research are not completely unexpected given that one of the symptoms of
depression is either psychomotor retardation or agitation (DSM-IV-TR, 2000). The finding in the
current study that learned helplessness animals show hyperactivity in the open field test at least
for three minutes lends support to the assertion that learned helplessness causes psychomotor
agitation and is truly a model of depression. The hyperactivity of the learned helplessness
animals is in excess to what control rats exhibited on days 2, 3, and 7 of testing indicating that
this effect lasts at least one week following a session of inescapable shock.

There are many elements of the current experiment that could have led to some of the
non-significant results and many things that could be improved upon for future research. The
most notable error in our study was an unexpected difficulty with the light-dark cycle. For the
first two weeks of habituation the lights failed to turn off at 7:30 pm as they were supposed to.
Therefore, the animals were exposed to light continuously for approximately two weeks. This
certainly was an extreme stressor on all 36 animals and could have caused irreparable harm to
the data collected during this experiment. However, the differences noted between the animals
on the various behavioral measures could still represent some true differences given that all
animals were equally stressed. Another possible source of error in this study could be the fact
that a total of three researchers performed the IP injections on the animals. Two researchers were
present each day of injections. The animals were split in half so that each researcher injected 18
animals. One of the researchers was present on all days of injections and rotated between which
half of the animals she injected. The other two researchers rotated on a schedule of every other
day in assisting with injections. It is possible that the various researchers caused varying amounts
of stress to the animals when they injected them and this could have had some deleterious effect on the collected data.

Another possible downfall of the current study is that I did not check to make sure the learned helplessness animals actually experienced an escape deficit. In an effort to keep all behavioral tests the same among animals, I decided that it would be an additional stressor on the learned helplessness animals to have an additional shock session which would distinguish them from the CMS and control animals. However, I also could not apply the escape task across all of the animals in the experiment because the CMS and control animals had never been exposed to shock and this would have been an extreme stressor for them. Therefore, the current study decided to eliminate the escape testing and instead observe the behavioral effects that resulted from the uncontrollable shock session regardless of whether or not the animals actually developed and escape deficit. This does present an issue with regards to the validity of our learned helplessness results, and therefore a future study should perhaps utilize the escape testing to ensure that a significant portion of the animals are actually experiencing the helplessness syndrome that Seligman and Overmier discovered.

Though the current study and previous research in this lab have not been able to reproduce the decrease in sucrose consumption associated with the CMS model of depression, there is still hope that this model could be a valid and useful model of depression. Its construct, face, and predictive validities are extremely promising as is the duration of its symptoms and onset of antidepressant action. The theoretical rationale behind CMS is that mild everyday stressors can build up over the course of time and cause depression. This model could provide an alternative explanation of why some individuals are depressed when they cannot identify a particular traumatic event that caused the depression and find their depression is not biochemical
in nature. If a reliable measure could be discovered to represent the anhedonia that develops in the animals, this measure could still prove to be of great use to the clinical community. Future research should investigate alternate measures of anhedonia such as place preference conditioning and Intracranial Brain Stimulation. Previous studies have found that CMS causes decreases in responding to pleasurable stimuli and it would be interesting to investigate whether the lack of reliability in CMS stems from sucrose testing or the CMS procedure as a whole. Another possible realm to explore is the effect of age on the progression of the CMS procedure as mentioned previously. Future studies should examine the differences in how juvenile and adult animals react to the CMS procedure. Additionally, it is also possible that something needs to be altered within the CMS procedure in order to produce more robust results. Future research should investigate the stressors involved in the CMS procedure and also look for alternate stressors which may increase the reliability of the procedure as a whole.

Another avenue that should be pursued in the future is looking at the clinical efficacy of the antidepressants and their ability to reverse the behavioral effects of CMS and LH. The current study examined the preventative ability of imipramine which does not parallel a clinical setting. Humans are not prescribed antidepressants ahead of time in order to prevent the onset of depression. Instead, they are given these drugs once symptoms of depression appear. Future studies should address this fact and attempt to examine how effective antidepressants are at reversing the symptoms produced by CMS and LH. In addition, research should utilize other antidepressants than imipramine when studying animal models of depression such as Selective Serotonin Reuptake Inhibitors and Monoamine Oxidase Inhibitors to ensure that they also reverse the behavioral effects of the two models of depression.
References


