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A Rat Model of Cognitive Bias and the Effect of Acute Corticosterone

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

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ABSTRACT

In humans, affective states can influence cognitive processes, resulting in a phenomenon referred to as “cognitive bias”. Rodents exhibit similar biases during the interpretation of ambiguous cues. It has been shown that cognitive bias shifts towards the negative valence (pessimism) when animals are under chronic stress manipulations. However, the effects of acute stress on cognitive bias have not been well established in an animal model. Here, a non-operant appetitive task using fluid rewards and distinct visual/tactile cues was developed to examine cognitive bias in male rats. Corticosterone was used to mimic stress levels similar to acute restraint stress. It was shown that under basal conditions, rats exhibited a positive cognitive bias (optimism), and that acute corticosterone administration resulted in a shift towards negative cognitive bias (pessimism). In all, this thesis provides a novel animal model to examine cognitive bias and the effects of acute stress manipulations.

Keywords

Cognitive Bias, Affective Disorders, animal model, corticosterone

CO-AUTHORSHIP STATEMENT

This section defines the contribution from various authors for the work described in chapters 2, 3, and 4.

Chapter 2 and 3: K.Wang, K.P. Ossenkopp, M. Kavaliers. “A translational model of cognitive bias and the effect of acute corticosterone.” Article in preparation for submission to PLOS One.

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Kai Wang primarily conducted all the study design, experimental work, data analysis, and the interpretation of the results. Dr. Klaus-Peter Ossenkopp and Dr. Martin Kavaliers further contributed to the project design, manuscript editions, and general study supervision.

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1 Thessalonians 5:16-18

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1 INTRODUCTION AND LITERATURE REVIEW

1.1 Affective Cognition

Human emotional states have a major influence on cognitive processes, specifically in the way information is processed and decisions are made. For example, when we are feeling angry, we may lash out and act irrationally. Cognition can be understood as the way by which we process and act on the information from the environment (Paul, Harding, & Mendl, 2005). This process involves many neurobiological processes, ranging from sensory perception, associative learning, and cognition. Emotions, on the other hand, are associated with physiological and neural mechanisms that allows humans to consciously and subjectively experience the “feeling” of the emotion. In both human and non-human animals, emotions can: i) structure perception, ii) direct attention, iii) provide preferential attention to certain functions and meaning, and iv) alter judgements and decision making. “Affect” is a term commonly used interchangeably with emotion, and while some researchers will give the two terms distinct meanings, this thesis will be using them synonymously.

Cognition and emotions are strongly linked. In particular, how information with ambiguous emotional overtones can be perceived by an individual is determined by their tendencies towards specific emotional valence (ie. positive or negative mood) during cognitive processing (Richter et al., 2012). Studies of perception and memory in humans showed that the processing of emotionally salient information has an integral role in the production of appropriate cognitive responses (Elliott, Zahn, Deakin, & Anderson, 2011; Schwarz, 2010). For example, individuals are likely to overestimate the likelihood of positive, and to underestimate the likelihood of negative outcomes if they are in a happy (or positive) mood, with the reverse occurring for individuals who are in a sad mood (see Nygren, Isen, Taylor, & Dulin, 1996). This propensity to base cognitive evaluations and decisions on the associated emotional tendency (e.g. “How did I feel about this?”) is

specifically termed “affective cognition”. It can be defined as the interface by which emotional and cognitive processes integrate to generate behavior (Elliott et al., 2011).

1.1.1 Positive and Negative Cognitive Biases

When mood valences (i.e. positive or negative mood) bias the way emotionally ambiguous information is cognitively interpreted and processed, the resultant changes in affective cognition are called “cognitive biases”. Under these emotional influences, affective cognition can lead to either positive or negative cognitive biases. As such, this fluid relationship between affect-influenced expectation and actual outcome is one of the defining features of cognitive bias. Positive and negative cognitive biases occur as the result of a difference between the expectation and the resultant outcome. If the expectation is more positive than the outcome, the bias is termed “positive cognitive bias”; the opposite is true if the expectation is more negative than the outcome.

In positive cognitive bias, there is typically a significant overestimation of the likelihood for positive events and underestimation of negative events (Sharot, 2011). Such trends can be seen in novel situations requiring probability evaluation and choice making. Positive cognitive bias is one of the most prevalent biases documented and is typically used synonymously with the term “Optimism bias”. In general, healthy humans display an overall positive cognitive bias when facing an ambiguous situation. For example, we typically underestimate our chances of being in a car accident, or having a serious illness. At the same time, we might overestimate our likelihood of winning the lottery, or success in our respective career paths. It has been proposed that positive cognitive bias occurs because a positive affective state typically calls on positive memories in thinking and decision-making. An individual who is happy can retrieve thoughts about the positive aspects of a neutral situation more readily, leading to a more favorable evaluation and behavioral outcome (Isen, 2001; Nygren et al., 1996). As well, because having a positive cognitive bias is also associated with a motivation to maintain this positive state, individuals tend to be more conservative in choosing riskier options when there is a potential of loss (Arkes, Herren, & Isen, 1988). Therefore, positive emotions interact with

cognition during evaluations of emotionally ambiguous information to create and maintain a positive cognitive bias. A positive cognitive bias has a highly beneficial effect on physical and mental health by decreasing stress and anxiety, reducing recovery time after major illness, as well as promoting a healthy lifestyle (Kivimäki et al., 2005; Sharot, 2011). It is widely accepted that positive cognitive bias is advantageous in comparison to an unbiased cognition.

Results of studies on positive cognitive bias have shed light on the mechanisms and outcomes of its opponent, negative cognitive bias (used synonymously with the term “pessimism”). This is crucial in the healthcare field because of one large population of humans who do not show positive cognitive bias: those with stress related neuropsychiatric mood disorders (i.e. depression and anxiety disorders). Clinical depression and anxiety are common stress-related psychiatric disorders that are associated with impaired interpersonal abilities, extensive emotional distress, and significant psychological impairments (Ingram, Trenary, Odom, Berry, & Nelson, 2007). These are considered two of the most common affective disorders, and the disruption of affective processing constitutes a core aspect of these psychiatric disorders. While positive cognitive bias is a common trait in healthy humans, a negative cognitive bias seems to take over in these mood disorders. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM V), negative cognitive bias (or “pessimism”) is one of the key symptoms of Major Depressive Disorder (MDD). A seminal study by Strunk *et al.* (2006) correlated individuals’ optimism and pessimism levels with their scores on the Beck Depression Inventory-II (BDI-II) test, a highly reliable and valid measure of depressive symptoms (Strunk, Lopez, & DeRubeis, 2006). A higher BDI-II score indicated a greater severity of depressive symptoms. It was found that healthy individuals, who scored low on the BDI-II, have a generally positive prediction of the future, or an “optimism bias”. On the other hand, individuals who scored in the middle of the BDI-II scale exhibited more of an unbiased cognition, and individuals who scored high on depressive symptoms exhibited a negative cognitive bias, or often referred to in literature as “pessimism bias” (Strunk et al., 2006).

It has been well established that a negative affective state alters thinking processes in reaction to certain stimuli, and presents a major factor in the maintenance and etiology

of affective disorders. In fact, there is a strong correlation between negative affective state and a high risk of relapse/recurrence of depression (Beck, Rush, Shaw, & Emery, 1979; Bouhuys, Geerts, & Gordijn, 1999; Kloke et al., 2014). Depressed individuals display a high level of hopelessness and a distorted negative perception of self/others, the world, and their future (Beck et al., 1979; Beck, Weissman, Lester, & Trexler, 1974). They tend to have a more pessimistic outlook/attitude in comparison to healthy controls, in addition to superior efficiency and thoroughness when they process negative information (Beck, Riskind, Brown, & Steer, 1988; Dozois & Dobson, 2001). For example, these individuals displayed a faster response toward negative faces during emotional categorization tasks where the valence of affective stimuli, positive or negative facial expressions, were presented (Yoon, Joormann, & Gotlib, 2009). They would also interpret ambiguous statements (e.g. “That’s an interesting choice of outfit”) and ambiguous homophones (e.g. die/dye, pain/pane) in a more negative (“pessimistic”) manner than the controls (Eysenck, MacLeod, & Mathews, 1987). During interpretation of ambiguous scrambled sentences (e.g. “winner born I am a loser”), depressed individuals would perceive the more negative interpretation (as “I am a born loser”) rather than the positive (“I am a born winner”) (Amir, Beard, & Bower, 2005; Wells & Matthews, 1996).

While positive/negative affective state and optimism/pessimism, respectively, are often used interchangeably in literature, it is understood that they are independent and are sometimes used specifically. Optimism/pessimism are key indicators of positive and negative affective states respectively, but these affective states does not exclusively lead to optimism or pessimism. However, since the purpose of this thesis is to examine affective state in terms of affective cognition, these two terms will be used interchangeably here.

1.2 Evaluating Affective States in Non-Human Animals

Evaluations of affective states in humans relies mostly on subjective language-based measures, such as self-reporting or verbal communication. However, such direct measurements of these subjective experiences are not applicable in animals. Currently methodologies for evaluating affective states in animals, specifically rodents, have largely been based on extrapolations of behavioral and physiological measures. By utilizing specific “indicators” of stress and anxiety, these methods have been effective in evaluating the intensity of emotion, and the level of arousal in the animals. Common evaluations have included observations of approach/avoidance behaviors (considered to be indicative of anxiety-like behaviors and basic emotions) and measurements of stress markers associated with hypothalamic-pituitary adrenal (HPA) axis activity (Paul et al., 2005).

Based on the widely-accepted belief that animals do experience sufferings or pleasure, these indicators are important in animal welfare research, as affective states are typically used as representations of animals’ well-being (Mendl, 2008). For instance, animals can grow in what appears to be perfect physical health, yet still subjectively “suffer” from the lack of mobile space or stable housing. Thus, due to the nature of the field, most of the animal welfare studies have focused on examining improved animal welfare by studying indicators of a positive affective state. Environmental enrichment is a widely accepted method to enhance positive affect states in laboratory animals. Some commonly used environmental enrichment manipulations typically included adding physical resources (e.g. play wheels, chewing wood blocks) or cognitive stimulations (e.g. introducing cage-mates to increase social interactions; Simpson & Kelly, 2011). Behaviorally, it has been found that animals (specifically rodents) demonstrate superior ability to perform and learn tasks, and show a decrease in poor welfare behaviors (i.e. aggression). These results can be attributed to either the positive stimulations or the prevention of the development of negative emotions (for review see Boissy et al., 2007).

However, the use and interpretation of these measures is limited and difficult in animals, as they cannot always give an accurate depiction of emotional valence (i.e. whether the animal is in a positive or negative affective state). For instance, HPA axis activity is often measured by examining the level of plasma corticosteroids and other

markers as an index of stress, where higher levels of these markers indicate elevated stress. However, these markers tend to represent fleeting changes or symptoms, and thus incorrectly indicate long term sufferings when they are actually in a temporary state of distress (Rushen, 1986). Another drawback to this method is the fact that HPA axis activity can increase in either positive (e.g. sexual reproduction) or negative (e.g. confronting a predator) situations, making an accurate assessment of the emotional valences difficult (Michael Mendl, Burman, Parker, & Paul, 2009). Therefore, since the relationship between emotional states and physiological/behavioral measures are not always clear, the extrapolation and interpretation of these measures to infer an emotional state is typically limited and unreliable. Considerations must be given to alternative methods to measure affective states in animals. A more objective and standardized method is needed in order to examine emotional processes and to clearly elucidate specific changes in animals' affective states.

1.2.1 Applications of Cognitive Bias in Non-Human Animals

With the increasing understanding of cognitive bias through human studies, animal welfare research found that it can be used in non-human animals as well. From human studies, it has been well established that as an indicator for affective states, cognitive bias allows for discrimination between emotional valences (i.e. positive and negative moods). This presents a valuable and clear evaluation of how specific emotion can affect cognition and behavior. Thus, cognitive bias can be assessed by utilizing the anatomical, physiological, and behavioral similarities in cognitive and affective processes between humans and common laboratory animals. As part of animal welfare research, changes in cognitive bias have been examined on chronic scales as an index of affective states in animals (such as Brilot et al., 2010; Burman et al., 2009; Harding et al., 2004; Salmeto et al., 2011). Cognitive bias has been studied in a wide range of species, such as in honeybees (Bateson, Desire, Gartside, & Wright, 2011), chicks (Salmeto et al., 2011), starlings (Brilot et al., 2010), dogs (Kis, Hernadi, Kanizsar, Gacsi, & Topal, 2015; Michael Mendl et al., 2010), rhesus macaques (Bethell, Holmes, MacLarnon, & Semple, 2012), and rodents (Burman et al., 2009). A common method used to establish cognitive bias is to first train

the animals to distinguish between two reference conditions, a “positive condition” and a “negative condition”. These two reference conditions are typically represented by distinct and opposite cues (e.g. color, sound, tactile) that are reinforced with specific positive or negative outcomes (e.g. highly rewarding chocolate versus aversive quinine-soaked food pellets, etc). Once the animals have learned the two distinct conditions, they are then subjected to different affective manipulations, such as environmental enrichment or unstable housing. These conditions are designed to elicit either positive or negative mood in the animals. Animals are then exposed to a third “ambiguous” condition where the cues are intermediate to the trained reference conditions. The animals’ responses to these cues are taken as indicators of their mood. Operationally, a negative (or “pessimistic”) response would show an increased likelihood of responding to the ambiguous cues with a prediction of a negative outcome, and a positive (or “optimistic”) response would be opposite. It has been proposed that a further breakdown of a response indicative of a negative affective state may entail an increased anticipation of the negative outcome, and/or a decreased anticipation of the positive outcome (Bateson et al., 2011; Matheson, Asher, & Bateson, 2008).

These studies have shown that while the baseline affective state is represented by a positive cognitive bias, negative affective states tend to shift it to a negative cognitive bias (Chaby et al., 2013; Papciak, Popik, Fuchs, & Rygula, 2013). Animals undergoing chronic mild stress related manipulations have shown a shift in their interpretation of ambiguous stimuli towards a more negative manner. These manipulations have included events such as unstable housing (i.e. Unpredictable light/dark cycles, sudden noises, irregular food and water delivery, etc.) and the absence of environmental enrichment (i.e. isolation). For example, rats chronically housed in unstable housing have shown a decreased response to an ambiguous stimulus that signaled a positive outcome (Harding et al., 2004). As well, rats subjected to social isolation showed a decrease in anticipation for a highly palatable sucrose reward that persisted up to 3 months after the cessation of the social isolation (Von Frijtag et al., 2000). Taken together, these results suggest that chronically stressed rats show a shift towards a negative cognitive bias by exhibiting increased expectation of a negative outcome, or a decreased expectation of a positive outcome. This further confirms the application of cognitive biases as indices of affect state in animals.

1.2.2 Development of a Cognitive Bias Model in Rodents

The development of an animal model that could effectively elucidate these subjective changes in cognitive bias is still in its infancy. Harding et al. (2004) were the first to examine how affective state influences cognitive bias in rodents. Chronic mild stress was used to induce a negative affective state, and the manner in which it changed rats' responses to ambiguous stimuli was examined. They adopted a go/no-go task format, where rats either had to make an active choice in response to one stimuli, or to refrain from taking an action in response to a different stimulus. Specifically, rats were trained to press a lever when they heard a tone associated with a positive event (receive a food pellet), which represented the positive condition. In contrast, during the negative condition, the rats had to ignore the lever to avoid a negative event (white noise) when they heard a different tone. Once the appropriate responses were learned, the rats were placed in unpredictable housing environments for a prolonged period of time to elicit a mild "depression"-like state. This included random daily interventions that were aversive to rats, such as damp bedding and reversal of light/dark cycles. The rats were then exposed to unfamiliar ("ambiguous") tones that had frequencies intermediate to the two training tones. The animals demonstrated negative or positive cognitive biases that gave indications to their affective states. Chronically stressed rats showed slower response times, as well as lower responses to tones that were closer to the positively reinforced (food related) tone, indicating a negative cognitive bias, or "pessimism" (Harding et al., 2004). On the other hand, control rats that did not receive the stress manipulations displayed a positive cognitive bias. This paradigm was able to successfully show how pessimistic rats tend to have a negative interpretation of ambiguous events, which was partly consistent with that of depressed or anxious humans. Therefore, it demonstrated the potential to be adapted for assessing both the positive and the negative affective states in animal welfare. In order to further understand the underlying neurobiological basis of cognitive bias in affective neuropsychiatric disorders, there is a need to develop a reliable and objective non-human animal test.

Although this work by Harding et al. (2004) in the development of a cognitive bias task allowed for the interpretation of non-human animals' affective states, there was no

evidence for an enhanced anticipation of the negative event, which is another key indicator of a negative affective state. Further, the nature of this original go/no-go task presents a problem in the interpretation of the results, where it is difficult to distinguish go or no-go as an active response or an active omission. A go-go task was suggested to resolve these problems, as it requires an active response from the animals to both the positive and negative stimuli. This would give clear indications of specific responses that correspond to either a positive or negative cognitive bias. Subsequent studies have built upon the initial Harding et al (2004) study and used variants of food-reward paradigms to further develop animal models of cognitive bias. For example, Matheson, Asher, & Bateson (2008) developed a choice procedure in the form of a go-go task. European starlings were first trained to discriminate between 2 different lengths of light cues as the positive (2 seconds) and negative (10 seconds) conditions. Two distinct colors were used at two separate choice buttons that birds could peck to receive reinforcements. During the positive condition, instant food delivery was given if the bird pecked at one choice button, and delayed food delivery was given if the bird pecked at the other choice button during the negative condition. Then, the birds were placed into enriched and unenriched housing environments for 2 weeks, followed by the evaluation of their affective states. During the evaluation, they were exposed to light cues ranging between 2 and 10 seconds as the intermediate “ambiguous” stimuli (e.g. 5 seconds). A positive cognitive bias was indicated by the bird choosing to peck at the colored button associated with the positive condition (instant food delivery) following the ambiguous light cue. Results showed that non-enriched birds had a lower association of the intermediate light duration to the positive outcome (instant food delivery). In comparison, the enriched birds showed a higher expectation of the positive condition when the ambiguous cue was given. These results demonstrated a shift in cognitive bias following affective manipulations towards the positive valence. Further, because animals had to respond actively to both the positive and negative stimuli to receive food rewards, the ambiguity of whether there were any reductions in motivation and general activity was eliminated (Matheson et al., 2008). These results, along with a number of ensuing studies (Chaby et al., 2013; Kloke et al., 2014; Mendl et al., 2010; Salmeto et al., 2011, etc), not only established the effectiveness in using cognitive bias, but also confirmed the value of a go-go task format to discriminate distinct emotional valences.

Although the use of cognitive bias in animal welfare has provided a valuable and objective measure of animals' affective states, they've primarily been used with chronic manipulations to mimic welfare-related issues for animals in captivity. In comparison, very little research has been done on short-term mood manipulations, such as acute stress challenge. Burman and colleagues (2009) used a food reward choice procedure and spatial location cues in an eight-arm radial maze to examine the effect of short-term emotional state manipulation on rats. Only five arms were used, where two reference arms contained the positive and negative goals, flanked by 3 ambiguous locations. The reward location (black circle) contained highly palatable food pellet, whereas the aversive location (white circle) contained a pellet soaked in quinine (aversive to rats). The locations were paired with high or low light levels. After the rats learned to differentiate between the two reference locations, they were allowed access to only the three middle locations. Light intensity was used to induce short-term stress during this stage, where brighter light intensities generated more anxiety than darkness. This led to differential reactions towards the ambiguous stimuli. It was found that increased anxiety (brighter lights) led to negative cognitive bias, and a decrease in the speed by which the rats reach the goal. However, it was also found that, while the rats demonstrated clear differences in their cognitive bias under different anxiety levels (ie. brighter light inducing higher stress levels), the locations in the open arms maze were too easily distinguishable by the rats. Specifically, the angles by which the arms were placed in relation to each other may have presented the three middle locations similarly as containing unknown outcomes rather than distinct ambiguous locations (Burman et al., 2009). It has been suggested that an open-arena may resolve this issue (Burman et al., 2009; Richter et al., 2012). This study confirmed the use of cognitive bias in evaluating changes in affective states following acute manipulations, in addition to chronic manipulations typically seen before.

In response to this development, Brydges and colleagues (2011) were the first to adopt a semi-open arena paradigm containing olfactory cues to model a shift toward an increase in optimism as result of environmental enrichment in rats. Rats were first trained to associate a specific coarseness of sandpaper with specific olfactory and visual cues on positive and negative trials. In the positive trial, coarse sandpaper was paired with a chocolate reward (high reward value) in a cinnamon-scented bowl on the left side of the

open arena. On the other hand, in the negative trial, fine sandpaper was paired with a less-rewarding cheerio in a coriander-scented bowl on the opposite side of the open arena. When the rats learned to distinguish between the positive and the negative trials, their responses toward an intermediate sandpaper coarseness (ambiguous stimuli) were assessed before and after one week of environmental enrichment. While the unenriched rats stayed in the original cages, the enriched rats were moved to larger cages that had deep wood shavings and a slew of physical enrichment tools (e.g. cardboard tubes, cardboard houses, and wooden blocks). It was found that both enriched and the unenriched rats showed similar levels of optimism (choosing the location previously paired with the chocolate) pre-treatment. However, the enriched rats showed significantly more optimistic responses after environmental enrichment. In comparison, the control rats (no environmental enrichment) maintained a baseline level of optimism throughout the experiment. These results demonstrated that chronic positive manipulations can induce a shift towards a more positive affective state, further confirming the advantage of using cognitive bias to tease out emotional valences. More importantly, these results also suggested the effectiveness of developing a task in an open arena to evaluate cognitive bias (Brydges, Leach, Nicol, Wright, & Bateson, 2011).

In all, because animal welfare issues tend to exist on a long term scale with consistent mild negative experiences, animal models of cognitive bias have been based on a chronic time scale. To date, there is still very little research on the effect of acute stress in cognitive bias, but it needs to be examined in order to further our understanding of the neurobiological basis for cognitive bias (Chaby et al., 2013; Papciak et al., 2013). An open arena, when combined with ambiguous cues, has shown to be effective in portraying positive cognitive bias in rats following chronic manipulations. Thus, it would be worthwhile to examine the effects of acute stress manipulation on cognitive bias with an ambiguous cue go/go task in an open arena.

1.3 Rationale, Hypothesis, and Objectives

Therefore, the rationale for this thesis was founded upon three major premises:

1. **Stress and anxiety have a negative effect on cognitive bias, leading to pessimistic behavioural outcomes in chronic stress studies.**
2. **There is currently very little research on the effects of acute stress on cognitive bias**
3. **The development of the cognitive bias animal model is still in its infancy.**

The present paradigm used highly salient spatial location cues to model cognitive bias in adult rats. These stimuli (texture and visual information) greatly increased the ease by which rats learned to discriminate between the positive and negative contextual visual/tactile cues (see Brydges N. M., 2011 for a similar group approach). This paradigm also overcame a key limitation from previous cognitive bias models by using an open testing arena to ensure that the rats avoid viewing all the ambiguous choice spouts similarly, as one collective “ambiguous outcome”, but instead viewing them as distinct ambiguous choice locations (Burman et al., 2009). More importantly, unlike the initial go/no-go tasks, this paradigm required active responses to the ambiguous contextual visual/tactile cues, eliminating the difficulty of interpreting no-go responses. Therefore, in view of the limitations of prior investigations of cognitive bias, a new animal model that could be rapidly utilized was developed using rats. This first involved the establishment of a task that can be used to show changes in cognitive bias in response to ambiguous cues. In addition, the effects of an acute stress challenge by the administration of a physiologically relevant level of corticosterone, was examined. **We hypothesized that an acute stress challenge will lead to a shift in baseline positive cognitive bias to a negative cognitive bias.**

Specifically, the cognitive bias paradigm was adapted from that of Brydges et al (2011) to model changes in affective states following acute manipulations. The first objective (chapter 2) was to develop a cognitive bias paradigm using visual and tactile cues paired with an appetitive/ingestion task. This task was designed in an open arena apparatus to examine changes in the rats’ behaviors following exposure to ambiguous visual/tactile

cues, and to establish the validity of such a task in evaluating cognitive bias. Rats learned to associate specific visual and tactile cues with either reward/neutral or neutral/aversive outcomes presented in lickometer spouts. Rewarding and neutral reinforcements was used because they have been found to be the most ideal for detecting positive affect (Mendl et al., 2009). Cognitive bias was measured by the response of the rats during ambiguous trials, which consisted of the presentation of intermediate visual and tactile cues. The proximity association between the ambiguous choice spouts to the previously paired reference locations (higher or lower reward value outcomes) allowed the generalized interpretation of the choices to be that of “optimistic” or “pessimistic”. The second objective (chapter 3) was to demonstrate that similarly valenced, but distinct, emotional states may be differentiated through this paradigm. Here, the rats’ responses to the environmental cues following an acute stress challenge was evaluated using the intraperitoneal administration of a physiologically relevant level of corticosterone. In chapter 4, the two components of the ambiguous condition, ambiguous environmental cues versus ambiguous choice location cues, were separated to examine whether they work together or independently during the evaluation of cognitive bias shifts. It was predicted that under ambiguous conditions, rats without any affect manipulations would display a tendency towards positive cognitive bias in our apparatus. As well, with an acute “stress” manipulation (corticosterone injection), the baseline optimism would shift towards pessimism.

1.4 References

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2 DEVELOPMENT OF A COGNITIVE BIAS TASK

2.1 Introduction

There is a close relationship between cognition and emotions of affective states. The way information is perceived by an individual is determined by their underlying tendencies towards specific emotional valence (i.e. whether individual is in a positive or negative mood) during cognitive processing (Richter et al., 2012). When underlying emotional valences bias the way ambiguous information is interpreted and processed, the resultant changes in affective cognition is termed “cognitive bias” (Hales, Stuart, Anderson, & Robinson, 2014). If the expectation is more positive than the outcome, the bias is a positive cognitive bias; conversely, it is a negative cognitive bias if the expectation is negative. In humans, the evaluation of affective cognition and cognitive bias is largely based on verbal self-reporting, with such information being generally accepted as an accurate indicator of affective states. In general, healthy humans display an overall positive (optimistic) cognitive bias when facing an ambiguous situation, where the likelihood of positive events are overestimated and the likelihood of negative events underestimated (Sharot, 2011). This baseline positive cognitive bias has also been widely established in animal models as part of animal welfare research. By utilizing the anatomical, physiological, and behavioral similarities in cognitive and affective processes between humans and common laboratory animals, one can assess the underlying processes in cognitive bias using non-human animals (for discussion see section 1.1.3). However, the development of animal models to examine changes in affective states are still in its infancy. So, in order to further understand the underlying neurobiological basis of cognitive bias in affective neuropsychiatric disorders, there is a need to develop a reliable and objective non-human animal test.

The examination of affective states in animals has been established since the pioneering work by Harding, et al, 2004, mostly as part of animal welfare research by using chronic stress manipulations. Typically, a task used to evaluate cognitive bias first establishes two distinct reference conditions, a “positive condition” and a “negative

condition”. These two reference conditions are typically indicated by distinct and opposite cues (e.g. color, sound, tactile) and specific positive or negative reinforcements (e.g. highly rewarding chocolate versus aversive quinine-soaked food pellets, etc). Once the animals have learned to distinguish between the two reference conditions, they are then subjected to different affective manipulations, such as environmental enrichment or unstable housing. These conditions are designed to elicit either positive or negative mood in the animals. Animals are then exposed to an “ambiguous” condition where the cues are intermediate to the trained reference conditions. The animals’ responses to these cues are taken as indicators of their mood. A negative (or “pessimistic”) response will show an increased likelihood of responding to the ambiguous cues with a prediction of a negative outcome, and a positive (or “optimistic”) response would be the opposite. It has been proposed that a response indicative of a negative affective state may entail an increased anticipation of the negative outcome or punishment, and/or a decreased anticipation of the positive outcome (Bateson et al., 2011; Matheson et al., 2008).

Using this general framework, studies have shown that while the animals’ baseline affective state is represented by a positive cognitive bias, negative affective states tend to shift cognitive bias to a negative valence in response to ambiguous stimuli (Burman et al., 2009; Hales et al., 2014; Harding et al., 2004; etc). For example, Harding et al (2004) developed a cognitive bias task that allowed for the interpretation of rodents’ affective states following chronic stress manipulations. However, the nature of this original go/no-go task presents a problem in the interpretation of the results. Such a task creates difficulties in distinguishing go/no-go as an active response or an active omission. A go-go task would resolve this problem, as it requires an active response from the animals to both the positive and negative stimuli. As well, by requiring active responses for both stimuli, potential problems like motivation and general activity reductions could also be minimized (Matheson et al., 2008).

Ensuing studies have improved upon this pioneering work to further develop tasks of cognitive bias that can objectively elucidate animals’ affective state. Through these studies, it has been shown that cognitive bias can accurately show specific shifts in emotional valences (positive or negative affective states) from the baseline “optimism” that

is typically seen in humans. For example, environmental enrichment (i.e. adding physical play toys or social cage mates) shifts cognitive bias to a more positive valence than in the baseline. In contrast, unstable housing (i.e. irregular lighting schedule, damp bedding, etc) leads to a shift in cognitive bias towards the negative valence. Despite these advances, there are still a number of limitations for using cognitive bias to accurately measure affective states in non-human animals. One important issue is that animal welfare research typically studied changes in mood on a chronic scale, where affective manipulations (e.g. environmental enrichment or stressful housing) occur on the scale of weeks to months (Burman, Parker, Paul, & Mendl, 2008). In contrast, there is relatively little research on the effects of acute stress on shifts in affective states. One such study was done by Burman and colleagues (2009), where a go-go task in an eight-arm radial maze was used to examine the effect of short-term emotional state manipulation. Ambiguous cues at specific locations were used to test changes in cognitive bias. While rats did demonstrate shifts in affective states under different anxiety levels (ie. brighter light inducing higher stress levels), it was found that the locations in the open arms maze were too easily distinguished by the rats. Thus, it was suggested that an open arena might be used for testing (Burman et al., 2009; Richter et al., 2012).

Therefore, in order to examine the effect of acute stress challenge on cognitive bias shift, a novel rat model of cognitive bias using visual and tactile cues in an open arena apparatus was first developed. Ambiguous visual/tactile cues were used to test shifts in cognitive bias. In groups, rats first learned to associate specific visual and tactile cues with either a positive or a negative condition. Rewarding and neutral enforcements were used because they have been found to be the most ideal to elicit positive affect (Michael Mendl et al., 2009). Cognitive bias was then measured by the response of the rats during ambiguous trials, which consisted of the presentation of intermediate visual and tactile cues. It was hypothesized that without any affect manipulations, rats would display a positive cognitive bias in the ambiguous testing cue task.

2.2 Materials and Methods

2.2.1 Animals

Twelve adult male Long-Evans rats (Charles River, Quebec, Canada), weighing between 300 - 450g at the start of the experiment were used. The rats were pair housed in standard polypropylene cages (45cm × 22cm × 20cm), in a colony room with ad libitum access to both food (ProLab RMH3000 rat chow) and tap water when applicable. The colony room was maintained at $21 \pm 2^\circ\text{C}$, on a 12:12 hour light/dark cycle with the lights on from 07:00 to 19:00 h. All experiments were carried out during the light phase. For the entire duration of the experiment, each rat was water deprived for 15 hours starting at the beginning of their dark cycle (starting at 19:00h), and then given one-and-a-half-minute access to tap water at the end of the water deprivation period. The rats were observed during the one-and-a-half-minute water access to ensure that each rat drank from the water bottle. This method was used so that rats would be motivated to explore and drink from the liquid reinforcements that were used to indicate differences between positive and negative conditions. Each rat was given identification markings on their tails using a permanent marker (Black Sharpie pen). All procedures were performed according to the Canadian Council on Animal Care guidelines and were approved by the Western University Animal Care Committee.

2.2.2 Cognitive Bias Apparatus

The apparatus consisted of a rectangular start box (20cm x 20cm) attached to a rectangular arena (100cm long, 90cm wide, 25cm height) made of clear Plexiglas with a transparent lid, set on top of a white board (fig. 2.1). A manually operated transparent guillotine door opened into the arena from the start box. This apparatus was set up in a designated testing room, on a table 1m above floor level. The wall colors of the open arena were created using white or black cardboards, attached to the outside of the clear Plexiglas. Plastic lighting sheets (2'×2' Replacement lens for Metalux Recessed Troffer, Cooper

Lighting, GA, USA) were used to line the floor of the arena to create rough floors, and the smooth back of the sheets were used to create smooth floors. All behaviours were videotaped with a video camera positioned approximately 1.5 m directly above the apparatus on the ceiling.

Five automated lickometers, each consisting of a stainless steel spout attached to a glass graduated drinking tube, were used (Contact 108 lick analysis system, Dilog Instruments, Tallahassee, FL). The ends of the drinking spouts were mounted 5cm above the arena floor. The 5 spouts were evenly spaced 15cm apart from each other, as well as from the sides of the apparatus, on the opposite side to the start box (Fig. 2.1 and 2.2). The two outer spouts were designated “reference locations”, and were used during the training stage (described in section 2.2.3). Each spout was centered on a specific colored cue card that could be changed to give different visual cues. The spouts were accessible through an oval opening, only big enough for the rats’ tongues without altering the natural facial movements of drinking. To monitor the licks, a computer-controlled lickometer (DiLog instruments, Tallahassee, FL) passed a low, non-detectable current ($\sim 60\text{nA}$) through the spouts. The electric circuit was completed each time the rat’s tongue came into contact with the spout, and the signals were amplified before being recorded for licking measures (QLick, version 4.0). These recordings allowed for the analysis of the rats’ licking frequency. The volume of solutions consumed was also quantified by manually reading the changes in fluid amounts of the graduated drinking tubes.

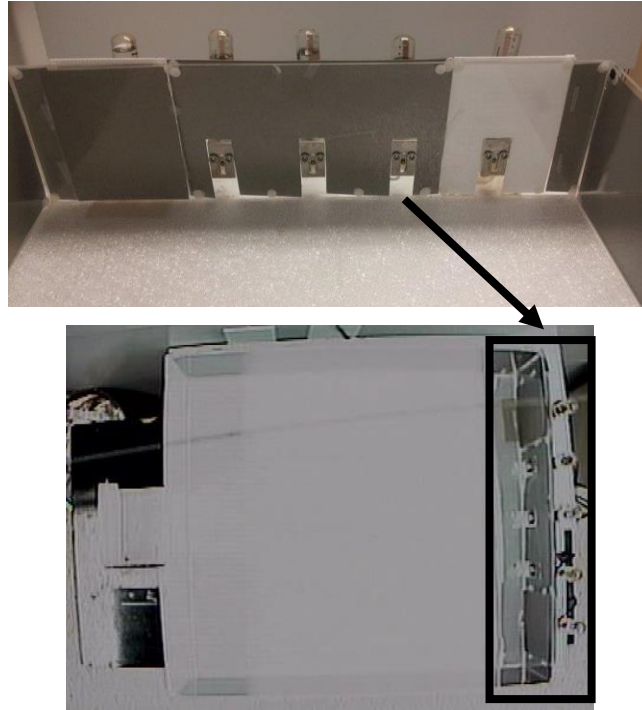


Figure 2.1. Photograph of the cognitive bias apparatus, five lickometer spouts (top) are evenly spaced apart on the far wall of the apparatus, each centered on a visual cue card.

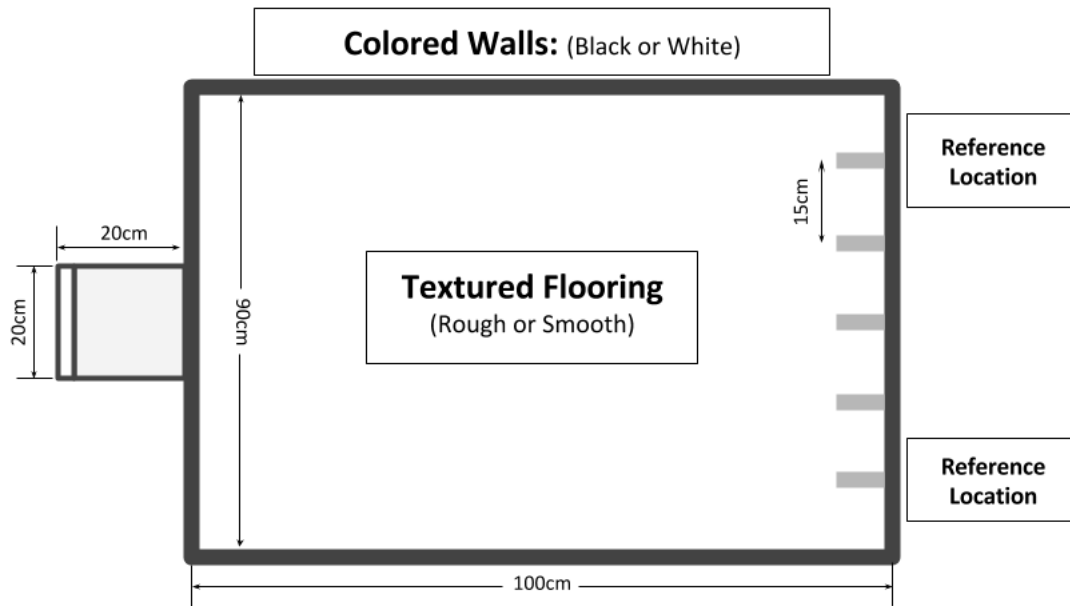


Figure 2.2. Diagram of the cognitive bias apparatus, showing the dimensions of the start box (20cm x 20cm) and the open testing arena (100cm long, 90cm wide, 25cm height). The light grey lines at the right side of the diagram indicate the locations of the lickometer drinking spouts.

2.2.3 Experimental Procedure

All of the procedures are summarized in figure 2.3. Details of each stage are provided below.

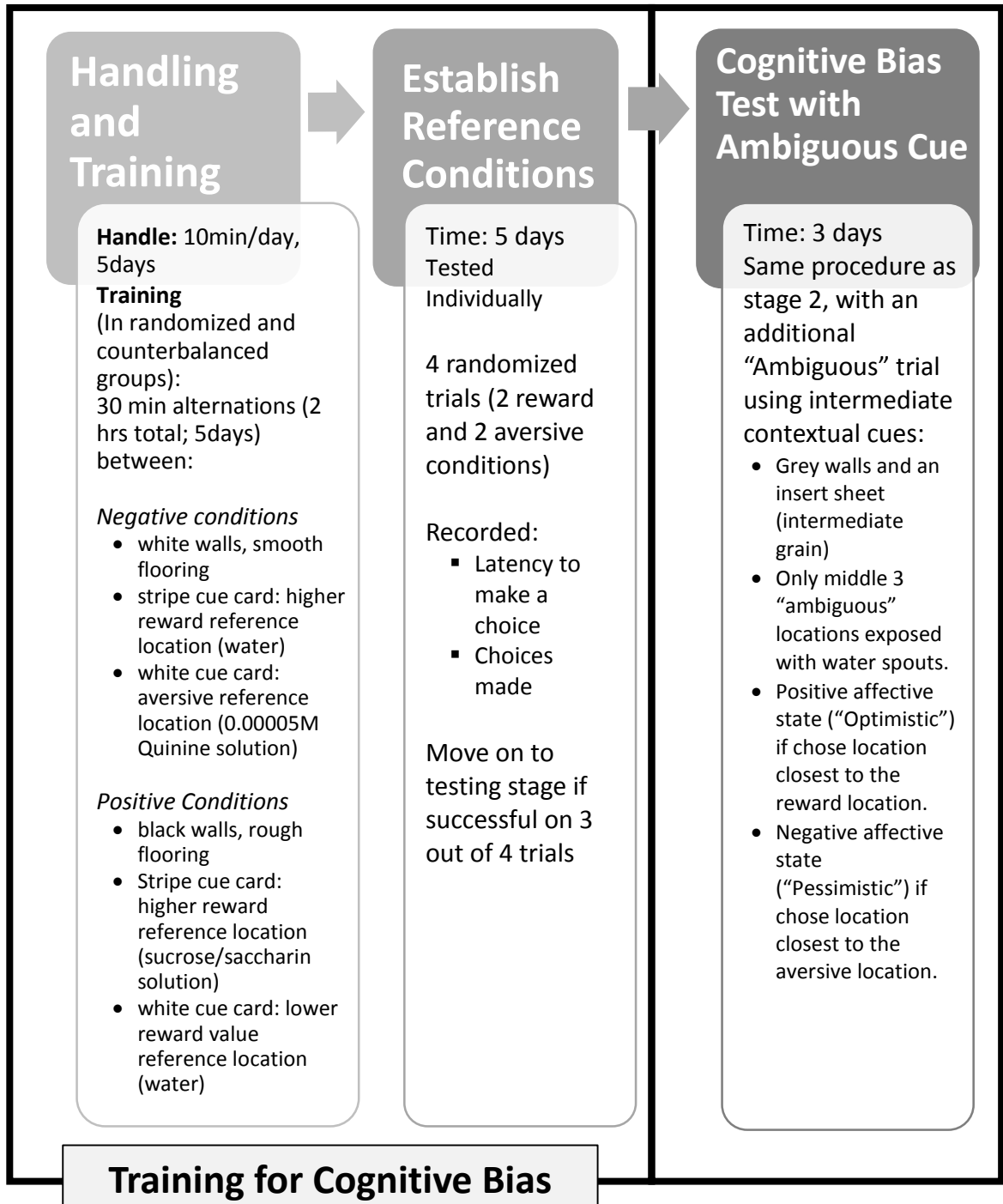


Figure 2.3. Outline of procedures for experiment 1.

2.2.4 Stage 1 – Handling and Training

During the first 5 days of the experiment, each rat was handled daily for 10 minutes during the light phase. 24 hours after the final handling day and for the subsequent 5 days, all of the rats were placed into the open arena in groups of 6 for training. Group learning facilitates the acquisition of cognitive tasks more effectively and efficiently than individual training (Krasheninnikova & Schneider, 2014). During this time, visual (black or white walls) and tactile (smooth or rough flooring) cues were placed in the arena to indicate the positive or negative condition. As well, visual cue cards (black-and-white stripe or white) were used to indicate the higher/lower reference location spouts, respectively. These two reference locations were located at the outermost 2 locations out of the 5 possible locations; they were 15cm from the sides of the goal box, and 60cm apart from each other.

The rats alternated between a “positive” condition and a “negative” condition for 30 minutes each, for a total of 2 hours a day in groups. The positive condition was represented by environmental cues consisting of black walls and a rough floor. Here, a highly palatable sucrose/saccharin solution (3% sucrose with 0.125% saccharin dissolved in distilled water) represented the higher reward-value choice location (associated with the black-and-white stripe visual cue card), whereas tap water represented the lower reward value choice location (associated with the white visual cue card; figure 2.4a). In contrast, the negative condition was represented by environmental cues consisting of white walls and a smooth floor. Here, a quinine solution (0.00005M) represented an aversive reference location (associated with the white visual cue card), whereas tap water represented a comparably higher value reference location (associated with a black-and-white visual cue card; Figure 2.4b). The concentration of quinine used in this task was a very low concentration, so that rats would find it aversive but not so much as to cause stress or major changes in appetitive behaviour. The reward and aversive reference locations were counterbalanced between rats to eliminate side-bias. The specific reference locations paired with a particular visual and tactile condition environment were consistent for each rat throughout the experiment (ie. sucrose/saccharin solution were always at the right side of the goal box).

On the other hand, the 3 middle locations (figure 2.4) were blocked off by a smooth board with a color that was the same as the walls, thus leaving only the 2 reference location spouts exposed. All rats in the group had free access to explore both reference locations (and the accompanying lickometer spouts) in the arena during this stage (figure 2.4). Since all rats were identified by an ID marking on their tails, their exploration and consumption of the various reference fluids were verified by video recording.

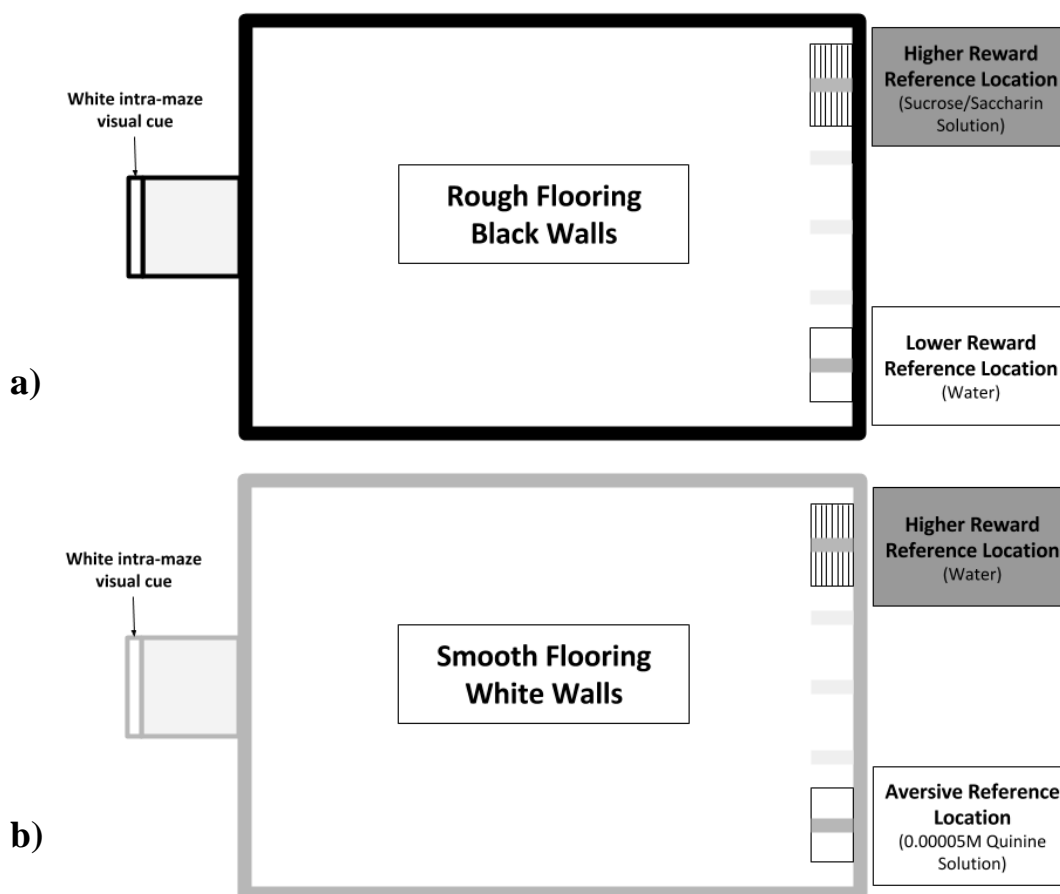


Figure 2.4. Diagram of training apparatuses in either positive (a) or negative (b) condition. a) The rough floor and black walls (positive) environment was reinforced with the higher value sucrose/saccharin solution (3% sucrose and 0.125% saccharin dissolved in distilled water) at the left side of the goal box (stripe cue card) and tap water on the right side (white cue card). b) The smooth floor and white walls (negative) environment was associated with the lower value water at the left side of the goal box (stripe cue card) and 0.00005M quinine at the right side (white cue card). The middle 3 locations (light grey lines) were blocked off by a card with the same color as the walls.

2.2.5 Stage 2 – Establishing reference conditions: Discrimination between positive and negative conditions

In order to determine whether the rats had acquired the task, 24 hours after the last training day, the rats were tested individually to see if they were able to determine the more rewarding location in the positive and negative conditions. In this stage, each rat individually received 4 consecutive trials in a randomized order (2 trials in the positive condition and 2 trials in the negative condition) each day for five days. Between each trial, the apparatus was cleaned thoroughly with 20% alcohol solution. The time taken (in seconds) for the rat to exit the start box and choose one of two reference locations was recorded. The specific reference location spouts that the rat chose and drank from was recorded as well. The rat was considered to have made a correct choice if it chose to drink from the reference location spout associated with the higher reward value outcome immediately after exiting the start box. For the positive condition, the correct choice was the sucrose/saccharin solution, whereas in the negative condition, the correct choice was the water. A rat was considered to be successfully trained and could advance to the next stage if it consistently made the correct choice at least 3 times out of the 4 trials over the 5 testing days.

2.2.6 Stage 3 – Cognitive Bias Testing with Ambiguous Cue

24 hours following the trials to test the establishment of cognitive bias, each rat individually received three consecutive days of cognitive bias testing with ambiguous cues. In this stage, the trials proceeded in the same method as the previous trials, but with one additional “ambiguous” trial included. During the ambiguous condition, an insert sheet, intermediate in texture to the two conditioned floor textures, was used as the flooring (Acrylic Lighting Panels Cracked Ice Clear, Plaskolite Inc., Ohio, USA). In addition, grey cardboard was attached to the walls of the apparatus to give an intermediate wall color (figure 2.5). These two conditions created an environment where the visual and tactile cues were intermediate to the ones used for the training trials (i.e. black walls/rough flooring for positive condition, white walls/smooth flooring for the negative condition).

During these trials, the individual rats were exposed to only the 3 middle “ambiguous” locations. The two reference location spouts (for the positive and negative context conditions) were blocked off by a card in the same shade of grey as the walls. The graduated drinking tubes contained water, and were placed in each of the three middle ambiguous locations (figure 2.5). Ambiguous position 1 (O1) was located 15cm to the right of the reward reference location, while the ambiguous position 3 (P3) was located 15cm to the left of the aversive reference location. Ambiguous location 2 (M) was in the middle, 30cm to either of the two reference locations. Measurements were made and recorded for the: i) time taken for the rat to exit the start box, ii) first choice from the three middle lickometer spouts. If the rat selected the O1 location, it was recorded as making a choice reflective of a positive cognitive bias; if the P3 location was chosen, it was recorded as a choice reflective of a negative cognitive bias (figure 2.5). 30 seconds after the rat cease to drink from their initial choice spout, they were removed from the apparatus. During this stage, each rat received 1 trial in the ambiguous cue condition plus 2 trials each of the previously trained positive and the negative conditions.

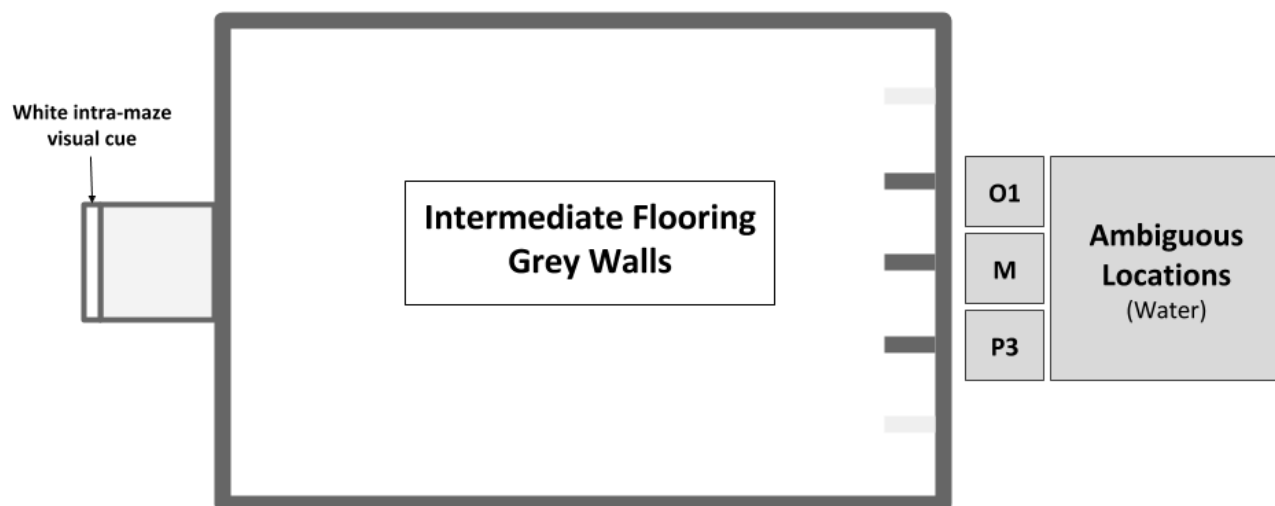


Figure 2.5. Diagram of experimental apparatuses in the ambiguous task. The smoothness/roughness of the flooring was in between the smooth and rough flooring of the training trials. The walls were a solid grey color. The two reference locations were blocked off and inaccessible using a smooth board with the same grey color as the walls. The drinking tubes of the ambiguous locations (dark grey lines) contained tap water. The O1 ambiguous location was located closest to the previously trained more rewarding location, whereas the P3 ambiguous location was located closest to the previously trained less rewarding/aversive location. The M ambiguous location was in the middle between the O1 and P3 ambiguous locations.

2.2.7 Statistical Analysis

In this experiment, all data generated were analyzed using SPSS (Version 21, SPSS Inc., Chicago, IL, USA). The treatment groups were equally counterbalanced across the conditions. For the training stage, the data for correctly and incorrectly choosing the higher reward value outcome for each condition were compared separately using the chi-square test, as this was needed to determine when the rats were ready to proceed to the next stage. The positive or negative choices across all three testing days during the ambiguous condition testing were also analyzed with the chi-square test of independence. The latency to approach choice spouts during the cognitive bias testing with ambiguous conditions were analyzed using one-way Analysis of Variance (ANOVA). Specifically, this measure compared the three ambiguous locations (O1, M, and P3). All significant effects and interactions were further examined using Tukey's HSD. A significance level of $p < 0.05$ was used throughout the experiment.

2.3 Results

2.3.1 Establish Reference Conditions

Performances during the individual trials to evaluate whether rats have learned to choose the more rewarding location in either positive or negative conditions, were combined across all training days for analysis (refer to section 2.2.5; figure 2.6). In the positive condition (sucrose saccharin solution/water and black walls/rough floor), the correct response was choosing to drink from the sucrose (more rewarding) location when the rat exited the start box. In the negative condition (water/quinine with white walls/smooth floor), the correct response was choosing to drink from the water (more rewarding) location first when the rat exited the start box. Chi-square test revealed significance between correct choices and incorrect choices, where rats made significantly more correct choices than incorrect choices in both the positive and the negative conditions ($X^2(1, n=12) = 81, p < 0.001$) (Figure 2.6).

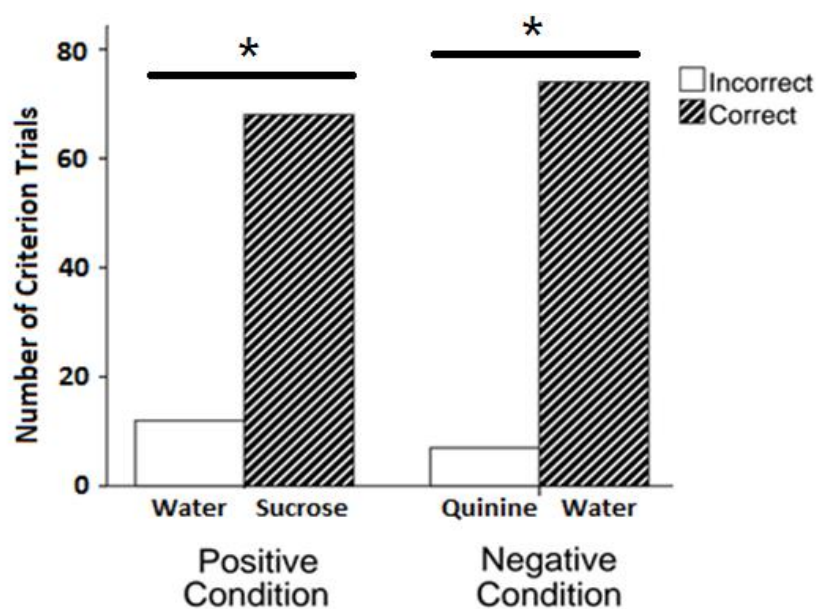


Figure 2.6. Comparison of choices that were correct vs. non-correct during the establishment of cognitive bias trials (described in section 2.2.5). “Number of Criterion trials” represent accumulation of all training trial results for all rats. Rats were able to successfully make the distinction between the choices locations by choosing the higher reward choice location in both positive (sucrose/saccharin vs. water) and negative (water vs. quinine) conditions across all five training days. * $p < 0.05$

2.3.2 Cognitive Bias Testing with Ambiguous Cues

In the ambiguous condition, there was significance between the choice for the optimistic location (O1) to the middle (M, $p < 0.01$) and the pessimistic location (P3, $p < 0.01$). Rats chose the O1 location significantly more than the P3 location (O1, $p < 0.01$, figure 2.7).

The mean latency from the time that the rat exited the start box until it made a choice was compared among the positive, negative, and ambiguous conditions across all three testing days (Figure 2.8). ANOVA analysis yielded a significant interaction between the latency and the condition. Further analysis revealed a significant difference for latency during ambiguous trials versus positive conditions ($F(2, 201) = 7.265, p < 0.01$), as well as with the negative condition ($F(2, 201) = 7.265, p < 0.05$). Rats took significantly longer time to make a choice in the ambiguous condition in comparison to the trained positive or negative conditions. There was no significant difference between time spent making a choice when the rat was exposed to the trained positive and negative conditions ($p > 0.05$). The ambiguous environmental and choice location cues presented in the ambiguous condition did have an effect on the choices made by the rat in the present paradigm.

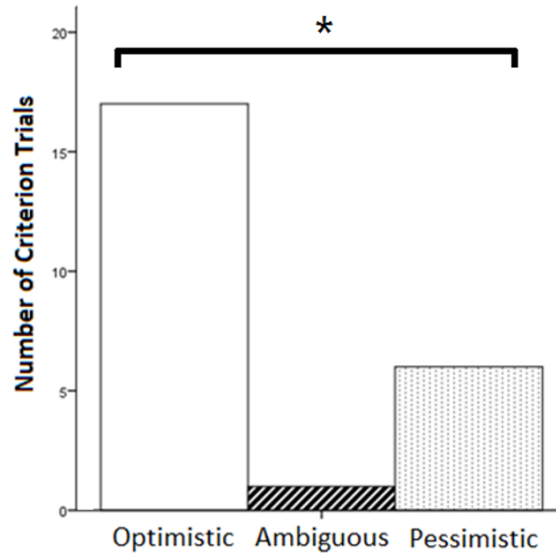


Figure 2.7. Choices in response to the ambiguous contextual (visual/tactile) cues. “Number of Criterion trials” represent accumulation of all training trial results for all rats. Choices made over three days of ambiguous cue testing are shown. There was a significant difference between the positive choices (choosing the O1 location first) and the negative choices (choosing the P3 location first). Rats chose the O1 location significantly more. * $p < 0.05$

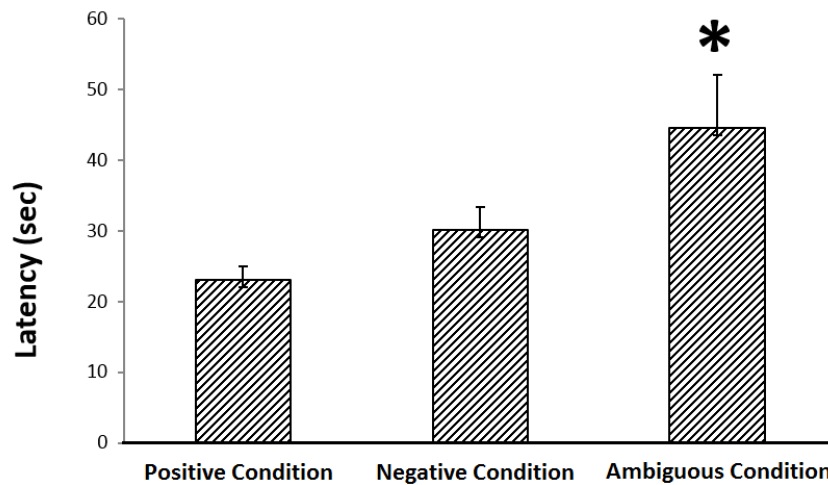


Figure 2.8. Latency of making a choice during the Ambiguous condition trials as compared to during cognitive bias training in positive/negative conditions (in seconds). In the trained conditions, higher reward locations consisted of sucrose and water as the enforcements, while lower reward value locations consisted of water and quinine instead. Error bars indicate S.E.M. * $p < 0.05$ (males: $n=12$).

2.4 Discussion

The aim of this experiment was to establish a relatively rapid task to examine cognitive bias in rats that offers advantages over previously established tasks. Here, solutions of different reward values were used in conjunction with different visual and tactile cues to provide indices of positive and negative cognitive biases. The results of using this task showed that adult male rats displayed a positive cognitive bias (optimism bias) when facing ambiguous cues under basal conditions.

Rats were first trained to discriminate between a positive and a negative condition (using wall color and textured flooring) that were reinforced with either higher reward value (water or sucrose/saccharin solution), or lower reward value outcomes (quinine solution or water) choice outcomes, respectively. After the rats demonstrated a clear ability to distinguish between the positive and the negative conditions, ambiguous cues were used to determine whether there were any changes in the underlying cognitive bias. Both environmental cues (grey walls and intermediate grain flooring), as well as choice location cues (grey visual cue cards) were used to create the ambiguous condition. The results of the present experiment showed that rats were able to accurately acquire the task under the absence of any significant behavioral manipulations (i.e. stress induction). They were able to correctly discriminate between the different environmental visual/tactile cues in the positive and the negative conditions by consistently choosing the higher reward choice spout to drink from. Upon further testing and analysis with the presentation of ambiguous cues (intermediate textured flooring and wall color), it was found that the rats preferentially chose the ambiguous location more closely associated with the rewarding reference location. This suggested that rats anticipated positive outcomes when they were exposed to affectively ambiguous cues, indicative of a positive (or “optimistic”) affective state. Such results are consistent with previous studies that used other tasks of cognitive bias in adult male rats and starlings, where it was found that an optimistic baseline typically exists in animals without any mood manipulations (Brydges et al., 2011; Matheson et al., 2008).

These results suggested that the current cognitive bias model can be a highly effective and valuable tool in the assessment of altered affective processing and emotional

valences (i.e. positive or negative mood). This paradigm has a number of advantages over previously established tasks. First, an open arena apparatus was adopted to overcome the limitations presented by previous studies. This ensured that the rats avoided viewing all the ambiguous choice locations similarly, as one collective “ambiguous outcome”, but instead viewed them as distinct ambiguous choice locations (Burman et al., 2009). Thus, when paired with fluid outcomes that have discrete reward values (sucrose/saccharin solution is highly palatable, water is neutral, and quinine solution is aversive), allowed the different locations to represent distinct significances to the rats. The proximity association between the ambiguous choice spouts to the previously paired reference locations (higher or lower reward value outcomes), allowed the generalized interpretation of the choices to be that of “optimistic” or “pessimistic”. Further, unlike the initial go/no-go tasks (such as the one presented by Harding, et al, 2004), this paradigm required active responses to the ambiguous contextual visual/tactile cues, eliminating the difficulty of interpreting no-go responses. As well, by requiring active responses to different conditions, problems such as motivation or locomotor reductions could be minimized (Matheson et al., 2008). In the Harding et al.’s task, if one result of the chronic stress manipulation was a decline in activity level or a decreased motivation to press the lever, the observed reduction in responses during the ambiguous condition could be confounded and inaccurate. This was improved upon by using a go-go task format in the current model.

Another advantage of the present task was that it allowed for quick cognitive bias testing in non-human animals without the requirement of complex processing and skills. Specifically, it used highly salient spatial location cues (tactile and visual information) to model cognitive bias in adult rats. These stimuli greatly increased the ease for rats to learn to discriminate between the positive and negative contextual visual/tactile cues (see Brydges N. M., 2011 for a similar approach). In addition, the cues and reinforcements used allowed for the potential to titrate changes in cognitive bias with different strengths of aversive (i.e. quinine concentration) and positive (i.e. sucrose/saccharin solution concentration) reinforcements. Variations in these parameters can help to tease out putative underlying mechanisms associated with the establishment of cognitive bias. Thus, the results of the present study demonstrated that similarly valenced, but distinct, emotional states may be differentiated through this paradigm.

It is important to note that while the majority of rats chose the O1 location, demonstrating a positive cognitive bias that is indicative of a positive affective state, there were some rats that chose to drink from the P3 location. This can be attributed to a number of variable factors. First, individual differences in responding to the task over the course of the experiment could have affected the acquisition and performance in the cognitive bias task. The rats were allowed to explore the apparatus for 30 seconds following the cessation of drinking from the choice spout to avoid potential conditioned pairing of specific spouts with handling. Since the ambiguous locations were all reinforced with water, the rats that went on to explore the other ambiguous choices would have found that all spouts gave equal outcomes, which may then potentially interfere with their responses in succeeding trials. However, the presentation of water as an outcome in the ambiguous choices could also have been a potential confounder in the ambiguous cue testing trial, depending on whether a positive or negative condition was proceeding it (Trials with ambiguous cues were administered in addition to a randomized order of positive and negative conditions). Thus, because the water was trained as either the higher reward value outcome in the negative condition or lower reward value in the positive condition, receiving water in the ambiguous condition could have led to a biased recall of conditioned choices in the ambiguous condition.

The differences in rats' performances in the ambiguous cue testing could also be attributed to the differences in acquisition of the task during the establishment of cognitive bias stage. Although all rats were observed to ensure drinking from the water spout following water deprivation, as well as drinking and exploring the lickometer locations during training, individual differences could have affected how well they acquire the task during these two stages. Further, although the use of group learning was adopted to enhance the acquisition of the task, the hierarchal social nature of rats allows for the possibility of stress associated with dominate versus subordinate roles. In all, the saliency and time-effective nature of the current task presented a promising method to further elucidate changes in cognitive bias in rats.

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3 THE EFFECT OF CORTICOSTERONE ON COGNITIVE BIAS

3.1 Introduction

Affective disorders such as depression and anxiety are serious wide-spread medical conditions that are complex and multi-dimensional, involving both genetic and environmental factors. At the same time, depression and anxiety are comorbid conditions, where symptoms of depression are often made worse by co-existing symptoms of anxiety. These symptoms include anhedonia (decreased reward value of highly palatable foods), irritability, sleep disturbances, nervous dread of the future, and psychomotor agitation (Gregus, Wintink, Davis, & Kalynchuk, 2005). In fact, pure depression without symptoms of anxiety occur infrequently, and have been widely shown to be related to stress. Stress is a physiological response to threatening or novel stimuli, characterized by the activation of the hypothalamic-pituitary-adrenal (HPA) axis. When the HPA axis is activated, it elicits a cascade of events leading to the elevation of adrenal glucocorticoids (Dinan, 1994). In humans, cortisol is the circulating glucocorticoid, while corticosterone (CORT) is the equivalence in other species, notably rats. A normal HPA response is necessary for survival as it maintains physiological homeostasis in the body. However, when the HPA axis is repeatedly activated, problems can arise in the body and the brain. For example, repeated glucocorticoid exposure can lead to downregulation of hippocampal regulatory pathways, leading to abnormal secretion of the hormone to the rest of the body (for review see Dinan, 1994). Thus, it is not surprising that depression and anxiety has been strongly associated with elevated stress responses, in particular an increase in HPA axis activation and cortisol levels.

One aspect of cognition that seems to be disrupted by this abnormal stress response takes shape behaviorally as cognitive bias. In fact, it has been well established that negative cognitive bias (or “pessimism”) is a key characteristic typically exhibited by stressed individuals (Beck et al., 1988; Strunk et al., 2006). There is a strong correlation between negative affective state and a high risk of relapse/recurrence of depression (Bouhuys et al.,

1999). These individuals tend to have negative views of self and others, as well as increased hopelessness and more negative thoughts/attitudes in comparison to healthy controls (Beck et al., 1988). For example, these individuals would interpret ambiguous statements (e.g. “That’s an interesting choice of outfit”) in a more negative (“pessimistic”) manner than the controls (Eysenck et al., 1987). As well, during emotional categorization tasks where the valence of affective stimuli (positive or negative facial expressions) were to be categorized, these individuals showed faster responses toward negative faces (Yoon et al., 2009). Such negative cognitive bias is not limited to humans. In fact, non-human animals, under chronic stress manipulations, have been shown to exhibit similar tendencies when facing ambiguous situations (Brydges, Hall, Nicolson, Holmes, & Hall, 2012; Burman et al., 2009; Harding et al., 2004). Therefore, there is great value in examining how negative affective state can affect behavior in animal models to further our understanding of the underlying neurobiological mechanisms of affective disorders. In the previous (chapter 2), a novel task was developed using an open arena with tactile and visual cues in an attempt to overcome some of the limitations presented by existing animal models. It was shown that healthy rats with no significant stress influences exhibited a positive cognitive bias, consistent with existing research. In the current study, we built upon this cognitive bias framework by adding an acute stress manipulation to examine whether it would lead to a similar shift to negative cognitive bias as that found in chronic stress studies.

There are a wide range of manipulations used in chronic mild stress studies that can elicit abnormal stress responses to shift cognitive bias. The most common methods in animal welfare research are the lack of environmental enrichment and restraint stress (Burman et al., 2009). However, while these methods do mimic the repeated stress exposure, they have not been able to produce consistent and robust changes in depression symptomatology (for review, see Zhao et al., 2008). This may be attributed to procedural differences between experiments and lack of control over individual differences in response to manipulations (Zhao et al., 2008). As a result, there may be variable corticosterone levels between animals when exposed to the same stressor, leading to the inconsistency of studies on stress. As well, chronically repeated restraint stress may allow for habituation to the adverse effects. For example, it has been shown that corticosterone levels in male rats declined over the course of 21 days following physical restraint, where

the levels are significantly lower on day 14 when compared to day 1 and 7 (Galea et al., 1997). One way to avoid these problem in traditional rat stress models is by using exogenous corticosterone administration to study the effect of elevated corticosterone levels. In the current study, in order to examine the effect of acute stress challenge on changes in cognitive bias, daily injections of exogenous corticosterone were administered for three days. The dose of corticosterone was chosen because it was shown to approximate physiological stress levels associated with aversive responses following physical restraint (Ossenkopp et al., 2011).

Thus, the present study examined the effects of physiologically relevant levels of corticosterone on cognitive bias. It was hypothesized that corticosterone administration would lead to a negative (pessimistic) cognitive bias when rats face ambiguous conditions. The ambiguous cues consisted of grey walls and intermediate grain flooring, with the 3 middle (“novel”) choice spouts open (previously inaccessible to rats). Whether the rats chose to drink from the spout that was more closely associated with the previously paired higher reward value outcome location, or vice versa for the lower reward value outcome location, would give insight on how an acute stress challenge could affect cognitive bias.

3.2 Materials and Methods

3.2.1 Experimental Procedure

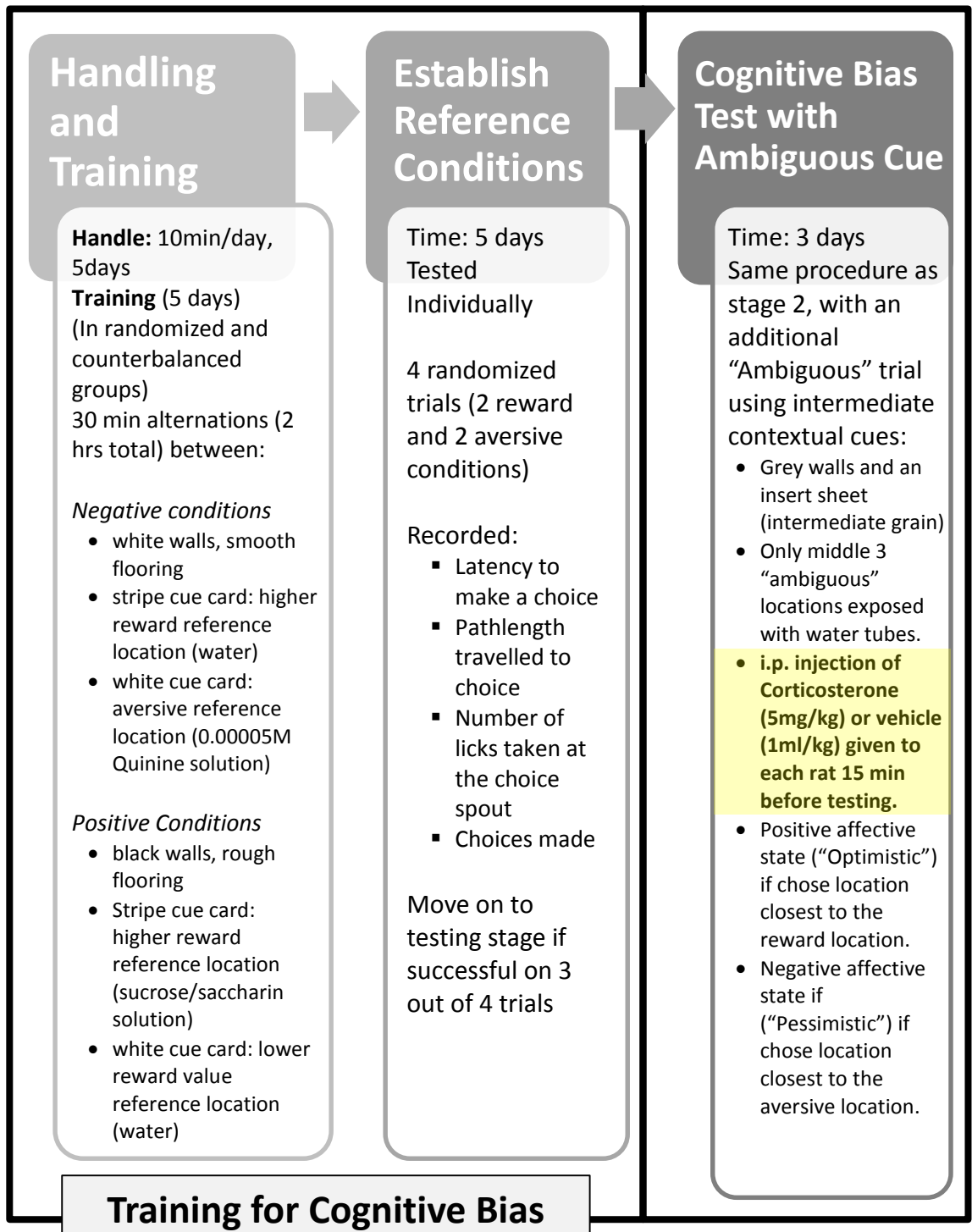


Figure 3.1. Outline of procedures for experiment 2. Procedural differences from experiment 1 are highlighted.

3.2.2 Animals

Twenty-three adult male Long-Evans rats (Charles River, Quebec, Canada), weighing between 300 - 450g at the start of the experiment were used. The rats were pair housed in standard polypropylene cages (45cm × 22cm × 20cm), in a colony room with ad libitum access to both food (ProLab RMH3000 rat chow) and tap water when applicable. The colony room was maintained at 21± 2°C, on a 12:12 hour light/dark cycle with the lights on from 07:00 to 19:00 h. All experiments were carried out during the light phase. For the entire duration of the experiment, each rat was water deprived for 15 hours starting at the beginning of their dark cycle (starting at 19:00h), and then given one-and-a-half-minute access to tap water at the end of the water deprivation period. The rats were observed during the one-and-a-half-minute water access to ensure that each rat drinks from the water bottle. This method was used so that rats would be motivated to explore and drink from the liquid reinforcements that were used to indicate differences between positive and negative conditions. Each rat was given identification markings on their tails using a permanent marker (Black Sharpie pen). All procedures were performed according to the Canadian Council on Animal Care guidelines and were approved by the Western University Animal Care Committee.

3.2.3 Drugs

Corticosterone (Cort; Sigma, Toronto, ON) was used as the acute stress challenge, as it is typically released internally by the rat as part of the hypothalamic-pituitary-adrenal (HPA) axis's adaptive response to stress (I. Z. Mathews, Wilton, Styles, & McCormick, 2008). CORT was dissolved in 45% hydroxypropyl-β-cyclodextrin to a dose of 5mg/mL. The vehicle (VEH) used in the control group was 45% hydroxypropyl-β-cyclodextrin. Corticosterone and vehicle were administered via intraperitoneal (i.p.) treatment at a volume of 1.0 mL/kg, 15 minutes before the start of the test. This Corticosterone administration procedure was used based on previous studies where it mimicked physiological levels of acute stress 15 minutes following the injection (Kent, Cross-Mellor, Kavaliers, & Ossenkopp, 2000).

3.2.4 Cognitive Bias Apparatus

The apparatus consisted of a rectangular start box (20cm x 20cm) attached to a rectangular arena (100cm long, 90cm wide, 25cm height) made of clear Plexiglas with a transparent lid, set on top of a white board (fig. 3.2). A manually operated transparent guillotine door opened into the arena from the start box. This apparatus was set up in a designated testing room, on a table 1m above floor level. The wall colors of the open arena were created using white or black cardboards, attached to the outside of the clear Plexiglas. Plastic lighting sheets (2'x2' Replacement lens for Metalux Recessed Troffer, Cooper Lighting, GA, USA) were used to line the floor of the arena to create rough floors, and the smooth back of the sheets were used to create smooth floors. All behaviours were videotaped with a video camera positioned approximately 1.5 m directly above the apparatus on the ceiling.

Five automated lickometers, each consisting of a stainless steel spout attached to a glass graduated drinking tube, were used (Contact 108 lick analysis system, Dilog Instruments, Tallahassee, FL). The ends of the drinking spouts were mounted 5cm above the arena floor. The 5 spouts were evenly spaced 15cm apart from each other, as well as from the sides of the apparatus, on the opposite side to the start box (Fig. 3.2 and 3.3). The two outer spouts were designated “reference locations”, and were used during the training stage. Each spout was centered on a specific colored cue card that could be changed to give different visual cues. The spouts were accessible through an oval opening, only big enough for the rats' tongues without altering the natural facial movements of drinking. To monitor the licks, a computer-controlled lickometer (DiLog instruments, Tallahassee, FL) passed a low, non-detectable current (~60nA) through the spouts. The electric circuit was completed each time the rat's tongue came into contact with the spout, and the signals were amplified before being recorded for licking measures (QLick, version 4.0). These recordings allowed for the analysis of the rats' licking frequency. The volume of solutions consumed was also quantified by manually reading the changes in fluid amounts of the graduated drinking tubes.

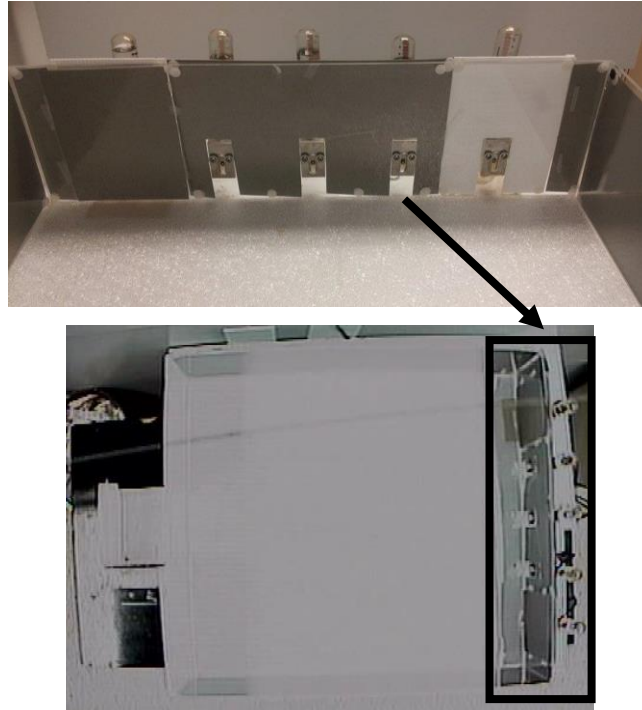


Figure 3.2. Photograph of the cognitive bias apparatus, five lickometer spouts (top) are evenly spaced apart on the far wall of the apparatus, each centered on a visual cue card.

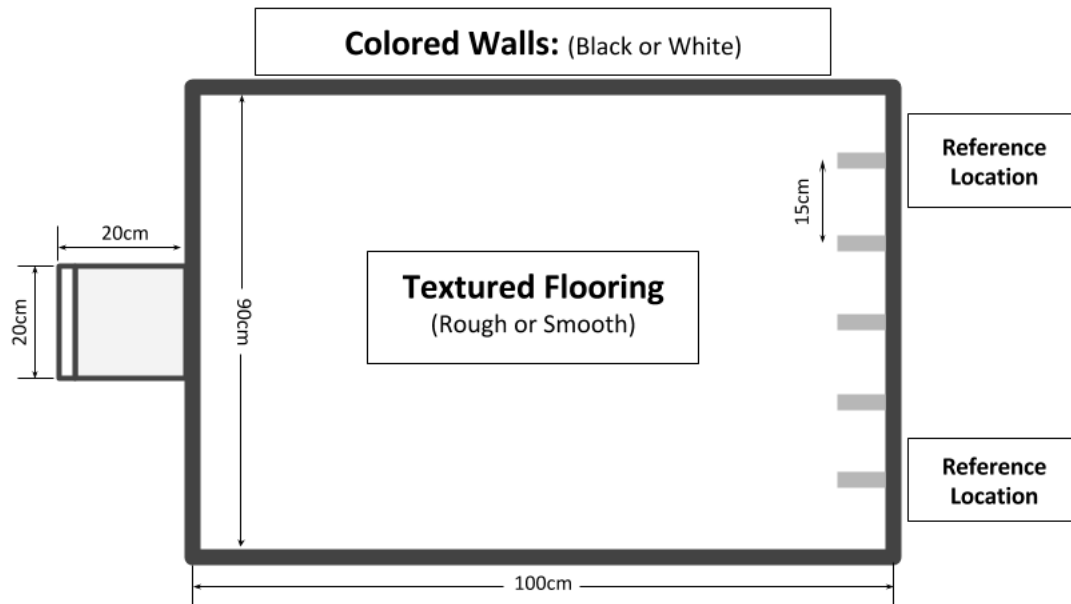


Figure 3.3. Diagram of the cognitive bias apparatus, showing the dimensions of the start box (20cm x 20cm) and the open testing arena (100cm long, 90cm wide, 25cm height). The light grey lines at the right side of the diagram indicate the locations of the lickometer drinking spouts.

3.2.5 Training and the establishment of reference conditions

During the first 5 days of the experiment, each rat was handled daily for 10 minutes during the light phase. 24 hours after the final handling day and for the subsequent 5 days, all of the rats were placed into the open arena in groups of 6 for training. Group learning facilitates the acquisition of cognitive tasks more effectively and efficiently than individual training (Krasheninnikova & Schneider, 2014). During this time, visual (black or white walls) and tactile (smooth or rough flooring) cues were placed in the arena to indicate the positive or negative condition. As well, visual cue cards (black-and-white stripe or white) were used to indicate the higher/lower reference location spouts, respectively. These two reference locations were located at the outermost 2 locations out of the 5 possible locations; they were 15cm from the sides of the goal box, and 60cm apart from each other.

The rats alternated between a “positive” condition and a “negative” condition for 30 minutes each, for a total of 2 hours a day in groups. The positive condition was represented by environmental cues consisting of black walls and a rough floor. Here, a sucrose/saccharin solution (3% sucrose with 0.125% saccharin dissolved in distilled water) represented the higher reward-value location (associated with the black-and-white stripe visual cue card), whereas tap water represented the lower reward value location (associated with the white visual cue card; figure 3.4a). In contrast, the negative condition was represented by environmental cues consisting of white walls and a smooth floor. Here, a quinine solution (0.00005M) represented an aversive reference location (associated with the white visual cue card), whereas tap water represented a comparably higher value reference location (associated with a black-and-white visual cue card; Figure 3.4b). The concentration of quinine used in this task was a very low concentration, so that rats would find it aversive but not so much as to cause stress or major changes in appetitive behaviour. The reward and aversive reference locations were counterbalanced between rats to eliminate side-bias. The specific reference locations paired with a particular visual and tactile condition environment were consistent for each rat throughout the experiment (ie. sucrose/saccharin solution were always at the right side of the goal box).

On the other hand, the 3 middle locations (figure 3.4) were blocked off by a smooth board with a color that was the same as the walls, thus leaving only the 2 reference location spouts exposed. All rats in the group had free access to explore both reference locations (and the accompanying lickometer spouts) in the arena during this stage (figure 3.4). Since all rats were identified by an ID marking on their tails, their exploration and consumption of the various reference fluids were verified by video recording.

In order to determine whether the rats had acquired the task, 24 hours after the last training day, the rats were tested individually to see if they were able to determine the more rewarding location in the positive and negative conditions. In this stage, each rat individually received 4 consecutive trials in a randomized order (2 trials in the positive condition and 2 trials in the negative condition) each day for five days. Between each trial, the apparatus was cleaned thoroughly with 20% alcohol solution. The time taken (in seconds) for the rat to exit the start box and choose one of two reference locations was recorded. The specific reference location spouts that the rat chose and drank from was recorded as well. The rat was considered to have made a correct choice if it chose to drink from the reference location spout associated with the higher reward value outcome immediately after exiting the start box. For the positive condition, the correct choice was the sucrose/saccharin solution, whereas in the negative condition, the correct choice was the water. A rat was considered to be successfully trained and could advance to the next stage if it consistently made the correct choice at least 3 times out of the 4 trials over the 5 testing days.

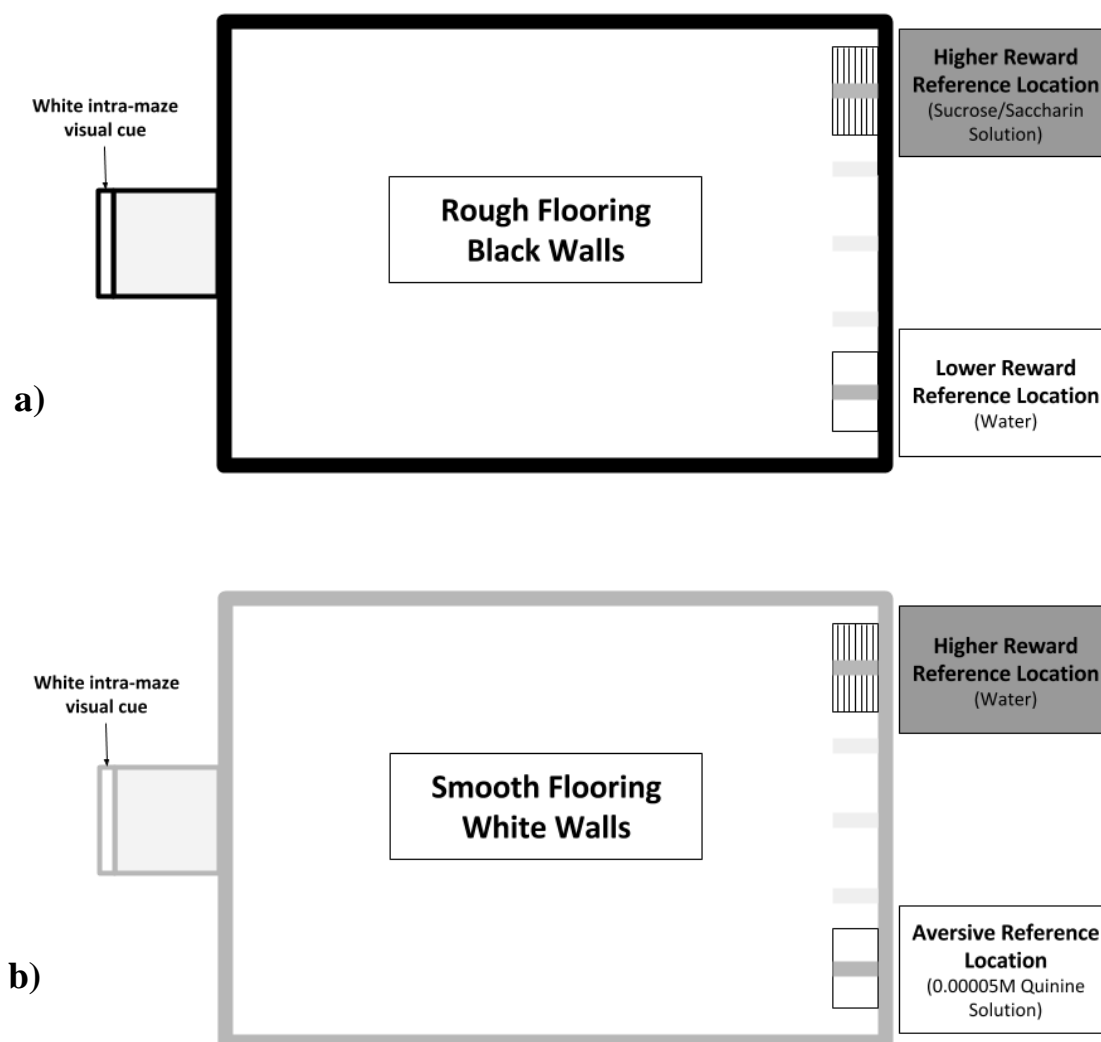


Figure 3.4. Diagram of training apparatuses in either positive (a) or negative (b) condition. A white intra-maze visual cue was presented in the start box. a) The rough floor and black walls (positive) environment was associated with the higher value sucrose/saccharin solution (3% sucrose and 0.125% saccharin dissolved in distilled water) at the left side of the goal box (stripe cue card) and tap water on the right side (white cue card). b) The smooth floor and white walls (negative) environment was associated with the lower value water at the left side of the goal box (stripe cue card) and 0.00005M quinine at the right side (white cue card). The middle 3 locations (light grey lines) were blocked off by a card with the same color as the walls.

3.2.6 Cognitive bias test with ambiguous cues under acute stress challenge

24 hours after the trials for establishing cognitive bias, each rat received three consecutive days of individual cognitive bias testing with ambiguous cues. In this stage, the trials proceeded in the same method as the previous trials, but with one additional “ambiguous” trial included where the testing was done with an acute stress challenge using Corticosterone. Thus, the trials were in a randomized order across animals, where each rat received 1 trial of ambiguous cue condition plus 2 trials each of the positive and the negative conditions. During the ambiguous condition, an insert sheet, intermediate in texture to the two conditioned floor textures, was used as the flooring (Acrylic Lighting Panels Cracked Ice Clear, Plaskolite Inc., Ohio, USA). In addition, grey cardboard was attached to the walls of the apparatus to give an intermediate wall color (figure 3.5). These two conditions created an environment where the visual and tactile cues were intermediate to the ones used for the training trials (i.e. black walls/rough flooring for positive condition, white walls/smooth flooring for the negative condition).

At the start of this stage, each pair of rats from the same cage were randomly allocated to two groups, acute stress group and control. Corticosterone was used to elicit the acute stress challenge. During the ambiguous cue test, either CORT (n=12), or VEH (n=11) were injected intraperitoneally 15 minutes before testing each day in the ambiguous cue context (refer to figure 3.1 for procedural outline). During these 15 minutes, the rats were placed back into their home cages. This 15 minutes waiting period was necessary for Corticosterone to reach peak physiological effect in the rat (Kent et al., 2000).

During the ambiguous cue trials, rats were individually tested by being exposed to only the 3 middle “ambiguous” locations. The two reference location spouts (for the positive and negative context conditions) were blocked off by a card in the same shade of grey as the walls. Graduated drinking tubes containing water were placed in each of the three middle ambiguous locations (figure 3.5). Measurements were made and recorded for the: i) time taken for the rat to exit the start box, ii) first choice from the three lickometer

spouts to drink from. iii) the number of licks made. If the rat selected the O1 location, it was recorded as making a choice reflective of a positive cognitive bias; if the P3 location was chosen, it was recorded as a choice reflective of a negative cognitive bias (figure 3.5). 30 seconds after the rat cease to drink from their initial choice spout, they were removed from the apparatus.

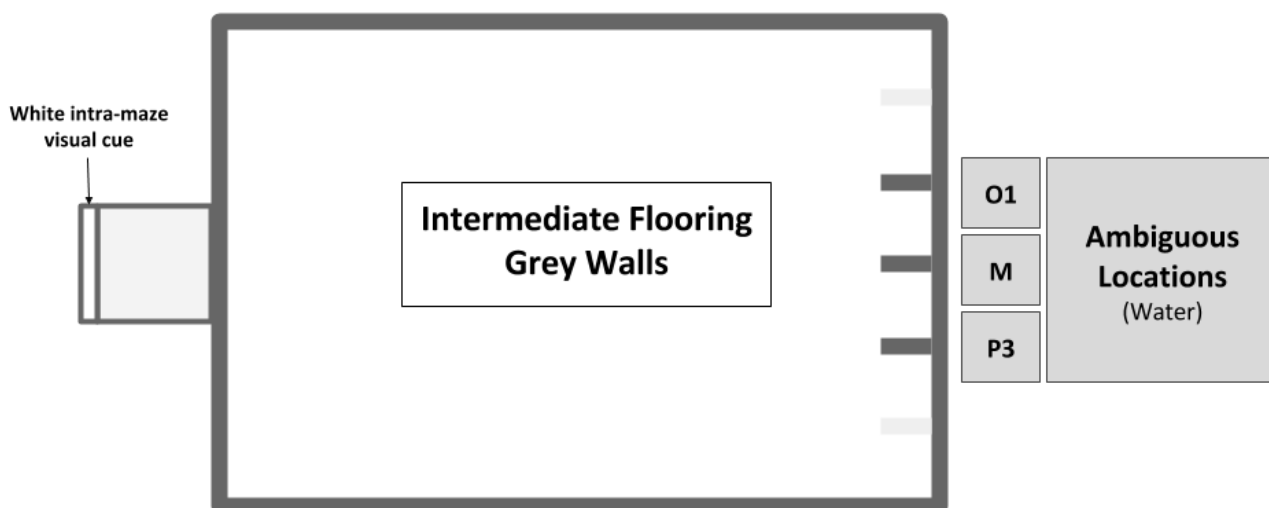


Figure 3.5. Diagram of experimental apparatuses in the ambiguous task. The smoothness/roughness of the flooring was in between the smooth and rough flooring of the training trials. The walls were a solid grey color. The two reference locations were blocked off and inaccessible using a smooth board with the same grey color as the walls. The drinking tubes of the ambiguous locations (dark grey lines) contained tap water. The O1 ambiguous location was located closest to the previously trained more rewarding location, whereas the P3 ambiguous location was located closest to the previously trained less rewarding/aversive location. The M ambiguous location was in the middle between the O1 and P3 ambiguous locations.

3.2.7 Statistical Analysis

In this experiment, all data generated were analyzed using SPSS (Version 21, SPSS Inc., Chicago, IL, USA). The replicates in this experiment and the treatment groups were equally counterbalanced across the conditions. The positive or negative choices across all three testing days during the ambiguous condition testing were analyzed with the chi-square test of independence. The behavioural measures (e.g. licking patterns, latency to approach choice spouts, and pathlengths to approach the choice spouts) during the cognitive bias testing with ambiguous conditions were analyzed using one-way Analysis of Variance (ANOVA). A significance level of $p < 0.05$ was used throughout the experiment.

3.3 Results

The total number of choices for the two treatment groups (VEH vs CORT) at each of the ambiguous test locations (O1/optimistic, M/Middle, P3/Pessimistic) across the three days of ambiguous cue testing is shown in figure 3.6. O1 represents the optimistic choice (choosing the location closest to the rewarding reference location), P3 represents the pessimistic choices (choosing the location closest to the aversive reference location). chi-square test for the number of choices made in the ambiguous cue test revealed significance between the locations chosen (i.e. Optimistic choices location (O1) and Pessimistic choice location (P3)) and treatment group ($\chi^2(2, n=23) = 13.746, p < 0.001$). Specifically, CORT treated rats chose the pessimistic location significantly more than then VEH treated rats. As well, VEH treated rats chose the optimistic location significantly more than the CORT treated rats. There was no significance found between the two treatment groups for choosing the middle location.

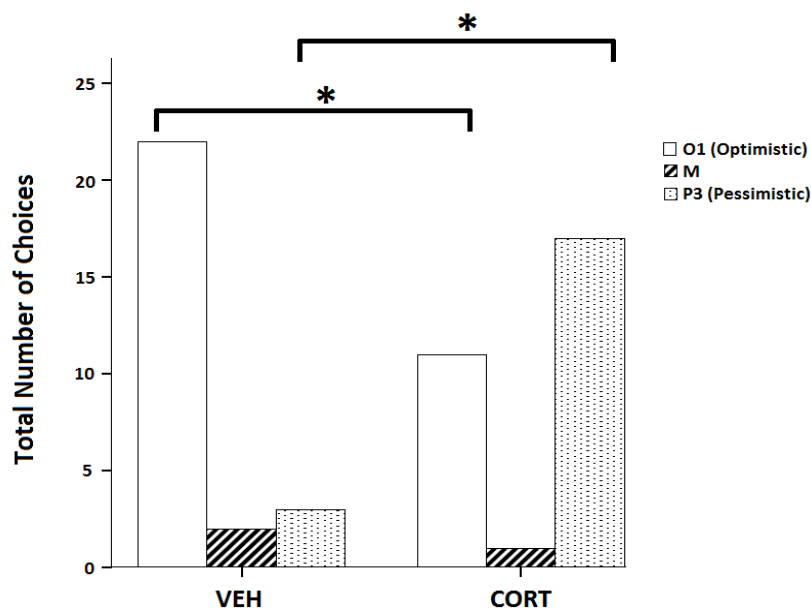


Figure 3.6. Comparison of choices made during the ambiguous cue testing (total) over 3 days. There was a significant association between the locations chosen (i.e. Optimistic choices location (O1) and Pessimistic choice location (P3)) and treatment group. Specifically, CORT treated rats chose the pessimistic location significantly more than then VEH treated rats, and the VEH rats chose the optimistic location significantly more than the CORT treated rats. There was no significant difference between the two treatment groups for the middle location. * $p < 0.001$

The ambiguous choices across 3 days of ambiguous cue testing was also broken down to be analyzed by day, shown in figure 3.7-3.9. Overall, the chi-square test revealed that there were no statistical significances between the choices made by the two treatment groups on days 1 and 3, but there was a significant interaction between the treatment groups and the ambiguous location choices on day 2.

Specifically, on day 1, Chi-Square test revealed that no statistical significance between the two treatment groups for the choice locations ($\chi^2 (2, n=23) = 3.893, p > 0.05$). Control rats did not differ from the acute stress animals in their choices of the three ambiguous locations. However, the VEH group exhibited a significant difference between the O1 location to the M ($\chi^2 (1, n=23) = 4.278, p < 0.05$) and the P3 location ($\chi^2 (1, n=23) = 4.278, p < 0.05$). The control rats chose the optimistic location significantly more than the pessimistic location.

On day 2, Chi-Square test revealed statistical significances between the two treatment groups for the choice locations ($\chi^2 (2, n=23) = 7.987, p < 0.05$). Specifically, the control rats preferentially chose the optimistic location over the pessimistic location ($\chi^2 (1, n=23) = 11.000, p = 0.001$). On the other hand, the acute stressed rats preferentially chose the pessimistic location ($\chi^2 (1, n=23) = 12.000, p = 0.001$). No animals chose to drink from the middle (M) location.

On day 3, chi-square test revealed no statistical significance between the two treatment groups in terms of the location choices that the rats made ($\chi^2 (2, n=23) = 2.500, p > 0.05$). No animals chose to drink from the middle (M) location.

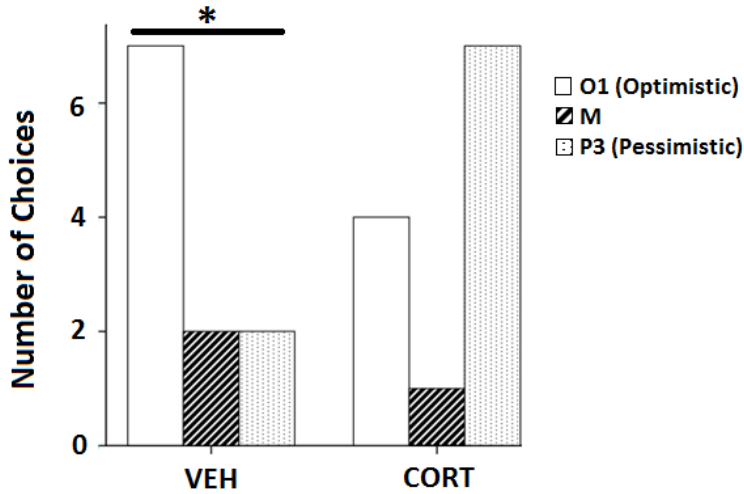


Figure 3.7. Comparison of choices in the ambiguous cue conditions between the two treatment groups on day 1. *p<0.05

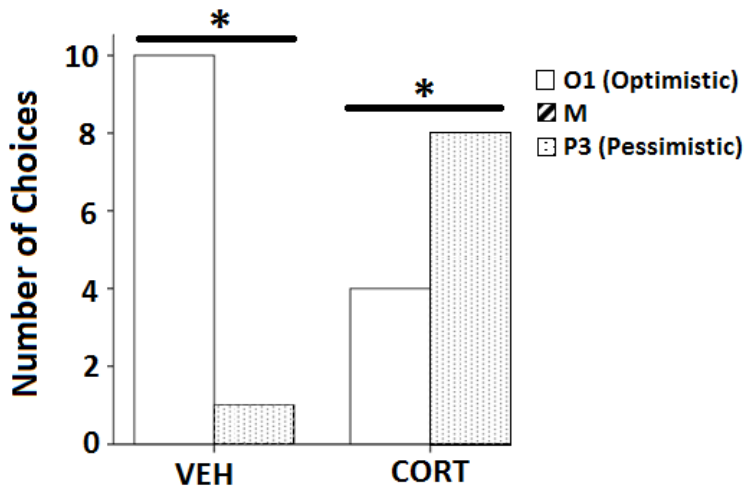


Figure 3.8. Comparison of choices in the ambiguous cue conditions between the two treatment groups on day 2. No animals chose to drink from the middle (M) location. *p<0.05

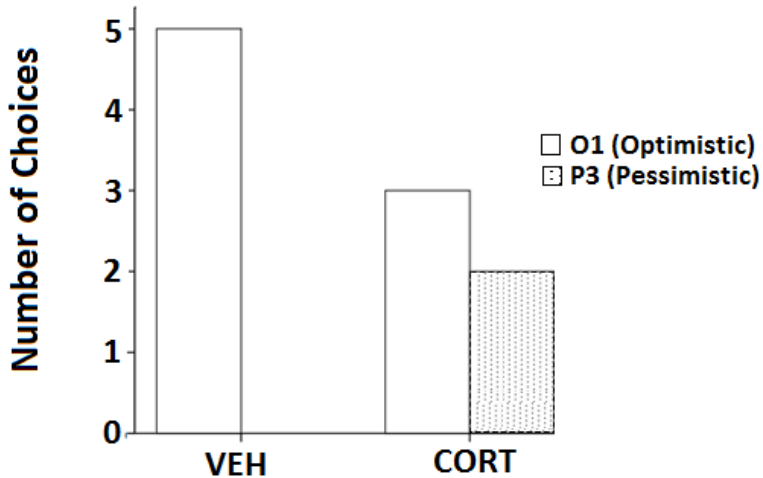


Figure 3.9. Comparison of choices in the ambiguous cue conditions between the two treatment groups on day 3. No animals chose to drink from the middle (M) location.

The mean path length taken to reach the choice spout and drink from it, as well as the number of licks taken, and latency to approach the choice spouts for the VEH and CORT groups are shown in Figure 3.10 – 3.12. One-way ANOVA analysis comparing VEH and CORT treated groups revealed no significance among any of the three locations with regards to the path length taken to reach the choice spout ($F(1,55) = 0.044, p > 0.05$) and the number of licks taken at the choice spout ($F(1,55) = 0.963, p > 0.05$). Both the control rats and the acute stressed rats took similar distance to reach their choice and took similar number of licks at their choices. However, there was a significant difference between the treatment groups in terms of latency to approach the choice spout. Specifically, ANOVA analysis revealed that the corticosterone treated groups displayed a significant increase in latency to make a choice in comparison to the control group ($F(1,32) = 4.114, p < 0.05$). However, no significance was found when comparing the VEH and CORT treated groups ($F(1,55) = 2.875, p > 0.05$) for the middle and pessimistic locations.

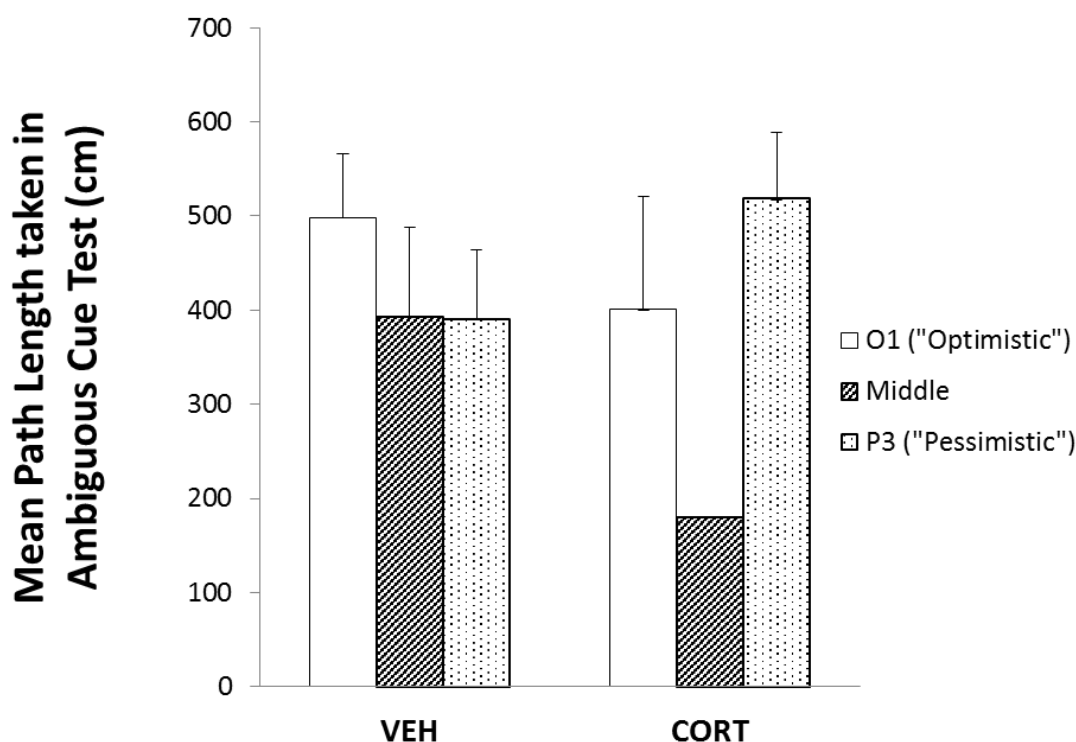


Figure 3.10. Path lengths taken to reach choice spout and drink from it during ambiguous cue testing trials. There were no significances between any of the three locations when comparing between VEH vs. CORT rats' performances ($p > 0.05$). No standard errors were generated ($n=1$) for the results from CORT rats at the M location.

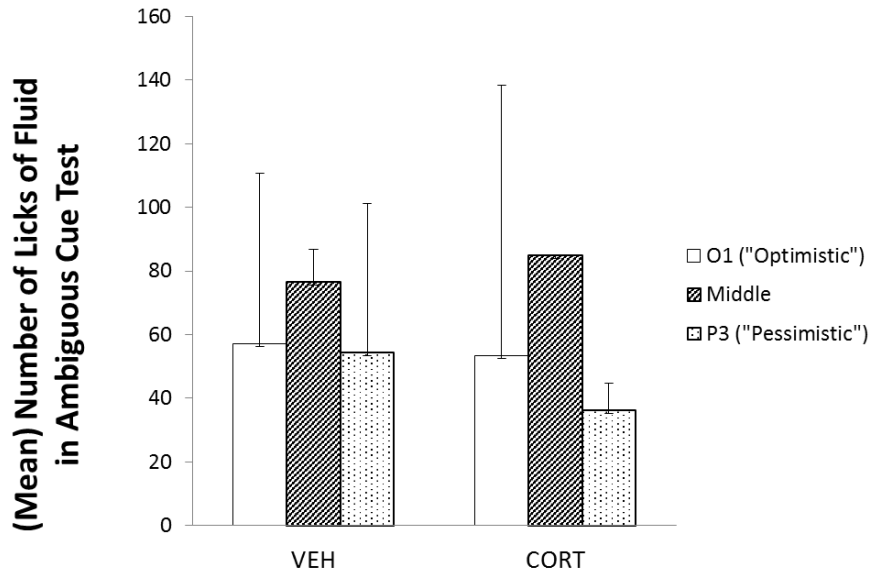


Figure 3.11. Number of licks taken at the choice spout during the ambiguous cue testing trials. There was no significance found between the VEH and CORT groups for the licking frequency ($p > 0.05$). No standard errors were generated ($n=1$) for the results from CORT rats at the M location.

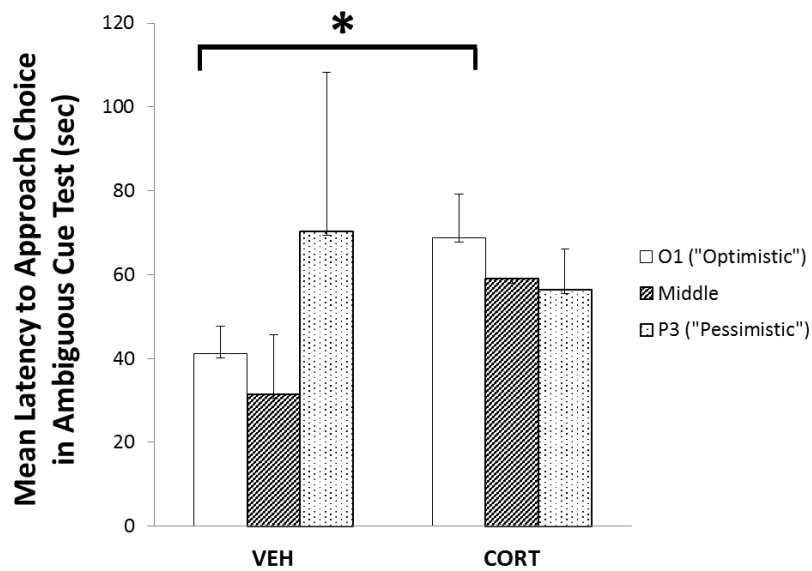


Figure 3.12. Latency to approach the choice spout in the ambiguous cue testing trials. No significance was found when comparing between the VEH and CORT treated groups ($p > 0.05$) for the middle and negative cognitive bias locations, but there was a significant difference between the CORT and the VEH groups when the rats approached the positive cognitive bias (O1) location, with the CORT treated rats taking significantly longer time to approach the location ($F(1,32) = 4.114, p < 0.05$). No standard errors were generated ($n=1$) for the results from CORT rats at the M location.

3.4 Discussion

The current study was able to build upon the previously established cognitive bias task by first demonstrating a positive cognitive bias at the basal level in rats, even with possible mild stress from injection. More importantly, this experiment demonstrated a shift to negative cognitive bias from the baseline positive cognitive bias following acute stress challenge using corticosterone.

Specifically, the baseline optimism that was observed in experiment 1 was shifted towards pessimism when rats were subjected to acute stress challenge from corticosterone treatment. Such pessimism is an indication of a shift towards negative cognitive bias, which is a key component of neuropsychiatric affective disorders such as depression. In this experiment, control rats that received the vehicle injection displayed an overall optimism in the ambiguous condition, similar to the findings from chapter 2, where rats demonstrated positive cognitive bias without any stress manipulations (i.e. injections). This showed that the injection procedure itself was not enough of a stressor to cause shifts in cognitive bias. As well, rats preferentially avoided the middle location across 3 days. In contrast, rats treated with Corticosterone displayed significant shift in cognitive bias to negative when presented with ambiguous stimuli. These rats preferentially chose to drink from the ambiguous choice location associated with the less-rewarding/aversive reference location (P3 location). Thus, the current study is consistent with previous research on chronic mild stress, and showed that negative emotional states will shift baseline optimism towards “pessimism” (negative cognitive bias) under ambiguous conditions.

However, the CORT treated rats exhibited a shift in their preferences across 3 days. This is shown by the comparison of the results from day 3 to those from days 1 and 2. Specifically, there was no significant interactions between the CORT treatment with the location choices on days 1 and 3. In contrast, the same CORT treated group demonstrated strong preference for the pessimistic (P3) location on day 2. This effect may be due to the acclimation of animals to the ambiguous condition through 3 days of testing. The animals were allowed a small window of time (30seconds) after making a choice to remain in the

open arena. This was done to reduce the possibility of conditioned pairing between specific spouts and handling stress. However, this did allow some of the rats to have extra time to further explore the other choices. Since the ambiguous locations were all reinforced with water, the rats that did go on to explore the other ambiguous choices would have found that all spouts gave equal outcomes, which may potentially have interfered with their responses in the succeeding trials.

Overall, the rats were generally not affected by locomotor confounders, as indicated by the lack of differences in latency, licking, and path length measures between the VEH and CORT groups. There was one exception, where acute-stressed rats took longer time (not path) to approach the O1 location (indicative of positive cognitive bias). This suggests that the acute stress rats showed a lack of an expectation of a positive outcome at that specific ambiguous condition. This increase in latency, when paired with the results showing significant difference in optimism and pessimism, suggested altered response to the reward value of the outcome. The rats showed an increased expectation of a negative outcome (indicative of anxiety), or a decreased expectation of a positive outcome (indicative of depression). While it may be difficult to understand why these animals are preferentially choosing the less rewarding outcome, it is important to note that these behaviors (anhedonia and behavioral despair) are indicators of a negative affective state for both humans and animal models. Specifically, anhedonia (i.e. reduced preference for sweet sucrose solution because of a decrease in its rewarding value) and behavioral despair have been well established in the human population as key characteristics of mood disorders (JP et al., 1972). As well, they are also well established in rodent models of depression (Harkin, Houlihan, & Kelly, 2002; Rygula et al., 2005; Willner, 1997). Such maladaptive, or “irrational”, behavior responses do reflect results from animal welfare research with chronic mild stress. The lack of environmental enrichment can elicit robust maladaptive behaviors, abnormal brain development, and impaired higher-order cognitive functioning (Simpson & Kelly, 2011; Würbel, 2001). In addition, acute administrations of corticosterone in rats have been shown to decrease fear in threatening situations, a maladaptive behavior (Skórzewska et al., 2007).

The present cognitive bias task also presents important advantages over previously established chronic stress paradigms. First, since acute corticosterone was administered only before undergoing ambiguous cue test (not throughout the whole experiment), differences in the task acquisition versus testing stages can be teased out. This differs from a number of animal welfare research, where chronic stress treatment was applied before the onset of training and testing, making it difficult to examine only the effect of ambiguous cues on affective cognition (Marks, Fournier, & Kalynchuk, 2009; Olausson, Kiraly, Gourley, & Taylor, 2013; Skórzewska et al., 2006). Another advantage of the current acute stress task is the potential to use it to study the effect of different levels of stress. Although only a physiological level of acute stress was used in this study, a number of variations could be used to examine the effect of low to high stress on cognitive bias by varying the concentration of corticosterone.

On the other hand, it is important to keep in mind that the ambiguous cues used in the current task were composed of two components. The environmental visual/tactile cues (grey walls and intermediate grain flooring) and the choice location cues (grey cue cards indicating the middle choice locations) were combined in this task to evaluate shifts in cognitive bias. However, whether each ambiguous component (i.e. ambiguous environment versus ambiguous choices) have a separate effect on cognitive bias is unclear. This is important to examine, and is the focus of the next chapter, in order to further tease out the mechanisms underlying cognitive bias.

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4 EFFECTS OF AMBIGUOUS ENVIRONMENTAL AND CHOICE CUES ON COGNITIVE BIAS

4.1 Introduction

In humans, cognitive bias has been well established as the result of an interplay between cognition and affect. The way information with ambiguous or unclear meanings is cognitively processed tends to be biased by the positive or negative mood (or “affective valence”) of the subject. As a result, decisional and behavioral outcomes are altered to reflect the propensity of the subject towards a specific affective valence. Specifically, a negative cognitive bias, characterized by indicators such as an increased anticipation of negative events or decreased anticipation of positive events, plays a key role in affective disorders such as depression and anxiety (e.g. Beck et al., 1979; A. Mathews & MacLeod, 2005; a Mathews & MacLeod, 1994; Mineka Sutton, Steven K., 1992; Rude, Valdez, Odom, & Ebrahimi, 2003).

When translated to animal models, such traits have also been found. Although tests of cognitive bias cannot unequivocally show whether animals consciously experience subjective affect changes, these do provide objective indicators for animals’ affective valences. This is commonly seen as an anticipation of either positive or negative outcomes in response to affectively ambiguous situations. The development of an objective cognitive bias model using non-human animals have generally adopted a similar framework. The animal is first trained to distinguish between a positive condition and a negative condition based on distinct contextual stimuli and outcome reinforcement (e.g. high-value reward or aversive outcome). Then, the animal is subjected to some affective manipulations designed to generate positive or negative affective states, followed by exposures to a new condition where the stimuli are ambiguous (i.e. intermediate to the trained conditions). The responses of the animals in this ambiguous condition would then be scored to show any shifts in cognitive bias by an increased anticipation for the positive or negative outcomes. A negative (or “pessimistic”) response would be accompanied by an increased likelihood of responding to the ambiguous cues with a prediction of a negative outcome, and a positive

(or “optimistic”) response would be opposite. In studies of negative cognitive bias, chronic mild stress has been extensively shown to cause an increased expectation of the negative event and/or decreased expectation of the positive event in a wide range of non-human animals such as dogs, bees, starlings, and rodents (Bateson et al., 2011; Burman et al., 2009; Matheson et al., 2008; Michael Mendl et al., 2010, 2009).

The development of an objective rodent model of cognitive bias is still in its infancy. Since the pioneering study by Harding et al (2004), various models have been developed in an attempt to improve upon the test specificity to better elucidate a clearer understanding of the mechanisms underlying cognitive bias. One major discovery based on the original Harding et al study revealed the advantages of using a go-go task format over a go/no-go format. By requiring active responses to the condition stimuli, the analysis of the animals’ responses in the ambiguous condition can eliminate confounders such as stimulus-related motivation and behavior changes, as well as give clearer indications of active responses versus active omission (Brydges et al., 2011; Harding et al., 2004). In addition, proceeding studies revealed disadvantages of using a testing apparatus with unclear distribution of condition stimuli. For example, a study by Burman et al (2009) using an adapted radial arm maze revealed that the specific locations of the arms prompted the rats to view all ambiguous choices together as a general “ambiguous outcome” rather than specific “ambiguous locations” with distinct values (Burman et al., 2009).

In the preceding studies, a cognitive bias model that could be used to evaluate the changes in affective state, such as that elicited by corticosterone induced acute stress, was developed. It was shown that male rats under acute stress displayed a negative cognitive bias when facing completely ambiguous (novel) condition cues. This was characterized by stressed rats making a slower response to the ambiguous condition and making more negative choices, indicating a reduced anticipation of reward (anhedonia). However, in order to further our understanding of this shift in cognitive bias in response to ambiguous cues, there is a need to refine the distinction between the ambiguous environmental/choice location cues. In particular, the ambiguous condition had 2 separate components. The first component was the background environmental cue, which consisted of intermediate grain flooring and grey walls (intermediate to the black wall of positive training condition, and

the white wall of the negative training condition). The second component was the ambiguous visual cues associated with the actual choice locations (hereafter referred to as “ambiguous choice location cues”). Thus far, in the present cognitive bias task developed in experiments 1 and 2, these two components of the ambiguous condition have been grouped together during the evaluation of cognitive bias.

Specifically, during the cognitive bias testing with ambiguous cues, the previously trained reference locations were blocked off, leaving only the three middle “novel” locations available to choose from. Each of these locations were centered on a grey visual cue card (ambiguous choice location cue), within an environment that was also intermediate to the previously trained backgrounds (grey walls and intermediate flooring). It was shown that when both environmental and choice location components were ambiguous, rats exhibited very distinct cognitive biases. The control rats displayed a positive (optimistic) cognitive bias, and acute stress treated rats displayed a negative (pessimistic) cognitive bias. However, whether these two components (environment vs choice location cues) interact with each other or act independently to influence affective states are still unknown. For example, if an ambiguous choice is offered in a positive environment, would the resultant behavior become more positive? Therefore, in order to further elucidate the mechanisms underlying cognitive bias, it is necessary to examine each separate component to see whether they have distinct effects on cognitive bias shifts.

In experiment 3, the mechanisms underlying shifts in cognitive bias as result of affective state changes were further examined. Specifically, the present study examined whether or not ambiguous environmental and ambiguous choice location cues interact with each other to influence behavioral outcomes of cognitive bias. This was achieved by creating two new conditions in the testing stage, where the presence of positive or negative environmental factors were coupled with ambiguous choice location cues (grey visual cue card at the choice spouts). The first condition, where positive environmental cues (black walls/rough flooring) were coupled with ambiguous choice location cues (grey visual cue card), will be referred to as the “positive ambiguous condition”. The second condition, where the negative environmental cues (white walls/smooth flooring) were coupled with the same ambiguous choice location cues, will be referred to as the “negative ambiguous

condition”. The purpose of using these two new conditions was to separate the two components of the ambiguous condition and evaluate whether or not they have different effects on cognitive bias. In order to properly evaluate this, responses were compared to the previously established, “original” ambiguous condition where the two ambiguous components were added together (grey walls, intermediate grain flooring, and grey ambiguous location visual cue cards). Thus, rats were also tested under the original ambiguous conditions without any stress manipulations (same as experiment 1), and with acute stress challenge using corticosterone (same as experiment 2).

It was hypothesized that the two components of the ambiguous condition work independently to generate cognitive bias. Specifically, environmental cues would have an effect on the choices made at the three ambiguous choice locations. After the establishment of the positive and the negative conditions during training, the associated environmental cues (wall colors and floor texture) would affect rats’ choices in response to the three middle novel “ambiguous” locations. Specifically, in the positive ambiguous condition (positive environmental cues with ambiguous choice location visual cue), rats would display a preference for the more positive location (O1), similar to that of the original ambiguous condition. Similarly, rats would respond in the same manner in the negative ambiguous condition (negative environmental cues with ambiguous choice location visual cue) as the previously trained negative conditions.

4.2 Materials and Methods

4.2.1 Experimental procedure

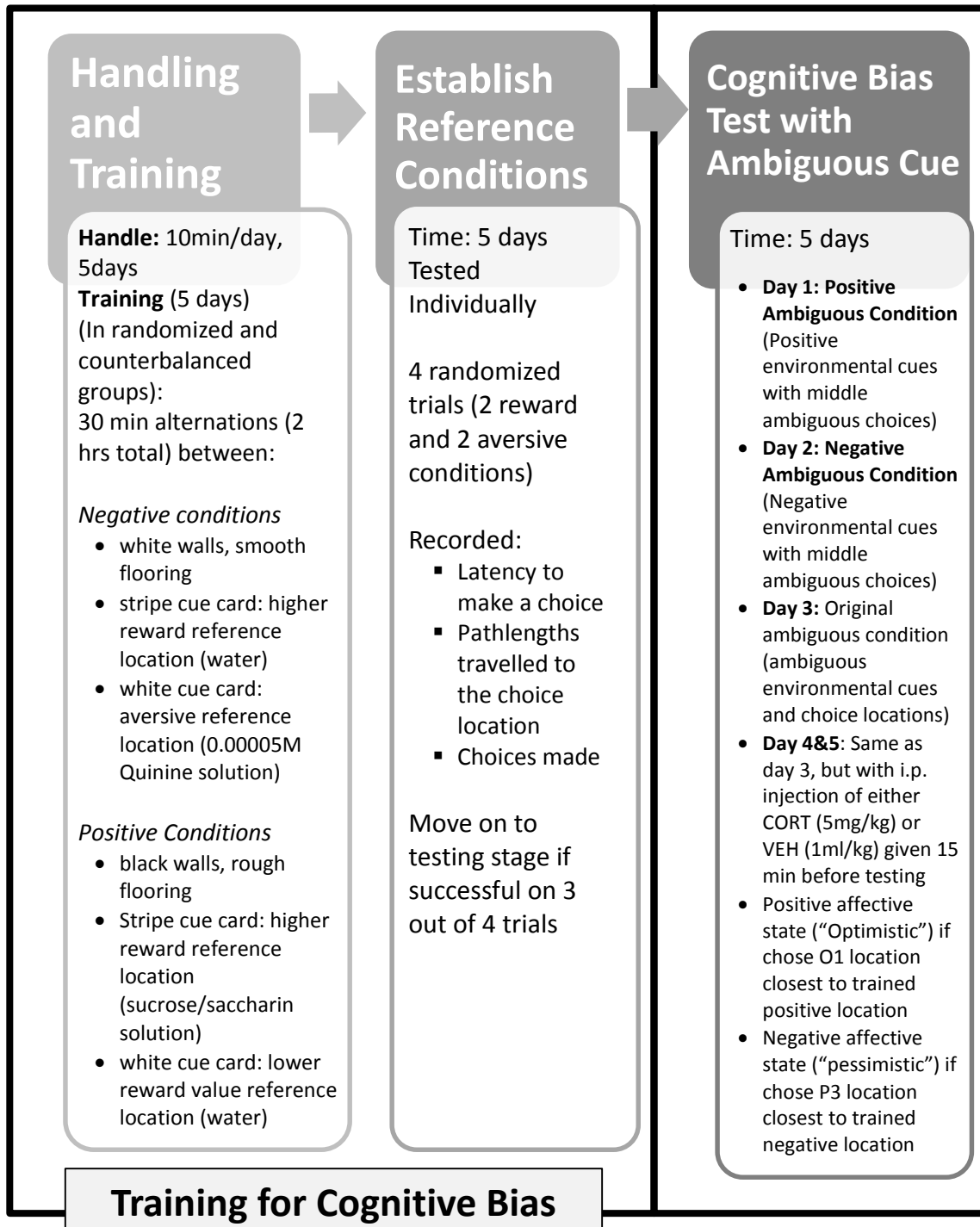


Figure 4.1. Outline of procedures for experiment 3.

4.2.2 Animals

Twelve adult male Long-Evans rats (Charles River, Quebec, Canada), weighing between 300 - 450g at the start of the experiment were used. The rats were pair housed in standard polypropylene cages (45cm × 22cm × 20cm), in a colony room with ad libitum access to both food (ProLab RMH3000 rat chow) and tap water when applicable. The colony room was maintained at $21 \pm 2^\circ\text{C}$, on a 12:12 hour light/dark cycle with the lights on from 07:00 to 19:00 h. All experiments were carried out during the light phase. For the entire duration of the experiment, each rat was water deprived for 15 hours starting at the beginning of their dark cycle (starting at 19:00h), and then given one-and-a-half-minute access to tap water at the end of the water deprivation period. This method was used so that rats would be motivated to explore and drink from the liquid reinforcements that were used to indicate differences between positive and negative conditions. The rats were observed during the one-and-a-half-minute water access to ensure that each rat drank from the water bottle. Each rat was given identification markings on their tails using a permanent marker (Black Sharpie pen). All procedures were performed according to the Canadian Council on Animal Care guidelines and were approved by the Western University Animal Care Committee.

4.2.3 Drugs

Corticosterone (Cort; Sigma, Toronto, ON) was used to mimic the effect of acute stress challenge on days 4 and 5. CORT was dissolved in 45% hydroxypropyl- β -cyclodextrin to a dose of 5mg/mL. The vehicle (VEH) used in the control group was 45% hydroxypropyl- β -cyclodextrin. Corticosterone and vehicle were administered via intraperitoneal (i.p.) treatment at a volume of 1.0 mL/kg, 15 minutes before the start of the test. This Corticosterone administration procedure was used based on previous studies where it mimicked physiological levels of acute stress 15 minutes following the injection (Kent et al., 2000).

4.2.4 Training and the establishment of reference conditions

During the first 5 days of the experiment, each rat was handled daily for 10 minutes during the light phase. 24 hours after the final handling day and for the subsequent 5 days, all of the rats were placed into the open arena in groups of 6 for training. Group learning facilitates the acquisition of cognitive tasks more effectively and efficiently than individual training (Krasheninnikova & Schneider, 2014). During this time, visual (black or white walls) and tactile (smooth or rough flooring) cues were placed in the arena to indicate the positive or negative condition. As well, visual cue cards (black-and-white stripe or white) were used to indicate the higher/lower reference location spouts, respectively. These two reference locations were located at the outermost 2 locations out of the 5 possible locations; they were 15cm from the sides of the goal box, and 60cm apart from each other.

The rats alternated between a “positive” condition and a “negative” condition for 30 minutes each, for a total of 2 hours a day in groups. The positive condition was represented by environmental cues consisting of black walls and a rough floor. Here, a sucrose/saccharin solution (3% sucrose with 0.125% saccharin dissolved in distilled water) represented the higher reward-value location (associated with the black-and-white stripe visual cue card), whereas tap water represented the lower reward value location (associated with the white visual cue card; figure 4.2a). In contrast, the negative condition was represented by environmental cues consisting of white walls and a smooth floor. Here, a quinine solution (0.00005M) represented an aversive reference location (associated with the white visual cue card), whereas tap water represented a comparably higher value reference location (associated with a black-and-white visual cue card; Figure 4.2b). The concentration of quinine used in this task was a very low concentration, so that rats would find it aversive but not so much as to cause stress or major changes in appetitive behaviour. The reward and aversive reference locations were counterbalanced between rats to eliminate side-bias. The specific reference locations paired with a particular visual and tactile condition environment were consistent for each rat throughout the experiment (ie. sucrose/saccharin solution were always at the right side of the goal box).

On the other hand, the 3 middle locations (figure 4.2) were blocked off by a smooth board with a color that was the same as the walls, thus leaving only the 2 reference location spouts exposed. All rats in the group had free access to explore both reference locations (and the accompanying lickometer spouts) in the arena during this stage (figure 4.2). Since all rats were identified by an ID marking on their tails, their exploration and consumption of the various reference fluids were verified by video recording.

In order to determine whether the rats had acquired the task, the rats were tested individually 24 hours after the last training day to see if they were able to determine the more rewarding location in the positive and negative conditions. In this stage, each rat individually received 4 consecutive trials in a randomized order (2 trials in the positive condition and 2 trials in the negative condition) each day for five days. Between each trial, the apparatus was cleaned thoroughly with 20% alcohol solution. The time taken (in seconds) for the rat to exit the start box and choose one of two reference locations was recorded. The specific reference location spouts that the rat chose and drank from was recorded as well. The rat was considered to have made a correct choice if it chose to drink from the reference location spout associated with the higher reward value outcome immediately after exiting the start box. For the positive condition, the correct choice was the sucrose/saccharin solution, whereas in the negative condition, the correct choice was the water. A rat was considered to be successfully trained and could advance to the next stage if it consistently made the correct choice at least 3 times out of the 4 trials over the 5 testing days.

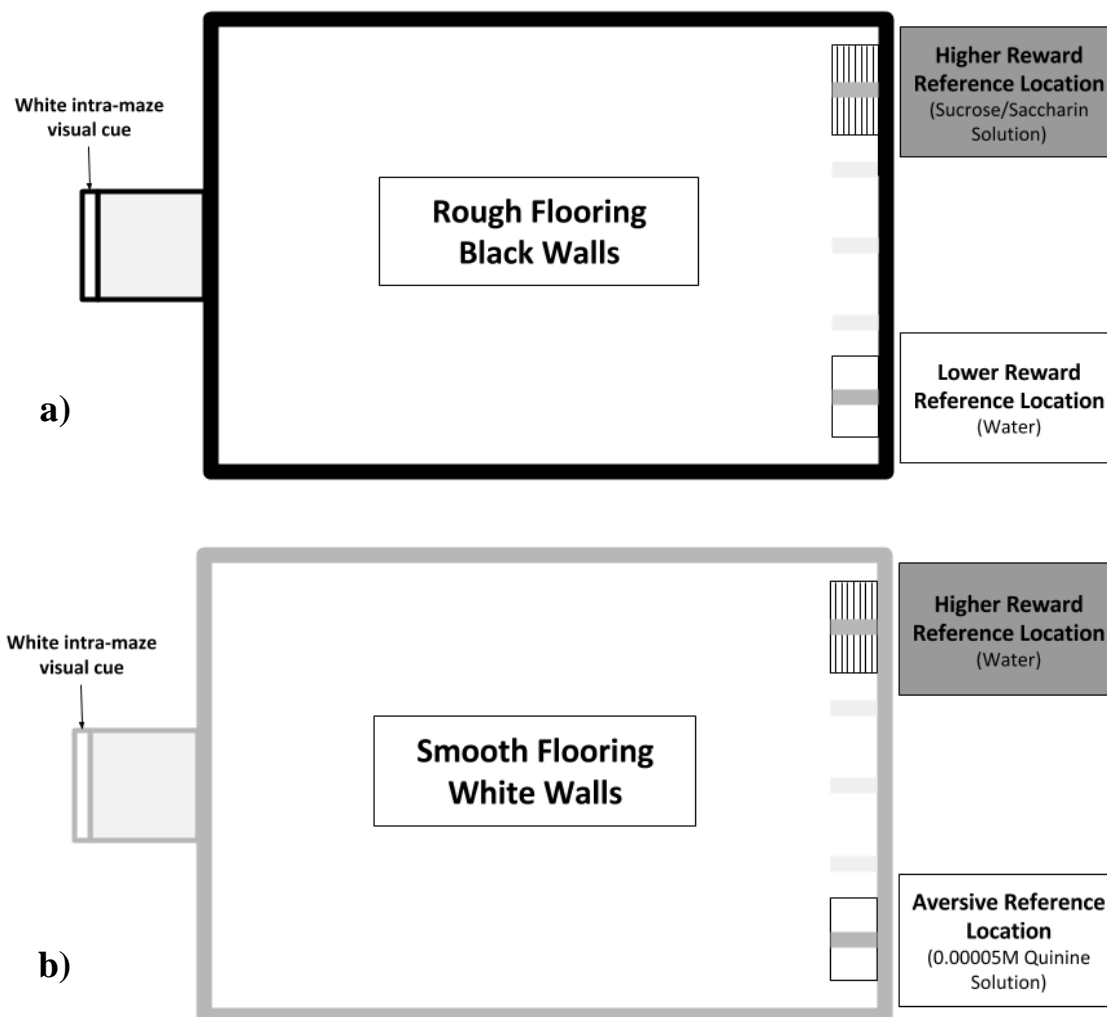


Figure 4.2. Diagram of training apparatuses in either positive (a) or negative (b) condition. A white intra-maze visual cue was presented in the start box. a) The rough floor and black walls (positive) environment was associated with the higher value sucrose/saccharin solution (3% sucrose and 0.125% saccharin dissolved in distilled water) at the left side of the goal box (stripe cue card) and tap water on the right side (white cue card). b) The smooth floor and white walls (negative) environment was associated with the lower value water at the left side of the goal box (stripe cue card) and 0.00005M quinine at the right side (white cue card). The middle 3 locations (light grey lines) were blocked off by a card with the same color as the walls.

4.2.5 Cognitive bias testing with ambiguous cues and previously trained environmental conditions

24 hours following the last stage, rats moved onto the cognitive bias testing with ambiguous cue condition, which lasted for five days. During this stage, the previously trained reference locations were now blocked off, leaving only the three middle “ambiguous” locations exposed (Figure 4.3 – 4.4). The lickometer drinking tubes contained water, and were placed in each of the three middle ambiguous locations. Ambiguous position 1 (O1) was located 15cm to the right of the reward location, and indicated a positive cognitive bias (optimistic choice) due to its close proximity to the previously trained positive reference location. The ambiguous position 3 (P3) was located 15cm to the left of the aversive location, and indicated a negative cognitive bias (pessimistic choice) due to its close proximity to the previously trained negative reference location. Ambiguous location 2 (M) was in the middle, 30cm to either of the two reference locations. The time was recorded for when the rat exited the start box and drank from one of the three lickometer spouts; the choice the rats made, as well as the number of licks taken were also recorded. If the rat selected the O1 location, it was recorded as making an optimistic choice; if the P3 location was chosen, it was recorded as a pessimistic choice. This was true for all five days of testing with ambiguous cues.

Day 1 and 2

On the first day of this testing stage, each rat was allowed access to only the 3 middle ambiguous locations, paired with a grey cue card at the lickometer spouts, in two separate trials each day for three days. The arena presented a positive condition (black walls and rough floor), with the two reference locations blocked off using black cardboard (Figure 4.3a). The ambiguous lickometer drinking spouts contained water as the outcome. This was referred to as the “positive ambiguous condition”. The second day followed the same procedure as the day one, except the arena was changed to show the negative condition (white walls and smooth floor), and the two reference locations blocked off using white cardboard (Figure 4.3b). This was referred to as the “negative ambiguous condition”.

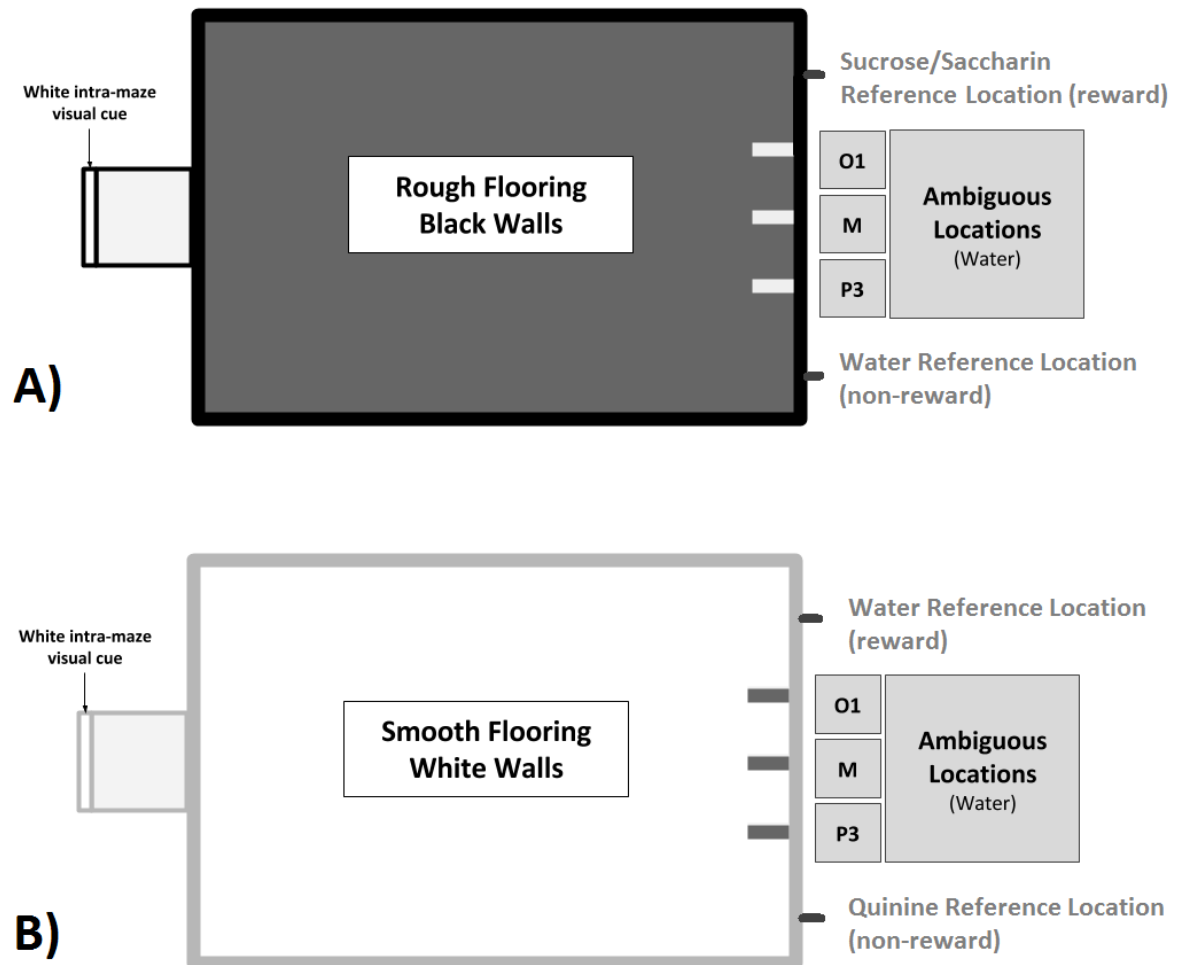


Figure 4.3. Diagram of the environmental conditions given on days 1 and 2. A) Positive Ambiguous Condition was used on day 1, where positive environmental cues were paired with ambiguous choice location cues (indicated by grey visual cue cards). **B) Negative Ambiguous Condition** was used on day 2, where negative environmental cues were also paired with the same ambiguous choice location cues. The reference locations from the training stage were now blocked off, but are shown in grey to indicate their locations for reference. The O1 ambiguous location was located closest to the previously trained more rewarding location, whereas the P3 ambiguous location was located closest to the previously trained less rewarding/aversive location. The M ambiguous location was in the middle between the O1 and P3 ambiguous locations.

Day 3

On day three, the arena showed a completely ambiguous condition, same as that used in chapters 1 and 2. Here, an “ambiguous” insert sheet, intermediate in texture to the two conditioned floor textures, was used as the flooring (Acrylic Lighting Panels Cracked Ice Clear, Plaskolite Inc., Ohio, USA). In addition, grey cardboard was used for the walls to give an intermediate wall color (Figure 4.4). Here, the rats were exposed to only the 3 middle “ambiguous” locations with the two reference locations blocked off by a smooth card (same shade of grey as the walls).

Day 4 and 5

In order to examine whether rats displayed shifts in cognitive bias during the positive and negative ambiguous conditions (days 1 and 2), they were then subjected to previously established ambiguous environmental/choice location cues with corticosterone-induced acute stress (figure 4.4). Here, on days four and five, the procedure (including the ambiguous condition) followed that of day three, except the rats were randomly divided into vehicle and CORT groups prior to receiving their respective injections 15 minutes before each rat underwent testing.

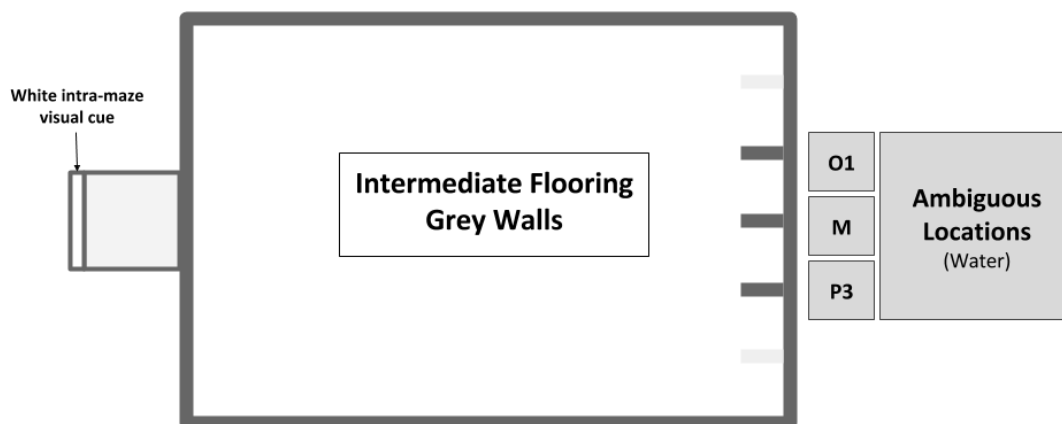


Figure 4.4. Diagram of experimental apparatuses in the ambiguous task for day 3.

The drinking tubes of the ambiguous locations (dark grey lines) contained tap water. Choosing to drink from the O1 location indicated optimism while drinking from the P3 location indicated pessimism.

4.2.6 Statistical Analysis

All data generated in this experiment were analyzed using SPSS (Version 21, SPSS Inc., Chicago, IL, USA). The replicates in this experiment and the treatment groups were equally counterbalanced across the conditions. For the training stage, the data for correctly and incorrectly choosing the higher reward value outcome for each condition were compared separately using the chi-square test, as this was needed to determine when the rats were ready to proceed to the next stage. The behavioural measures during the cognitive bias testing with ambiguous cue conditions (Licking pattern and latency to approach choice spouts) were analyzed using one-way Analysis of Variance (ANOVA). The specific choices that the animals made, the O1 (positive cognitive bias), P3 (negative cognitive bias), or the middle location was analyzed using chi-square test of independence to find whether the choices made at the three ambiguous locations were significantly different between the two treatment groups. A significance level of $p < 0.05$ was used throughout the experiment.

4.3 Results

4.3.1 Training and the establishment of Cognitive Bias

All rats were observed through live video-recording to have explored and drunk from each of the reference locations. Then, during the establishment of cognitive bias phase, each rat's performance was evaluated on whether they had successfully acquired the task in the positive and the negative training conditions. Correct choice on the positive and negative conditions required the rats to choose the more rewarding locations three out of four times (i.e. sucrose/saccharin for the positive condition, water for the negative condition). Chi-Square analysis did not reveal a significant difference between the choice (always choosing the higher reward value outcome) and the condition (positive or negative condition) ($\chi^2 (1, n=12) = 1.778, p > 0.05$). Where rats always chose the higher reward value outcome regardless of whether the condition was positive or negative. As well, Chi-Square test revealed a significance between the correct choices (drinking from the more reward location) and incorrect choices (drinking from the less rewarding/aversive location), where rats made the correct choices significantly more (figure 4.5).

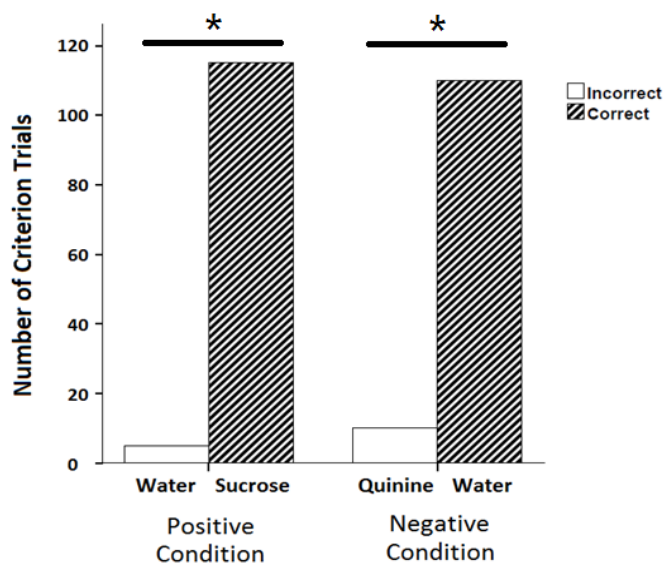


Figure 4.5. Comparison of correct and incorrect choices (accumulation of all trials for all rats) **made during the establishment of cognitive bias trials.** There were no interaction between accuracy of choice and the condition presented ($p > 0.05$), but rats made significantly more correct choices (more rewarding location). * $p < 0.05$

4.3.2 Cognitive bias testing with Ambiguous Cue

Choices made in the positive and the negative ambiguous conditions

Chi-Square analysis revealed no significance between the “optimistic” location (O1) and the “pessimistic” location (P3) in the positive ambiguous condition (day 1), the negative ambiguous condition (day 2), and with stress treatment (VEH and CORT) in the original ambiguous treatment (both ambiguous environmental cues and choice location cues) (χ^2 (4, n=12) = 3.145, $p > 0.05$; figure 4.6). Rats did not differ in their positive and negative choices of the three ambiguous locations when the environmental cues were the same as the previously trained positive and negative conditions. As well, following treatments with acute stress challenge, rats also did not preferentially choose positively or negatively when facing both the ambiguous environmental and choice location cues.

However, rats’ performances in the original ambiguous condition (both ambiguous environmental and choice location cues) without injection treatments showed a significant difference in the number of optimistic choices made (figure 4.6). Rats who did not receive any injections preferentially chose to drink from the O1 location (χ^2 (1, n=12) = 6.494, $p < 0.05$).

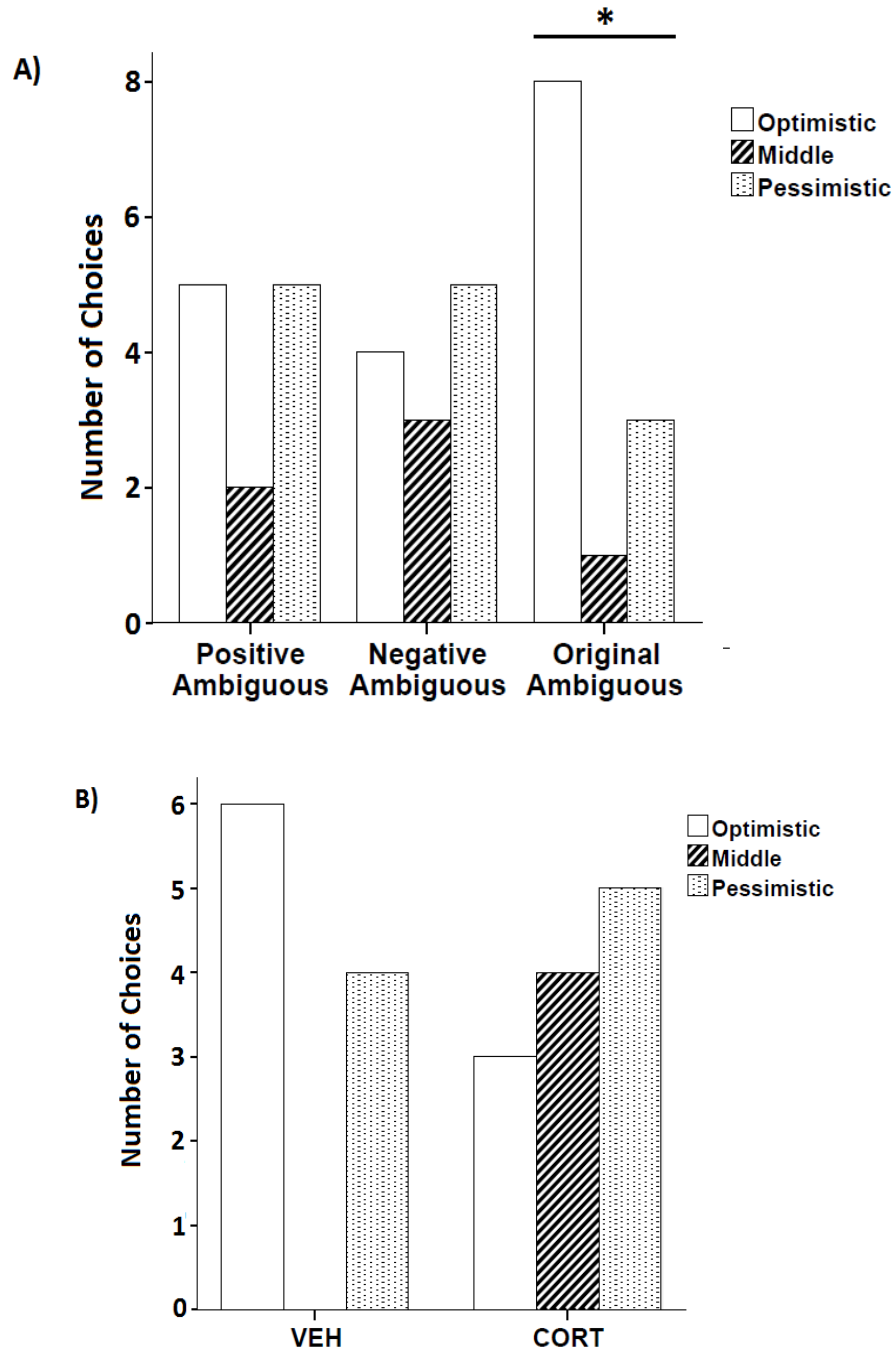


Figure 4.6. A) Choices made by rats in the positive ambiguous condition (day 1), negative ambiguous condition (day 2), and original ambiguous conditions (no injections). No significant differences were found between choices made in the positive and negative ambiguous conditions ($p > 0.05$). Only the original ambiguous without any treatment injections showed a significant difference in the positive and negative choices, with a strong preference for the positive (O1) location ($p < 0.05$). **B) Choices made with VEH and CORT treatments for comparison.**

Latency, licking, and path length measures

First, the latency for the animals to make a choice only in the original ambiguous conditions (grey walls and intermediate flooring), with and without stress treatment during days 1-3, was analyzed and compared. ANOVA analysis revealed no significant differences between the latencies of rats treated with a vehicle injection and rats that did not receive any injection treatments ($p > 0.05$; figure 4.7). The injection itself did not cause significant psychological changes to shift rats' positive or negative choices in the completely ambiguous condition. As well, no significance was found between the latencies of vehicle rats versus acute stressed rats in making a choice ($p > 0.05$; figure 4.7). However, there was a significant difference between the latency to make a choice between the rats that did not receive any injection treatments versus receiving corticosterone treatment ($F(4, 57) = 3.764, p < 0.05$). Rats that did not receive any treatments took significantly longer time to make a choice when compared with rats that were under acute stress.

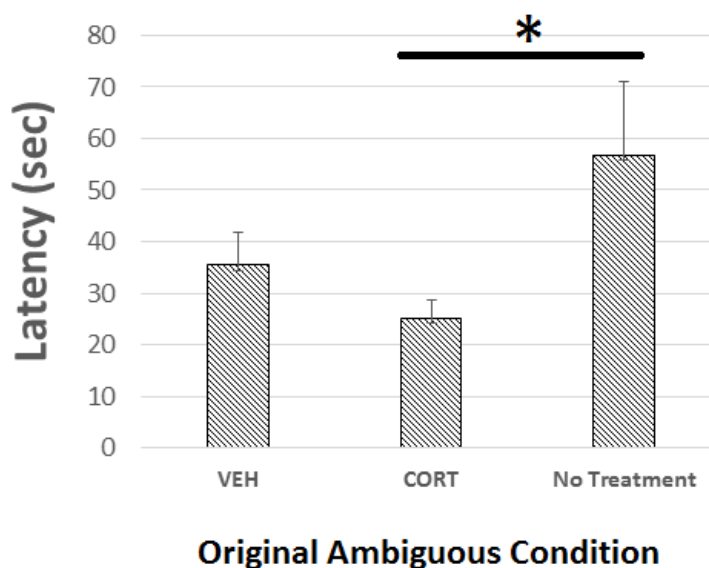


Figure 4.7. The latency for the animals to make a choice in the original ambiguous condition (grey walls, intermediate floor, grey visual cue indicating the choice spouts in the middle, see figure 25), with and without any acute stress treatment. One-way ANOVA revealed a significant difference between the latency to approach the choice between the acute-stress treated rats and rats that did not receive any injection treatments. There was no significant difference between rats' latency to approach choice between the vehicle control rats and rats that did not receive any injection treatments.

These results were then compared to the latencies of rats making a choice in the positive and negative ambiguous conditions on days 1 and 2 (refer to figure 4.3). The mean latency from the time that the rat exited the start box to the time it made a choice during the ambiguous cue test was compared between the positive ambiguous condition (positive environmental cues with ambiguous choice location cues), negative ambiguous condition (negative environmental cues with ambiguous choice location cues), original ambiguous conditions without injections (refer to figure 4.4). It was found that overall, there was a significant interaction between conditions and the latency for the rats to make a choice (Figure 4.8). Specifically, rats took a significantly shorter amount of time to make a choice in the positive ambiguous condition (black walls and rough flooring), than the negative ambiguous conditions (white walls and smooth flooring) ($F(1,22) = 17.117, p < 0.001$). When the choices in the positive ambiguous condition was compared to the original ambiguous condition without any treatment (ambiguous environmental and choice location cues), it was found that rats took significantly less time to make a choice in the positive ambiguous condition ($F(1,22) = 5.860, p < 0.05$). However, when comparing the negative ambiguous conditions with the original ambiguous conditions, ANOVA analysis revealed no significant differences in the latency to make a choice ($F(1,22) = 0.634, p > 0.05$).

ANOVA analysis did not reveal a significant interaction between the condition and the latency measures in the original ambiguous condition when rats were subjected to acute stress challenge (VEH or CORT) ($F(4,57) = 3.764, p > 0.05$) (figure 4.8).

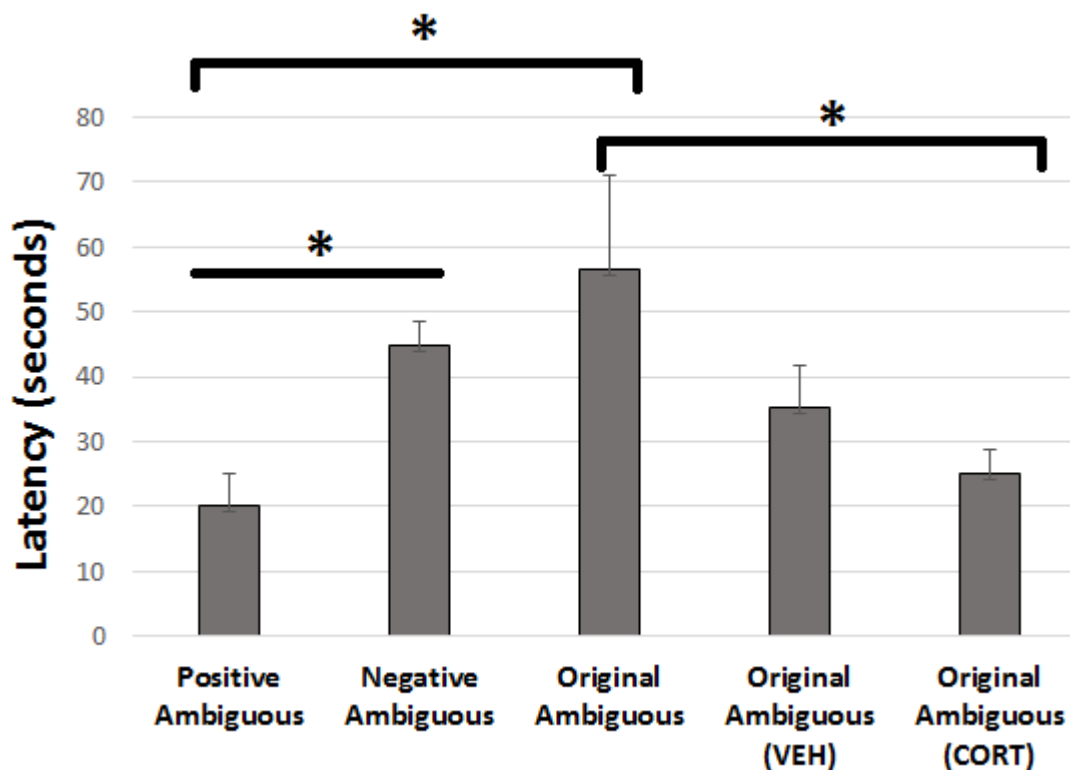


Figure 4.8. The latency for the animals to make a choice in the positive environmental condition with ambiguous choices, the negative environmental condition with ambiguous choices, and the original completely ambiguous condition. Rats took longer to make a choice in the positive ambiguous condition when compared to the negative ambiguous condition ($F(1,22) = 17.117$, $p = 0.000$), and the original condition with no stress manipulations ($F(1,22) = 5.860$, $p < 0.05$). As well, acute stressed rats took significantly shorter time to make a choice in the original ambiguous condition than rats without any stress manipulations in the same condition ($F(4, 57) = 3.764$, $p < 0.05$). No differences in latency to make a choice was found between all other conditions.

When the number of licks that the rats took in each of the testing conditions were compared, ANOVA analysis revealed a significant difference among the rats when they were in the positive and negative environmental conditions with ambiguous choice cues (Refer to figure 4.3; $F(4,57) = 3.450, p < 0.05$). Rats took significantly more licks in the negative ambiguous condition when compared to the positive ambiguous condition (figure 4.9). As well, we compared the rats' performance in the original ambiguous conditions (see figure 4.4) with the positive/negative environmental conditions with ambiguous choice cues (see figure 4.3). There was a significant difference between the number of licks taken while in the positive environmental conditions when compared with acute-stressed rats in the original ambiguous condition, where stressed rats took significantly more licks at their choices ($F(4,57) = 3.450, p < 0.05$). Similarly, the licking patterns in the positive environmental conditions were not significantly different from the rats with no treatment injection in the original ambiguous condition ($F(4,57) = 3.450, p > 0.05$) (Figure 4.9).

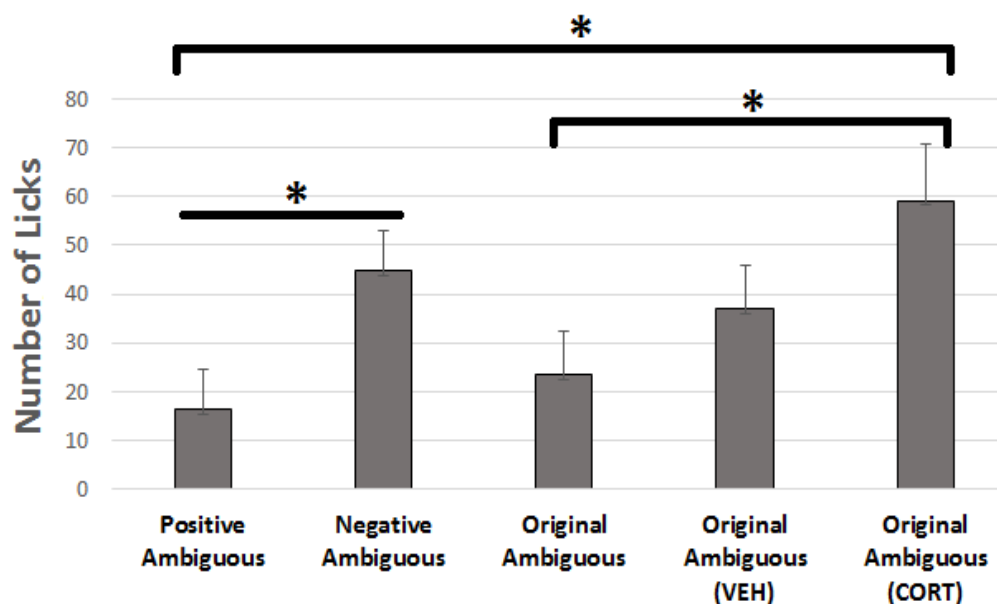


Figure 4.9. The number of licks that the animals took at the ambiguous cue tests. Rats took more licks in the negative environmental conditions with ambiguous choice cues rather than the positive environmental condition ($F(4,57) = 3.450, p < 0.05$). Rats also drank more when they were in the original ambiguous condition than when they were in the positive ambiguous condition. In contrast, the licking frequency of the rats was significantly lower in the positive ambiguous condition than the acute stressed rats ($F(4,57) = 3.450, p < 0.05$), but did not differ from the licking frequency of the un-treated or vehicle rats ($p > 0.05$).

4.4 Discussion

The aim of the present experiment was to determine whether the two ambiguous components, environmental (visual and tactile cues) and choice location cues, interact with each other to influence cognitive bias. Contrary to the hypothesis, the two components of the ambiguous condition (environmental cues versus choice location cues) did not work independently to produce changes in cognitive bias. That is, rats did not preferentially choose an ambiguous location depending on the environmental condition (positive condition or negative condition, respectively). In fact, rats demonstrated no differences in their choices of three ambiguous locations when the environmental cues were that of previously trained positive or negative visual/tactile cues. In other words, when the two components of the ambiguous condition were separated, there were no differences in optimistic vs pessimistic responses to the ambiguous location choices. In addition, the licking and latency measures both indicated an increased expectation of a lower reward value outcome for the positive and the negative ambiguous conditions. Rats preferentially took longer to approach and drank from the choice spouts in the negative ambiguous condition in comparison to the positive.

Specifically, when the positive ambiguous condition was presented (black walls/rough flooring with grey choice location cue at the middle 3 locations), the rats did not differ in their choices between the O1 location and the P3 location. The same trend was observed in the negative ambiguous condition (white walls/smooth flooring with only the 3 middle ambiguous choices to drink from). However, when the condition completely changed to one that was ambiguous (grey walls/intermediate grain flooring with 3 middle ambiguous choices) on day 3, the rats preferentially chose to drink from the O1 location, indicative of positive cognitive bias. With the same ambiguous conditions but under a treatment of either the VEH injection or the CORT acute stress challenge, their responses changed again. While the acute stressed rats did show a decrease in the number of optimistic choices made, the pessimistic choices did not differ from that of the VEH rats. The only similarity was that the control rats did not preferentially choose the middle location, which was also the same trend seen while in the positive ambiguous condition

and the original ambiguous condition without treatment injection. Both the negative ambiguous condition and the acute stressed rats demonstrated no significant preference between any of the three ambiguous locations (i.e. no negative cognitive bias was observed). These results differed from that of experiment 2, where it was found that CORT treatment induced a preferential choice at the pessimistic location (P3), indicative of a negative cognitive bias.

Such differences are unlikely to be due to chance or individual differences, as we do see a very clear positive cognitive bias as soon as the condition changed from partially ambiguous to completely ambiguous. This potentially indicates that both components of the ambiguous condition, environmental and choice location cues, must be “ambiguous” at the same time in order to accurately test cognitive bias. The lack of difference in positive and negative choices in the positive and negative ambiguous conditions could have been due to the novelty of the condition itself. The environmental visual and tactile cues were very salient to rats, and rats may therefore have been habituated to the environment enough to see it as a new “novel” condition to explore. Another interesting result to note is the subtle shift of cognitive bias from no treatment injection to the VEH treatment. There was a slight shift towards less positive choices and more negative choices. Although the differences were not statistically significant, this shift is still worth noting, as it may indicate the potential adverse effect of the act of injection on stress levels in the rat.

When the choices of the acute stressed rats were compared to that of the vehicle rats on days 4 and 5, the results showed a difference from that of chapter 2. In the previous study, acute corticosterone challenge elicited a negative response to the ambiguous stimuli in comparison to the control rats. In the present study, this interaction was not seen. Following exposures to a variety of ambiguous stimuli combinations, the administration of acute stress did not alter rat’s responses towards a negative cognitive bias. A possible explanation may be that the sequence of the ambiguous stimuli presented somehow changed the value and the ambiguity of the novel environmental to the rats. The rats were repeatedly exposed to the various ambiguous components before the onset of acute stress, first only the choice locations in days 1 and 2, then both environmental and choice location cues on day 3. This was different from the previous study, where the onset of acute stress

challenge was accompanied by the first exposures to the ambiguous condition. Here, as the primary objective was to elucidate any changes in rats' responses to only one component of the ambiguous condition, it was appropriate to expose them to the positive and negative ambiguous conditions first. As such, it is unclear whether rats' responses would change to reflect the pattern observed in the preceding study if the order of the ambiguous conditions presentation was altered.

The current experiment confirmed the importance of ambiguous cues by showing the necessity of combining both ambiguous environmental cues and choice location cues in the evaluation of cognitive bias. Previous studies that have established various rodent models of cognitive bias used both components to create the ambiguous condition. For example, Brydges et al (2011) used different coarseness of sandpaper and olfactory cues (cinnamon versus coriander) in conjunction with chocolate or cheerio reinforcements to create the different testing/training conditions. Thus, it was of interest to examine how different components of the ambiguous stimuli affect cognitive bias responses. In all, the current task demonstrated great potentials in evaluating and elucidating the mechanisms underlying cognitive bias in rats.

4.5 References

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5 GENERAL DISCUSSIONS

The aim of this thesis was to first develop a reliable model of cognitive bias. A task using a non-operant approach was successfully developed to model cognitive bias in rats, using visual and tactile cues in conjunction with higher/lower reward enforcements. This was developed so that changes in cognitive bias, such as that from an acute stress challenge, can be examined using this task. In experiment 1, an appetitive task was created using highly salient visual/tactile cues to evaluate cognitive bias. It was found that under normal, unaltered conditions, healthy male rats displayed a positive cognitive bias (“optimism”) in response to ambiguous conditions. Then, in experiment 2, the same task was used to evaluate the effect of acute stress challenge on cognitive bias. It was found that the baseline optimism was shifted towards pessimism (negative cognitive bias) in response to the ambiguous condition when a (physiologically relevant level of) corticosterone was administered. Lastly, in experiment 3, the ambiguous condition was dissected to further examine the mechanisms underlying cognitive bias. When the two components of the ambiguous condition (environmental visual/tactile cues and the choice location visual cues) were separated, there were no differences in optimistic and pessimistic responses. This indicated that both components of the ambiguous condition should be present in order to accurately evaluate cognitive bias.

The current model presents an improved task for the evaluation of cognitive bias in non-human animals. It overcomes a number of barriers presented by previously established tasks. For example, by using a go/go tasks, which requires the rats to respond actively to both the positive and negative stimuli, reduces possible confounds in the performance and interpretation of results (Enkel et al., 2010). In addition, adopting the use of an open arena enhances the acquisition and interpretation of the task by giving the different locations clearly distinct values (Brydges et al., 2011; Burman et al., 2009). As a result, the present model was successful in the evaluation of cognitive bias under acute stress, specifically in demonstrating a pessimistic shift in cognitive bias from a baseline optimism to reflect a negative affective state. Specifically, a key difference from previously establish tasks was

the use of acute stress challenge instead of chronic stress exposure to examine the shift to pessimism. The maladaptive effect of chronic stress manipulations on affective states have been repeatedly shown in non-human animals to model human neuropsychiatric affective disorders (e.g. Erickson, Drevets, & Schulkin, 2003). To date, few studies have examined cognitive bias and related acute affective disturbances with visual and tactile cues in animal models. Examining the changes in an animal's interpretation of ambiguous stimuli during a cognitive bias task can provide valuable information about their affective state, leading to the development and examination of cognitive bias in a variety of species and tasks. But despite the importance of cognitive bias in the understanding of affective changes, non-human studies of cognitive biases have been largely focused on the effects of chronic stress manipulations. Studying the shifts in cognitive bias as result chronic stress manipulations have high implications in the animal welfare field, as it allows the objective evaluation of "affect" in captive animals. As such, the acute or single exposure of stress on behavior by corticosterone administration have not been studied extensively. It has been shown that acute corticosterone treatments in rats resulted in behaviours that were indicative of anxiety, with behavioural and physiological effects that can last well after the acute stress exposure (Mitra & Sapolsky, 2008; Skórzewska et al., 2007). This, along with the lack of sufficient research on the shifts in cognitive bias as result of acute stress manipulations, makes it important to examine in order to further our understanding of this complex group of affective disorders. Thus, the current model improves upon and extends established studies on cognitive bias by providing a method of evaluating negative cognitive bias following acute stress challenge.

However, despite the advantages associated with the current study, a few important limitations still exist. First, whether the animals' responses to the ambiguous contextual cues would change due to the repeated corticosterone injections over three days was not accounted for. Although the novelty of the ambiguous environment was very clear on the first testing day, it was uncertain whether this ambiguity would diminish over the course of three testing days. As well, the repeated injections of corticosterone during the testing stage could have also impacted the animals' responses to the ambiguous contextual cues either physiologically or emotionally. Adding an extinction stage to the end of testing may help in a more accurate understanding of how an acute stress challenge can impact

cognitive bias. Another important limitation to examine is the possibility of a changed motivation state as result of the manipulations, for example as result of water deprivation. Existing research have shown that 15 hours of water deprivation do not produce sufficient stress on the animals to interfere with their performance, and that the one and a half minute free-access to water should be sufficient to decrease any remaining stress from water deprivation. To further reduce stress and facilitate learning, the rats were pair housed and group trained. However, it is still unclear whether sustained daily water deprivation over approximately three weeks may have an aversive effect on animals' responses.

It is clear that the investigation of cognitive bias in animal models would be conceptually and translationally beneficial to the study of affective disorders. Specifically, negative cognitive biases have been well established as a key characteristic in depression and other psychiatric affective disorders (Clark, Chamberlain, & Sahakian, 2009; Richter et al., 2012). In fact, negative cognitive bias has been shown to play a key role in the etiology, maintenance, and recurrence of depression and anxiety disorders (Beck et al., 1979; Kloke et al., 2014). For example, when anxious individuals enter a social setting, they tend to perceive the environment in a more negative manner, with a correspondingly negative affective state. Such negative affect is often associated with decreased social attention, heightened anxiety levels, poor performance in cognitive and affective tasks, and negative ruminative thinking (Rapee & Heimberg, 1997; Vassilopoulos & Moberly, 2013). Therefore, due to this prominent role of negative cognitive bias in neuropsychiatric disorders, it can be used as a predictor of negative emotional states for the evaluation of these disorders (Brydges et al., 2012). The availability of appropriate animal models for examining emotional disorder interventions has great value in furthering our understanding of human affective disorders. This is not only important on a pathophysiological level, but also for treatments and preventions. However, due to the lack of a comprehensive translational non-human assay for these disorders, the development of innovative therapeutic treatments has been severely limited (Berton, Hahn, & Thase, 2012). This is especially the case for the aforementioned depression and other affective disorders, of which identifying novel drug targets and the evaluation of new treatments are limited by the current availability of animal models (Stuart, Butler, Munafò, Nutt, & Robinson, 2013).

Furthermore, specific neurological systems that have been implicated in negative cognitive bias (i.e. endocannabinoid system and the dopaminergic system) can be explored using this paradigm to further our understanding of affective processing (Boyer, Lecrubier, Peuch, Dewailly, & Aubin, 1995; Che et al., 2013; Kregiel, Malek, Popik, Starowicz, & Rygula, 2016; Schoemaker et al., 1997). Changes in endogenous neuromodulator mechanisms were found to play a role in depression pathology (de Kloet, Joëls, & Holsboer, 2005). For example, Kukolja et al. (2008) found that with noradrenergic-glucocorticoid induced stress, the amygdala activation resulted in the shifting of cognitive processing toward negative stimuli (Kukolja, Klingmüller, Maier, Fink, & Hurlermann, 2011). Similar results were found in an ensuing study that used pharmacologically induced acute stress. Reboxetine (noradrenaline reuptake inhibitor) and Corticosterone manipulations shifted rats' responses away from optimism in a tone interpretation task that also used reward and aversive enforcements (Enkel et al., 2010). Using c-Fos immunoreactivity quantification, it was found that such behavioral variations in cognitive bias were accompanied by changes in the amygdala and dentate gyrus (Enkel et al., 2010). Therefore, these pharmacological challenges that mimic acute stress-like conditions can be applied to the present paradigm to further investigate the underlying neuronal mechanisms of cognitive bias. Future investigations can examine changes in behavioral outputs accompanying acute or chronic pharmacologically elicited stress for a more accurate and specific investigation of cognitive bias in targeted affective disorders. As well, the effect of anti-depressants or other affective state treatments can be applied to the present model to examine their efficacy and effectiveness.

Recent investigations have also been examining positive cognitive bias, specifically looking at positive expectation bias, in an attempt to further understand the mechanisms underlying affective cognition. Utilizing the link between neurohormonal regulatory mechanisms and human/non-human social cognition, it has been found that oxytocin administration induced positive judgement bias in dogs (Kis et al., 2015; Saphire-Bernstein, Way, Kim, Sherman, & Taylor, 2011; Yamasue et al., 2012). It would be beneficial to investigate whether similar effect can be seen in rodent models. These results suggest that the mechanisms underlying biased affective cognition may not be limited to one neurological process, such as neuromodulators. Instead, it may be a close interplay

between different modalities that collectively portrays affective cognition as a more complex process than what we know thus far. Therefore, it would be advantageous for the present paradigm to explore the effect of combining manipulations that elicit either positive or negative cognitive bias, and then measuring whether such interplay would diminish or increase symptoms of biased affective cognition.

Humans widely vary in how they interpret ambiguous events, as well as the extent to which they attend to negative or positive information (Hertel & Mathews, 2011). This phenomenon of positive and negative cognitive bias is the result of the interplay between our emotional states and cognition. Specifically, “pessimistic” behaviours, or a negative cognitive bias, has been found to be related to a negative emotional state. This is a core characteristic of depression and related affective psychiatric disorders. Although cognitive bias is well established in humans, research in translational animal model of cognitive bias is still in its infancy, limiting the development and innovation of therapeutic treatments. Much of the existing cognitive bias animal models studies animal welfare by using chronic stress (e.g. unstable housing) to induce changes in animals’ affective states. Therefore, the effect of acute stress have largely been left unexplored, but remains an important area to study to further understand the etiology of mood disorders. The present study overcomes this barrier by expanding the findings from previous cognitive bias animal models that were established to assess animal affective states (Brydges et al., 2012; Burman et al., 2009; Harding et al., 2004). These findings will be key in furthering our current understanding of the mechanisms underlying cognitive bias. Specifically, it allows for the examination of how a single or repeated acute stressors can impact psychological wellbeing in nonhumans. On the other hand, it also allows for new pharmacological advancements, as current methods to investigate depression-related behaviours (ex. Forced swim test) are limited to assessing only existing drugs (Berton et al., 2012). Overall, the study of affective cognition in animal models are still in its infancy. Therefore, the investigation of a novel behavioural approach to cognitive bias, specifically examining the effect of acute stress, would be beneficial both in the assessment of underlying mechanisms and the development of potential innovative therapeutic treatments.

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CURRICULUM VITAE

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Research Assistant – Ontario Jiangsu Student Exchange Program

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Nanjing University Model Animal Research Center

- Examined the effect of tamoxifen on a transgenic mouse model
- Extensive experience working with, as well as troubleshooting, PCR and Western Blot protein assay.
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Research Assistant

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Guelph Food Research Centre – Agriculture and Agri-Foods Canada

- Development of an effective and efficient method for the extraction and analysis of the chemical Protodioscin in asparagus plants
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- Worked with the gaping rat model to study the effects of extraneous anandamide in comparison to anti-emetic, dual FAAH/MAGL inhibitor JZL-195.
- Maintained and handled rodent populations in 12/12 light dark cycle rodent housing facilities.
- Performed intraperitoneal injections on rats, as well as the utilization of videotaping equipment to capture rats' activity for behavioural scoring.

PUBLICATION

Limebeer, C.L., Abdullah, R.A., Rock, E. M., Imhof, E., Wang, K., Lichtman, A.H. & Parker, L.A. (2014) Attenuation of Anticipatory nausea in a rat model of contextually elicited conditioned gaping by enhancement of the endocannabinoid system. *Psychopharmacology*, **231**:603-612

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CONFERENCE PRESENTATIONS

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Wang, K., Ossenkopp, K. P. & Kavaliers, M. (2015, October 16th)
Corticosterone elicits “pessimism” in rats in a novel cognitive bias task. Poster presented at the Society for Social Neuroscience (S4SN) annual conference in Chicago, Illinois

Wang, K., Ossenkopp, K.P. & Kavaliers, M. (2015, June 5-7) A translational Model of Cognitive Bias. Poster presented at the Canadian Society for Brain, Behaviour and Cognitive Science (CSBBCS) annual conference in Ottawa, Ontario

Wang, K., Ossenkopp, K.P. & Kavaliers, M. (2015, May). *A Novel Rat Model of Cognitive Bias*. Poster presented at the Southern Ontario Neuroscience Association (SONA) Annual Meeting in Hamilton, Ontario

AWARDS AND ACCOMPLISHMENTS

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