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Developing a Novel Touchscreen-based Test of Cognitive Judgement Bias

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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

Cognitive judgement bias (CJB) refers to the interpretation of ambiguous stimuli in a negative (pessimistic) or positive (optimistic) way. Negative CJB is observed in depression and anxiety, conditions that burden affected individuals and caregivers. Pre-clinical animal research is key to understanding CJB and developing therapies, so a translationally relevant CJB test would be a useful addition to the existing pre-clinical rodent touchscreen test battery. We pharmacologically validated a mouse touchscreen CJB task using bupropion and tetrabenazine, agents known to induce positive and negative CJB, respectively. Additionally, we validated the task using an ecologically relevant stressor (injection and handling). Bupropion produced an optimistic-like shift in CJB whereas both tetrabenazine and saline injection 30 minutes prior to testing produced pessimistic-like shifts in CJB. These results provide a validated task to assess CJB in mice, which will allow us to ask future questions surrounding its underlying neurobiology and develop treatments for affective disorders.

Keywords

Cognitive Judgement Bias, Cognition, Mouse, Affect, Pharmacological Validation, Bupropion, Tetrabenazine, Injection, Touchscreens, Translation

Summary for Lay Audience

Affect, which refers to the emotions and feelings that we experience, influences cognitive processes such as attention, memory and decision making. During positive affective states, ambiguous cues may be more likely to be interpreted in an optimistic manner, whereas in negative affective states they may be more likely to be interpreted in a pessimistic manner. This is referred to as cognitive judgement bias (CJB). Negative CJB is observed in depression and anxiety, conditions that burden affected individuals and caregivers. It is therefore important to develop novel ways to explore the factors that affect these processes in order to develop effective treatments to target these cognitive-emotional symptoms and disorders. Additionally, the use of pre-clinical animal models in parallel with clinical populations can enable us to translate findings between mice and humans. In humans, we can use self-report measures to assess affect; however, this is not possible in animals. Therefore, researchers have used physiological and behavioural measures to infer animal affective state. Many tests previously employed to assess animal affect are aversive or use methods that may not be relevant to humans. Automated touchscreen tests for animals provide a compelling way to implement identical tests of cognition and affect in animals and humans. Thus, the main objectives of this thesis were to validate a CJB task for mice using touchscreen-based technology. To do so, we used two pharmacological agents which have previously been shown to alter affect. Bupropion, an antidepressant, and tetrabenazine, an agent which induces depressive-like symptoms were used to induce positive and negative affect, respectively. We also investigated the effects of injection and handling (mild stressor) on CJB to test for ecological validity of the task. We found that bupropion administration caused an optimistic-like shift in CJB and tetrabenazine induced a pessimistic-like shift in CJB. Additionally, an injection of saline 30 minutes prior to testing induced a pessimistic-like shift in CJB. Overall, our results suggest that our mouse CJB task can detect both positive and negative shifts in CJB. This task can be used to further understand the mechanisms underlying CJB and for testing therapeutics to treat affect-related disorders.

Co-Authorship Statement

I was the primary person conducting experiments and writing the manuscript. All efforts were made to credit the appropriate sources. With assistance from my supervisors, Dr. Timothy Bussey and Dr. Lisa Saksida as well as my mentor, Dr. Paul Sheppard, I designed and executed experiments, performed statistical analyses, and produced the written thesis. I received assistance with certain aspects of my experiments. The following individuals assisted with my thesis project:

-Ryan Kim: Assisted with running touchscreen behavioural experiments and animal care (3x/week in undergraduate thesis course and 5x/week during summer internship)

-Dr. Paul Sheppard: Aside from my supervisors, Paul was the main source of guidance and assisted with various aspects of my thesis including the task design, pharmacological agent preparation, statistical testing and manuscript editing.

-Dr. Daniel Palmer: Modified training and testing task parameters in the first touchscreen task, developed and coded training programs for the second touchscreen task, developed and coded the testing probe for the third touchscreen task, provided input on parameters for touchscreen training.

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Table of Contents

Abstract ii

Keywords ii

Summary for Lay Audience iii

Co-Authorship Statement iv

Acknowledgments v

Table of Contents vi

List of Figures xi

List of Appendices xvi

List of Abbreviations xvii

1 Introduction 1

1.1 Measures of Affective State 1

1.1.1 Physiological Measures 2

1.1.2 Behavioural Measures 2

1.1.2.1 Learned Helplessness 3

1.1.2.2 Behavioural Despair 3

1.1.2.3 Anxiety 4

1.1.2.4 Anhedonia 5

1.1.2.5 Cognitive Affective Bias (CAB) 5

1.1.2.5.1 Affective Bias Test (ABT) 6

1.1.2.5.2 Judgement Bias Tests 6

1.1.2.5.3 Interpretation Bias 7

1.1.2.5.4 Cognitive Judgement Bias (CJB) 8

1.1.2.5.4.1 Spatial-based Tasks 10

1.1.2.5.4.2 Tactile-based Tasks 11

1.1.2.5.4.3 Olfactory-based Tasks 11
1.1.2.5.4.4 Auditory-based Tasks 12
1.1.2.5.4.5 Visual/Context-based Tasks 13
1.2 Translation of Rodent to Human 14
1.3 Automated Touchscreen Systems 14
1.4 Touchscreen CJB Tasks 15
1.5 Pharmacological Validation 16
1.5.1 Positive CJB 17
1.5.2 Bupropion (BUP) 17
1.5.3 Negative CJB 18
1.5.4 Tetrabenazine (TBZ) 18
1.6 Rationale and Objectives 19
2 Methods and Materials 21
2.1 Animals 21
2.2 Housing and Food Restriction 21
2.3 Touchscreen Apparatus 22
2.4 Pre-training 24
2.5 Task Design and Pharmacological Validation Experiment 24
2.5.1 CJB Task 1 (Lopez-Cruz Variant) 24
2.5.1.1 Habituation 1 25
2.5.1.2 Habituation 2a 25
2.5.1.3 Habituation 2b 25
2.5.1.4 Stimulus Touch 25
2.5.1.5 Target Stimulus Touch 26
2.5.1.6 1 Target (Positive Cue) and 1 Non-Target (Snowflake Cue) 26
2.5.1.7 1 Target (Positive Cue) and 1 Non-Target (Negative Cue) 27

2.5.1.8 1 CJB Task 1 (Lopez-Cruz Variant) Testing Probes 28
2.5.2 CJB Task 2 (Krakenberg Variant)..... 30
2.5.2.1 Initial Touch..... 30
2.5.2.2 Must Touch 31
2.5.2.3 Must Initiate 31
2.5.2.4 Punish Incorrect 1 31
2.5.2.5 Punish Incorrect 2 32
2.5.2.6 Discrimination Training..... 32
2.5.2.6.1 Discrimination 1..... 33
2.5.2.6.2 Discrimination 2..... 33
2.5.2.6.3 Discrimination 3..... 34
2.5.2.6.4 Discrimination 4..... 34
2.5.2.6.5 Discrimination 5..... 34
2.5.2.6.6 CJB Task 2 (Krakenberg Variant) Testing Probes..... 35
2.5.3 CJB Task 3 (2-Stage Novel Variant) 36
2.5.3.1 Visuomotor Conditional Learning (VMCL) Task 37
2.5.3.2 Pairwise Visual Discrimination (PVD) Task..... 38
2.5.3.3 CJB Task 3 (2-Stage Novel Variant) Testing Probes..... 41
2.5.4. Pharmacological Agents..... 42
2.5.4.1 Bupropion (BUP) 42
2.5.4.2 Tetrabenazine (TBZ)..... 42
2.5.5 Timeline and Design 42
2.5.6 Testing and Maintenance Training Washout Period..... 44
2.6 Handling, Transport, and Injection Stress Experiment..... 44
2.6.1 Timeline and Design 45
2.7 Data Collection and Statistical Analyses 46

2.7.1 CJB Task 1 (Lopez-Cruz Variant) 46
2.7.2 CJB Task 2 (Krakenberg Variant)..... 47
2.7.3 CJB Task 3 (2-Stage Novel Variant) 47
3 Results..... 50
3.1 CJB Task Comparison – Training Progression and Duration..... 50
3.1.1 CJB Task 1 (Lopez-Cruz Variant) 50
3.1.1.1 Baseline..... 50
3.1.2 CJB Task 2 (Krakenberg Variant)..... 52
3.1.2.1 Baseline..... 52
3.1.3 CJB Task 3 (2-Stage Novel Variant) 53
3.1.3.1 Baseline..... 53
3.1.4 Comparison of the Three CJB Tasks 55
3.2 Pharmacological Manipulations..... 55
3.2.1 Bupropion (BUP) (5 mg/kg) Produced a Trend Towards a Positive Shift in CJB 56
3.2.2 Bupropion (BUP) (7.5 and 10 mg/kg) Induced a Positive Shift in CJB 56
3.2.3 Tetrabenazine (TBZ) (6 mg/kg) Induced a Negative Shift in CJB 60
3.3 Handling, Transport, and Injection Stress Experiment..... 63
3.3.1 Training Progression..... 63
3.3.2 Baseline..... 64
3.3.3 Handling, Transport, and Injection Alone Were Sufficient to Induce a Negative Shift in CJB..... 65
3.3.4 Comparing Baseline and No-Injection Condition..... 69
4 Discussion 70
4.1 CJB Task Comparison – Training Progression and Duration..... 70
4.2 Advantages and Disadvantages of the CJB Task Designs..... 71
4.3 Experiments: CJB Task 1 (Lopez-Cruz Variant)..... 72

4.3.1 Bupropion (BUP) (5 mg/kg) Induced a Positive Shift in CJB 72

4.3.2 Tetrabenazine (TBZ) Induced a Negative Shift in CJB 73

4.3.3 Handling, Transport and Injection Alone were Sufficient to Induce a Negative Shift in CJB 74

4.3.4 Sex Differences 75

4.3.5 Single-Housed Animals 76

4.4 Limitations 77

4.5 Future Directions 78

4.6 General Conclusions 80

References 81

Appendices 106

Curriculum Vitae 118

List of Figures

Figure 1. Table outlining the most common constructs and tasks related to depression and anxiety in rodents. 7

Figure 2. Environmental enrichment included in the mouse home cages. 22

Figure 3. The Bussey-Saksida mouse touchscreen operant chamber (Lafayette Instrument Company & Campden Instruments Ltd, 2021). 23

Figure 4. A mouse completing a task in the touchscreen chamber (Lafayette, 2024). 24

Figure 5. The "1 Target (Positive Cue) and 1 Non-Target (Snowflake Cue)" stage in which animals learned how to discriminate between the positive cue and snowflake cue. In this example, the positive cue was rotated 45° right from vertical. 27

Figure 6. The "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)" stage in which animals learned how to discriminate between positive and negative cues. In this example, the positive cue was rotated 45° right from vertical and the negative cue was rotated 45° left from vertical. 28

Figure 7. Order of pre-training and discrimination stages in CJB Task 1 (Lopez-Cruz Variant). 28

Figure 8. The five cues used during testing probes. 29

Figure 9. Depiction of optimistic and pessimistic responding to the true ambiguous cue. The mouse will have previously learned to touch the positive cue to receive reward and to not touch the negative cue to avoid a noise and light. Therefore, touching the true ambiguous cue was considered an optimistic response due to the mouse interpreting the ambiguous cue positively (where they were previously rewarded). Conversely, rejecting the true ambiguous cue was considered a pessimistic response due to the mouse interpreting it negatively (to avoid the noise and light). 30

Figure 10. Order of pretraining stages in CJB Task 2 (Krakenberg Variant). 32

Figure 11. An example trial in "Discrimination 2". The positive cue is rotated 45° right from vertical and the negative cue is rotated 45° left from vertical. In this example, when the positive cue is presented, the mouse must respond to the left side to receive a large milkshake reward and when the negative cue is presented, the mouse must respond to the right side to receive a small milkshake reward. Responding to the incorrect side in either condition results in a correction trial..... 35

Figure 12. Depiction of optimistic and pessimistic responding to the true ambiguous cue. The mouse will have previously learned that when they were presented with the cue rotated 45° right from vertical, they were given more milkshake reward on the left side than right side. Therefore, responding to the left side when encountering the true ambiguous cue would be an optimistic response because the mouse would interpret it positively and respond to the corresponding side (where they were rewarded higher). They would have also learned that when they were presented with the cue rotated 45° left from vertical, they were given milkshake reward on the right side and not the left. Therefore, responding to the right side when encountering the true ambiguous cue would be a pessimistic response because the mouse would interpret it negatively and respond to the corresponding side (where they were rewarded). 36

Figure 13. Easy version of VMCL. In this example, when the icicle is presented, the mouse must respond to the left side (rewarded) and when the equal sign is presented, the mouse must respond to the right side (rewarded). Correct side was counterbalanced between animals. 38

Figure 14. Hard version of VMCL. In this example, when the cue rotated 45° right from vertical is presented, the mouse must respond to the left side (rewarded) and when the cue rotated 45° left from vertical is presented, the mouse must respond to the right side (rewarded). Correct side was counterbalanced between animals. 38

Figure 15. Easy version of PVD. In this example, the fan cue is correct (rewarded) and the marbles cue is incorrect (house light on, time out). 39

Figure 16. Hard version of PVD. In this example, the cue rotated 45° right from vertical is correct (rewarded) and the cue rotated 45° left from vertical is incorrect (house light on, time out). 40

Figure 17. The order of training stages for CJB Task 3 (2-Stage Novel Variant)..... 40

Figure 18. Depiction of optimistic and pessimistic responding to the true ambiguous cue. The mouse will have previously learned that when they were presented with the cue rotated 45° right from vertical, they were rewarded in PVD, and needed to respond to the left side if they encountered this cue in VMCL. Therefore, responding to the left side when encountering the true ambiguous cue would be an optimistic response because the mouse would interpret it positively (where they were previously rewarded in PVD) and respond to the corresponding side. They would have also learned that when they were presented with the cue rotated 45° left from vertical, they were not rewarded in PVD, and needed to respond to the right side if they encountered this cue in VMCL. Therefore, responding to the right side when encountering the true ambiguous cue would be a pessimistic response because the mouse would interpret it negatively and respond to the corresponding side (where they were unrewarded in PVD). 41

Figure 19. Experimental timeline for CJB Task 1 (Lopez-Cruz Variant) animals. 43

Figure 20. Experimental timeline for handling, transport, and injection stress experiment. . 45

Figure 21. Baseline CJB responding for n = 11 animals on the CJB Task 1 (Lopez-Cruz Variant). 51

Figure 22. Baseline CJB Responding for n = 2 animals on the CJB Task 2 (Krakenberg Variant). 52

Figure 23. Baseline CJB Responding for n = 5 animals (females) on the CJB Task 3 (2-Stage Novel Variant). 54

Figure 24. Furthest Stage Completed for Each CJB Task. a) CJB Task 1 (Lopez-Cruz Variant): 100% of animals completed all stages (pre-training, discrimination training and testing probes). B) CJB Task 2 (Krakenberg Variant): 6.25% of animals completed all stages. c) CJB Task 3 (Novel 2-Stage Variant): 43.75% of animals completed all stages. 55

Figure 25. Survival Curve for Each CJB Task (Task 1 n = 14, Task 2 n = 16, Task 3 n = 16). CJB Task 1 (Lopez-Cruz Variant) had the shortest training time of all 3 tasks and all animals met criteria to proceed to testing probes. 55

Figure 26. Effect of BUP (5 mg/kg) on CJB Responding (n = 11). Lower dose BUP trended towards increasing response rate at the true ambiguous cue when compared to vehicle. 56

Figure 27. Effect of BUP (7.5 and 10 mg/kg) on CJB Responding (n =11). The 2-way repeated measures ANOVA found a main effect of drug treatment. *A priori* tests found that both 7.5 and 10 mg/kg BUP significantly increased response rate for the true ambiguous cue versus vehicle. 57

Figure 28. Effects of BUP (7.5 and 10 mg/kg) on CJB responding. a) BUP did not affect response latencies for the five cues b) BUP non-significantly increased response rate for positive cue c) BUP non-significantly increased response rate for the negative cue d) BUP did not affect discrimination sensitivity (d') e) BUP induced a more liberal response bias (c) at 7.5 mg/kg only. 60

Figure 29. Effect of TBZ on CJB Responding (n =10). The 2-way repeated measures ANOVA found a significant drug treatment x cue interaction. Post hoc tests reflected differences between TBZ and vehicle at the near-positive, true, and near-negative ambiguous cues. *A priori* tests discovered that TBZ significantly reduced response rates for the true ambiguous cue when compared to vehicle. 60

Figure 30. Effects of TBZ on CJB responding. a) TBZ did not affect response latencies for the five cues b) TBZ significantly reduced response rate for the positive cue c) TBZ significantly reduced response rate for the negative cue d) TBZ did not affect discrimination sensitivity (d') e) TBZ induced a more conservative response bias (c). 62

Figure 31. Comparing CJB Baseline to TBZ and BUP Vehicle Injections. Both types of vehicle injections appeared to decrease the response rate at the near-negative, negative, and true ambiguous cues. 63

Figure 32. Furthest Stage Completed. n =15 animals completed all discrimination training and probe testing sessions and n = 1 animal completed discrimination and part of probe testing sessions and was removed from further analyses..... 64

Figure 33. Baseline CJB responding (n = 15 Animals) for handling, transport, and injection stress experiment..... 65

Figure 34. Effect of Saline Injection and Saline + DMSO Injection on CJB Responding (n = 15). 2-way repeated measures ANOVA found a main effect of condition. *A priori* tests found that “Saline Injection” significantly decreased response rate for the true ambiguous cue versus “No-Injection”. 66

Figure 35. Effects of Saline Injection and Saline + DMSO Injection on CJB responding. a) Response latencies were higher with injection, but values fell within response window b) Condition did not affect response rate for positive cue c) Both injection conditions decreased response rate for the negative cue d) Condition did not affect discrimination sensitivity (d’) e) Both injection conditions induced more conservative response biases (c)..... 68

Figure 36. Baseline vs “No-Injection” CJB Responding (n = 15). There was a significant decrease in response rate at the true ambiguous cue for the no injection condition compared to baseline. 69

List of Appendices

Appendix A: Supplemental Results Figures : CJB Task 1 (Lopez-Cruz Variant) 106

List of Abbreviations

BUP – bupropion

CAB – cognitive affective bias

CJB – cognitive judgement bias

CPT – continous performance task

DMSO – dimethyl sulfoxide

EPM – elevated plus maze

FST – forced swim test

HCl – hydrochloric acid

IP – intraperitoneal

LDB – light-dark box

OF – open field

PVD – pairwise visual discrimination

SPT – sucrose preference test

TBZ – tetrabenazine

TST – tail suspension test

VMCL – visuomotor conditional learning

1 Introduction

Everyone views the world in their own unique way. Some people tend to view the world in a "glass half full" or optimistic manner whereas others view the world in a "glass half empty" or pessimistic manner. One's affective state, or emotional state, which reflects the "experience of feeling the underlying emotional state", can influence this perspective (Thompson et al., 2019). Some examples of affective states include anger, disgust, confusion, frustration, happiness, contempt, sadness, surprise, anxiety, boredom, eureka, fear, neutral and curiosity (D'Mello et al., 2010). In general, when faced with ambiguous stimuli or situations, people in positive affective states are more likely to make optimistic judgements and people in negative affective states are more likely to make pessimistic judgements (Paul et al., 2005). This pessimistic perspective can negatively impact quality of life especially for individuals suffering from conditions such as anxiety and depression (Everaert et al., 2017; Hirsch et al., 2016), and it can also burden their family members and caregivers (Balkaran et al., 2021; Z. Liu et al., 2020). Additionally, negative CJB can predict the onset of developing MDD and it relates to depressive symptom severity (Lee et al., 2016; Phillips et al., 2010). It is therefore important to develop novel ways to explore the underlying processes and factors influencing them, in order to develop effective treatments that can target these cognitive-emotional symptoms and disorders.

1.1 Measures of Affective State

Comprehensive ways by which to measure affective state are necessary for understanding the processes that underlie affective-based disorders in order to develop targeted therapies to promote overall mental health and wellbeing. Preclinical animal models can provide key insight to enable the development of targeted therapeutics for affective-related diseases and disorders (Zhang et al., 2022). There are many ways to measure affect in humans through tools such as the positive and negative affect schedule (PANAS) (Watson et al., 1988), discrete emotions questionnaire (DEQ) (Harmon-Jones et al., 2016), and multiple affect adjective check list (MAACL-R) (Zuckerman & Lubin, 1965) (Boyle et al., 2015; Wilhelm & Schoebi, 2007). Affective state can be measured in animals but, unlike in humans, it cannot be directly assessed, due to its subjective nature

and the tendency of human tests requiring explicit self-report measurements. Previous researchers have attempted to use physiological and behavioural methods to assess affect indirectly in animal models; however, they have been faced with various limitations and criticism (Jirkof et al., 2019). Therefore, it is important to have tools that can reliably and effectively measure affect in animals. It is essential to study both human and animal research in parallel to better understand the underlying neurobiology and relevant mechanisms.

1.1.1 Physiological Measures

One method of assessing affective state in animals has been through physiological measures. Some researchers have used measures including body weight loss and corticosterone (rodent stress hormone) levels to infer negative affective state related to pain and distress (Bodden et al., 2018; Häger et al., 2018; Pfeiffenberger et al., 2015). Others have used heart rate, breathing rate and body temperature to investigate the negative effects of certain housing situations and during post-operative recovery (Häger et al., 2018; Hohlbaum et al., 2018; Miller & Leach, 2015; Roughan et al., 2014; Späni et al., 2003; Van Loo et al., 2007). However, body weight could remain stable even if other measures suggest a negative affective state, and low levels of stress hormones do not necessarily indicate a positive affective state (Jirkof et al., 2019). Therefore, these measures may not be suitable for assessing positive affective state (Mellor, 2016). The ability to detect animal affective state is important for evaluating and improving animal welfare (overall quality of life), especially in research (Jirkof et al., 2019). In general, physiological measures are indirect and primarily study peripheral markers of stress as opposed to cognitive aspects of affect.

1.1.2 Behavioural Measures

A different way of measuring affective state is by using behavioural testing paradigms. These tests assess different constructs which are often seen in affective disorders which include learned helplessness, behavioural despair, anxiety, anhedonia, and cognitive affective bias (Gencturk & Unal, 2024).

1.1.2.1 Learned Helplessness

Learned helplessness is indicated by the subject withdrawing efforts to combatting a repeated inescapable situation or stimulus (Seligman & Maier, 1967). The original development of the learned helplessness test was in dogs (Seligman & Maier, 1967) and has since been adapted for mice (Braud et al., 1969). The mouse learned helplessness paradigm includes a control group (no aversive stimulus), helpless group (inescapable stressful situation) and non-helpless group (escapable stressful situation) (Chourbaji et al., 2005). The paradigm employs a shock chamber with two compartments (shock and no-shock). Mice first experience a training period during which they are repeatedly exposed to aversive stimuli, which are normally foot shocks. The non-helpless group learns to enter the no-shock compartment to avoid the shock, however the helpless group cannot escape the shock. They then encounter a test session during which they encounter the shocks, but now have an option to escape the stressor via the no-shock compartment. Mice display learned helplessness by their reduction in effort expended to escape the stressor, even if escape is available. Helplessness is assessed during the test session by the number of escape failures and the escape latency whereby a helpless mouse would have a high number of escape failures and a high escape latency. Learned helplessness is not only used as a behavioural test but also as a method by which to induce depressive-like behaviour, which confounds the measure of affect with the stress induced from the test itself (Becker et al., 2021; Gencturk & Unal, 2024). Overall, since this test is aversive for the animals and contains a key confound, alternative tests should be considered.

1.1.2.2 Behavioural Despair

Behavioural despair is similar to learned helplessness. Behavioural despair tasks take place in inescapable aversive situations during which after initial efforts to escape, rodents display decreased locomotion and increased immobility (Unal & Canbeyli, 2019). The forced swim test (FST) and tail suspension test (TST) assess behavioural despair (Castagné et al., 2011). In the FST, the animal is placed in an inescapable pool of water (Porsolt et al., 1977), whereas in the TST, the animal is suspended by its tail (Steru et al., 1985). Both FST and TST measure the time at which the animal surrenders effort, reflected by increased immobility time (Yankelevitch-Yahav et al., 2015).

The FST and TST have been used to screen the efficacy of antidepressant drugs (Can et al., 2012a; Can et al., 2012b). The construct validity of the FST and TST has been criticized, some authors suggesting that the test may not be measuring helplessness, but instead an adaptive learned response, and therefore may not truly be measuring underlying affective state (Armario, 2021; Nestler & Hyman, 2010; Yankelevitch-Yahav et al., 2015). Also, the nature of these tests is aversive, and they induce stress, which may confound the behavioural measure itself (de Kloet & Molendijk, 2016). Overall, due to the aversiveness and questionable construct validity, the learned helplessness test, FST and TST may not be ideal tests for measuring animal affect.

1.1.2.3 Anxiety

Other tests used as proxies for measuring affect include the light-dark box (LDB) (Crawley & Goodwin, 1980), elevated plus maze (EPM) (Pellow et al., 1985) and open field (OF) test (Hall & Ballachey, 1932), which are classical tests used to evaluate anxiety-like behaviours. In the LDB, animals are placed in an apparatus that has both light and dark compartments, linked through a passage (Crawley & Goodwin, 1980). Rodents are averse to bright and open spaces, therefore an increased time spent in the dark box would indicate increased anxiety (Bourin & Hascoët, 2003). In the EPM, the animal is placed in a cross-shaped structure which contains two open and two closed arms (Pellow et al., 1985). Increased time spent in, or number of entries into, the closed arms reflect increased anxiety levels (Gencturk & Unal, 2024). In the OF, animals are placed in a square-shaped arena box (Seibenhener & Wooten, 2015). Decreased time spent in the centre and increased time spent near the walls in the periphery would reflect increased anxiety (Gencturk & Unal, 2024; Seibenhener & Wooten, 2015). One of the main issues with tests such as the LDB and EPM is that it is difficult to distinguish between heightened exploratory/approach behaviour and reduced anxiety levels; a mouse would spend more time in the bright open areas in both scenarios (Cryan & Holmes, 2005). Therefore, while these tests may provide some insight into anxiety-like behaviours, behavioural interpretations may not be completely straight-forward.

1.1.2.4 Anhedonia

Another related construct is anhedonia, the impaired ability to experience pleasure (Papp et al., 1991). Anhedonia is a main feature of depression whereby individuals often display a reduced interest in activities they previously enjoyed (Cooper et al., 2018). This construct is measured in rodents using the sucrose preference test (SPT) (Willner et al., 1987). The SPT relies on the assumption that rodents have a natural preference for sweet foods (Liu et al., 2018). The test measures an animal's preference for sucrose solution compared to regular water, whereby an animal exhibiting anhedonia would prefer the water over the sucrose solution (Liu et al., 2018; Pothion et al., 2004). Researchers have criticized the SPT's poor construct validity. Different factors such as animal strain, test timing and sucrose solution concentration may contribute to discrepancies in results (Gencturk & Unal, 2024). The SPT has also been criticized for its poor ability to translate to humans. While anhedonia is commonly assessed in humans using questionnaires (Snaith et al., 1995), when participants were given a similar sweet taste test, those with major depressive disorder (MDD) did not differ from those without MDD in terms of sensitivity to sucrose, hedonic response, or designation as sweet-liking or sweet-disliking (Dichter et al., 2010). Additionally, rodent tests of anhedonia (SPT) use sugar as the main reinforcer, whereas human tests use monetary or social rewards as reinforcers (Fussner et al., 2018). In all, due to the variability of past results and lack of a translatable protocol, the SPT is not suited to measure affective state across rodents and humans.

1.1.2.5 Cognitive Affective Bias (CAB)

A promising alternative to traditional methods of assessing animal affective state is through cognitive affective bias (CAB) testing. CAB is an umbrella term that refers to how affective state influences cognitive processes including decision making, attention and explicit memory (Gencturk & Unal, 2024; Hales et al., 2014). CAB can be measured using dedicated CAB tasks. CAB tasks differ from tests that measure anhedonia and behavioural despair in that CAB tests focus on biased mental functions (i.e. attention, explicit memory and decision making) that are thought to contribute to the manifestation and persistence of depression/mood disorders whereas the other tests measure behaviours that are commonly seen as symptoms in depression (Elliott et al., 2011; Gencturk &

Unal, 2024). Two types of tasks used to measure CAB include the affective bias test (ABT) and judgement bias tests (Gencturk & Unal, 2024).

1.1.2.5.1 Affective Bias Test (ABT)

First, the affective bias test (ABT) is a bowl digging task that was developed for rats by Stuart et al. (2013) with later adaptations for mice (Graulich et al., 2016). The ABT employs a within-subjects procedure during which rodents learn to associate a food reward with a specific digging substrate (e.g. sawdust, sand, cloth) in two separate experiences (e.g. experience 1: sawdust predicting sugar pellet reward vs sand predicting no reward, experience 2: cloth predicting sugar pellet reward vs sand predicting no reward). During these learning sessions, rodents are exposed to either a neutral condition or a pharmacological condition where they are administered an agent intended to manipulate affect (e.g. fluoxetine in experience 1 and vehicle in experience 2). Afterwards, they complete a preference test where they are exposed simultaneously with both previously rewarded substrates (e.g. sawdust and cloth), and they must choose between them. These affective-based manipulations are used to change the perceived value of reward when given the choice between two equal-value digging substrates. Affective bias is then quantified based on the rodent's choices whereby digging more in the fluoxetine-paired substrate (sawdust) bowl versus the vehicle-paired substrate (cloth) bowl would indicate a positive affective bias.

1.1.2.5.2 Judgement Bias Tests

Second, the use of judgement bias tests, which is also referred to as interpretation bias testing in humans and cognitive judgement bias (CJB) testing in animals, reflects affective state based on the individual's interpretation of ambiguous stimuli in an optimistic or pessimistic manner (Bethell, 2015). Figure 1 represents a table outlining the different affect-related constructs and tasks.

Measures of Affect

Construct	Tasks
Behavioural Despair	• Forced swim test (FST) • Tail suspension test (TST)
Learned Helplessness	• Learned helplessness test
Anhedonia	• Sucrose preference test (SPT)
Anxiety	• Light-dark box (LDB) • Elevated plus maze (EPM) • Open field (OF)
Cognitive Affective Bias	• Affective bias test (ABT) • Cognitive judgement bias (CJB) tests

Figure 1. Table outlining the most common constructs and tasks related to depression and anxiety in rodents.

1.1.2.5.3 Interpretation Bias

An interpretation bias is a type of cognitive bias that refers to the tendency to interpret ambiguous stimuli in a consistent way, which can be either threatening/negative or positive (Schoth & Liossi, 2017). Examples of stimuli could include images, facial expressions, social scenarios and more (Miers et al., 2020). There are negative interpretation biases ("seeing the glass as half empty") and positive interpretation biases ("seeing the glass as half full") (Berna et al., 2011). A negative interpretation bias occurs when an individual is prone to interpreting an ambiguous entity in a negative or threatening way instead of neutral or positive way (Butler & Mathews, 1983). Previous work has linked anxiety with negative interpretation biases (Mathews & MacLeod, 2005). Eysenck et al. (1991) found that anxious individuals were more likely than non-anxious individuals to choose a threatening interpretation instead of neutral interpretation when resolving ambiguous sentences. Additionally, negative interpretation bias has been linked to clinical depression, depressed mood, and depression severity (Lee et al., 2016;

Rude et al., 2003). Interpretation bias has also been studied in cases such as chronic pain (Schoth & Liossi, 2016; Schoth et al., 2016) and cancer fear (Miles et al., 2009) (Schoth & Liossi, 2017). Previous studies found that individuals with chronic pain were more likely to make pain or illness-related interpretations of ambiguous information compared to healthy controls (Schoth & Liossi, 2016; Schoth et al., 2016). In addition, Miles et al. (2009) found that those with a high fear of cancer tended to make more negative interpretations of ambiguous cancer-related scenarios than those with a low fear of cancer.

There are many paradigms that assess interpretation bias (Schoth & Liossi, 2017). These tasks often differ by stimulus type and the response type (Puccetti et al., 2023). The task stimuli often fall into one of three main categories which include ambiguous scenarios, images, or words. The response type could require participants to rate the likelihood of one specific interpretation or they may have to choose between a set of predetermined interpretations (Schoth & Liossi, 2017). One example of an interpretation bias task is the word sentence association paradigm (WSAP) (Beard & Amir, 2009). In the traditional version, participants are presented by a prime word then an ambiguous sentence. Afterwards, they must decide if the prime word is related to the sentence. The prime word is unambiguous and either threatening or benign. For example, participants might have the sentence, “People laughed after something you said” with the prime words being “embarrassing” (threatening) or “funny” (benign) (Beard & Amir, 2009). In the modified version (Hindash & Amir, 2012), the sentence is presented before the unambiguous word.

1.1.2.5.4 Cognitive Judgement Bias (CJB)

Although there are many ways to evaluate cognitive affective biases in humans, there is a need for animal models to understand the underlying mechanisms of affective disorders. To expand upon the human research, work has been done to adapt these tasks for use in animals (Gencturk & Unal, 2024). Interpretation bias is evaluated in animals through cognitive judgement bias (CJB) tests (Zhang et al., 2022). There are a variety of reasons why CJB is a superior measure to other physiological and behavioural measurements. First, CJB allows the objective assessment of positive affect. The majority of past research has focused on behavioural and physiological measures of negative affect and

has neglected the investigation of positive affect (Mellor, 2015; Paul et al., 2005). Additionally, CJB distinguishes between emotion and arousal whereas physiological measures such as corticosterone levels may not distinguish between arousal from excitement or arousal from fear (Hemsworth et al., 2015; Mendl et al., 2010). CJB is also generalizable across a plethora of species and helps to assess non-human animal affective state, which is particularly important for animal care and welfare (Bethell et al., 2012; Bethell, 2015). For example, addition of environmental enrichment induced an optimistic CJB in rats (Brydges et al., 2011) and pigs (Douglas et al., 2012). Overall, CJB is considered to be the gold standard for investigating affective state in non-human animals (Bateson & Nettle, 2015; Mendl et al., 2009).

A landmark task in the CJB field was developed for rodents by Harding et al. (2004). Rats were trained to press a lever for a food reward in response to one auditory tone (positive condition) and to refrain from pressing the lever to avoid a white noise (negative condition) upon hearing a different tone. Afterwards, they were presented with different unrewarded and unpunished ambiguous tones (tones of frequencies in between those of the of positive and negative trained stimuli) and their responses to those tones were calculated. In their study, rats were divided into two groups, the ‘predictable’ housing group (regular conditions), and the ‘unpredictable’ housing group, where housing conditions were disturbed at random to induce depressive-like symptoms (Willner, 1997; Zurita et al., 2000). They found that those in the ‘unpredictable’ housing groups exhibited more pessimistic behaviour than those in the ‘predictable’ housing group through their higher latencies (slower) and fewer lever presses in response to the ambiguous tones close to the positive tone. They concluded that this task could provide insight into animal mood.

The work done by Harding et al. (2004) initiated more in-depth investigation of CJB in animals and its underlying mechanisms. Since then, many animal tasks of CJB have been developed which follow a similar format. In typical animal CJB tasks, animals are trained to discriminate between a positive (rewarded) condition and negative condition (unrewarded and/or punished). During testing, they then are presented with ambiguous stimuli which are intermediates or blends of the positive and negative stimuli. Their

interpretation of those stimuli in an optimistic way or pessimistic way provides an indication of their affective state. It is assumed that animals interpreting the ambiguous stimulus positively and therefore displaying a positive CJB, would be in a putative positive affective state. Conversely, animals interpreting the ambiguous stimulus negatively and therefore displaying a negative CJB, would be in a putative negative affective state (Lagisz et al., 2020). In many tasks, multiple ambiguous stimuli are evaluated, where three ambiguous stimuli are often used (near-positive stimulus, mid-point/true ambiguous stimulus and near-negative stimulus) (Lagisz et al., 2020).

As researchers develop and validate behavioural tasks to assess affect in animals, there are important factors to consider. Two important measures that characterize suitable tasks include construct validity and predictive validity. Construct validity refers to how effectively the task measures what it is supposed to measure and is used to determine whether the task is sensitive to deliberate manipulations of affective state (Lagisz et al., 2020; Neville et al., 2020; Resasco et al., 2021; Strauss & Smith, 2009). Predictive validity refers to how well the task reflects the expected outcome, where here it would assess if the task produced judgement bias shifts in the predicted directions based on the drug-induced change in affect reported in humans (De Vry & Schreiber, 1997; Neville et al., 2020). Overall, it is important to have a CJB task that is non-aversive, has both construct and predictive validity and that measures positive and negative shifts in CJB. In recent years, animal tasks have used stimuli of different modalities including spatial, tactile, olfactory, auditory, and visual (Lagisz et al., 2020; Nguyen et al., 2020).

1.1.2.5.4.1 Spatial-based Tasks

In spatial judgement bias tasks, animals are normally required to discriminate between different spatial locations (rewarded at one location and not at the other) and the bias is evaluated at one or various intermediate, ambiguous locations (Roelofs et al., 2016). Burman et al. (2008) found that rats living without environmental enrichment responded less optimistically than rats with enrichment, but only at the near-negative location. Also, Krakenberg et al. (2019a) developed a novel tunnel length discrimination paradigm. While they were able to generate the typical graded CJB response curve (internal validity), they did not attempt to validate this task using any affective manipulation, and

so construct validity remains to be demonstrated. Other versions of mouse spatial judgement bias tasks have been developed but either have not attempted (Hintze et al., 2018) or failed to demonstrate construct validity (Bailoo et al., 2018; Kloke et al., 2014; Novak et al., 2015; Novak et al, 2016a; Verjat et al., 2021). Based on these findings, the use of spatial tasks is not yet suitable for measuring CJB due to the lack of sufficient validation for the majority of tasks. Additionally, findings from spatial-based paradigms may not be generalizable or easily adapted for use in humans.

1.1.2.5.4.2 Tactile-based Tasks

In tactile judgement bias tasks, animals discriminate between different surfaces (e.g. grades of sandpaper) and the bias is probed using intermediate grade surfaces (Nguyen et al., 2020). Brydges et al. (2011) developed a tactile judgement bias task for rats. They found that rats moved from unenriched cages to enriched cages showed optimistic responding for the ambiguous stimulus whereas rats kept in unenriched cages showed pessimistic responding. However, when using their task to investigate the effects of juvenile stress on CJB, their findings were unclear and contrary to expectations; rats exposed to juvenile stress were more optimistic than unstressed rats (Brydges et al., 2012). Chaby et al. (2013) adapted the Brydges et al. (2011) task and found that adolescent rats exposed to chronic unpredictable stress (social and physical) displayed a significant negative CJB compared to control rats on the first day. However, no significant differences were seen between groups on subsequent testing days and more investigation is warranted. The task developed by Novak et al. (2016b) also failed to demonstrate construct validity. Overall, given the mixed findings, tactile tasks could be suitable for assessing CJB in rodents, but more investigation is needed. Additionally, like spatial-based paradigms, tactile-based tasks may not be generalizable or easily adapted for use in humans.

1.1.2.5.4.3 Olfactory-based Tasks

In olfactory judgement bias tasks, animals must discriminate between different scents, and they are evaluated on ambiguous scents (Lagisz et al., 2020). Few studies have employed olfactory-based paradigms. The olfactory-based task for mice developed by

Boleij et al. (2012) failed to demonstrate construct validity. When attempting to use the task, they found that 129P3J mice could not discriminate between cues and for their BALB/cJ mice, all animals interpreted the ambiguous cue pessimistically. Resasco et al. (2021) developed and successfully validated an olfactory digging task in mice. They found that animals exposed to environmental enrichment responded optimistically compared to those in standard housing conditions. They used the task further to investigate the potential effects of cancer on mood and found that male mice with tumours responded more pessimistically than controls. While their task demonstrated construct validity, predictive validity should be attempted for the future. In all, given the small number of studies, olfactory tasks could be suitable for assessing CJB in rodents, but more investigation is needed. Additionally, like spatial-and tactile-based paradigms, olfactory-based tasks may not be generalizable or readily adapted for use in humans.

1.1.2.5.4.4 Auditory-based Tasks

In auditory judgement bias tasks, animals must discriminate between different auditory tones and are then tested with ambiguous tones (Lagisz et al., 2020). Auditory-based tasks have been more extensively used and validated in rodents. The Harding et al. (2004) task was adapted by Enkel et al. (2010) via their ambiguous-cue interpretation paradigm. Rats learned to press one lever when hearing one tone to receive reward and press a separate lever when hearing a different tone to avoid a foot-shock. During testing, they were played ambiguous tones, and their responding was measured by their proportions of presses to the positive or negative levers. They found that congenitally helpless rats (animal model of depression), displayed a negative bias via increased negative and decreased positive responding.

Rygula et al. (2012) further modified the task by Enkel et al. (2010). In their 2012 study, they found that rats that were tickled (induction of positive affective state) that made more 50-kHz ultrasonic vocalizations (‘laughter’) responded more optimistically when faced with an ambiguous tone compared to rats that made fewer vocalizations or that were not tickled (handling). The same laboratory continued to use the modified task and found that rats who were exposed to chronic psychosocial stress were more pessimistic compared to controls (induction of negative affective state) (Papciak et al., 2013).

Additionally, their laboratory investigated the influence of pharmacological agents on CJB using their task. They induced a positive CJB using lower dose citalopram (antidepressant) and higher dose *d*-amphetamine (psychostimulant) (Rygula et al., 2014a). Therefore, this particular task demonstrated construct validity and predictive validity; however, the use of an aversive footshock as a negative reinforcer could introduce confounds.

Hales et al. (2016) also modified the Rygula et al. (2012) paradigm to remove the aversive footshock; therefore, the tone in the positive condition was associated with a high value food reward and the tone in the negative condition was associated with a low value food reward. They found that acute treatment with an anxiety-inducing drug FG-7142, and chronic restraint stress and social isolation both induced a negative CJB. In this case, they demonstrated construct and predictive validity using a non-aversive method. While this task appears to be promising for use in rodents, future work should investigate its use in humans. Overall, auditory-based tasks have been more extensively studied and validated in rodents, but the auditory-based methods may not be as convenient for use in humans.

1.1.2.5.4.5 Visual/Context-based Tasks

While animals may be better suited to olfactory, tactile, or auditory-based tasks, humans may be better suited to visual-based tasks. In visual based cognitive judgement bias tasks, animals must discriminate between different visual cues, and they are evaluated on ambiguous versions (Lagisz et al., 2020). In such tasks, different images and colours are often used as cues (Zhang et al., 2022). Hodges et al. (2022) developed a novel context-based discrimination paradigm in which rats were trained to discriminate between two different contexts (shock-paired and no-shock-paired) and tested on an ambiguous context. However, this task uses aversive footshock and they did not attempt to demonstrate construct or predictive validity. In general, surprisingly, a relatively low number of visual-based studies have been conducted in mice, and recent reviews have promoted further investigation (Nguyen et al., 2020).

1.2 Translation of Rodent to Human

While studying CJB in animals alone is important for welfare purposes, the ability to translate findings from animals to humans using the same or highly similar testing paradigms is essential when assessing affect. Much of the previous work has focused on using specific tasks solely in animals or solely in humans. However, some work has started to develop tasks in humans that are based on the pre-existing judgement bias tasks in animals. Anderson et al. (2012) adapted the rat auditory-based judgement bias task for use in humans and results in humans were similar as the rodent findings (Harding et al., 2004; Enkel et al., 2010). Additionally, Neville et al. (2021a) created a human version of their judgement bias task, based on the previously employed rodent task (Jones et al., 2018). Neville et al. (2021b) then adapted their task so that food was used as the main reinforcer. While these are interesting findings, more research should continue along this avenue and translate findings between rodents and humans. Specifically, touchscreen technology provides an appropriate method for doing so.

1.3 Automated Touchscreen Systems

One recent approach for investigating CJB has been the use of automated touchscreen technology. Previous work has employed the touchscreen operant chambers to allow rodents and humans to complete the same or highly similar tests that assess the same cognitive processes to translate finings across both species (Nithianantharajah et al., 2015). The touchscreens also enable the investigation of the underlying neurobiological basis of diseases and disorders using a combination of chemogenetic, optogenetic and pharmacological methods in combination with behavioural measures in rodents. Additionally, touchscreen chambers are fully automated, which ensures minimal experimenter interference, and they generate consistent and comparable results. This yields better translation to human research and can eliminate stressful confounds when evaluating mouse performance (Lopez-Cruz et al., 2021) making the use of touchscreen cognitive testing an ideal modality for CJB tasks.

1.4 Touchscreen CJB Tasks

Krakenberg et al. (2019a) developed a new visual-based active choice task using touchscreen-based technology for mice. For their cues, they used single horizontal lines, positioned at either at the top or bottom of the presentation window. The mice were required to discriminate between line locations and choose the appropriate side based on the reward contingencies. For example, when the bottom horizontal line was displayed, the mouse was trained to choose the right side to receive a high reward instead of a low reward on the left side. Conversely, when the top horizontal line was displayed, the mouse was trained to choose the left side to receive a low reward instead of no reward, time out and houselight activation on the right side. During testing, mice were presented with horizontal lines at ambiguous positions. When animals were presented with ambiguous cues, they would be making an optimistic choice if they chose the left side (high reward during training) and a pessimistic choice if they chose the right side (low reward during training). Overall, this task produced the typical CJB response curve, demonstrating internal validity.

In later uses of the 2019 touchscreen-based task, Krakenberg et al. (2019b) investigated changes in CJB in serotonin transporter (5-HTT) knockout mice. There were no differences in CJB between wildtype, heterozygous knockout and homozygous knockout mice despite finding higher anxiety levels in the homozygous knockout mice in standard anxiety tests. Also, Krakenberg et al. (2020) investigated the effects of different social experiences (adverse, beneficial, or neutral) on CJB. In the adverse experience, mice encountered a dominant opponent, where the confrontation with defeat is thought to induce anxiety (Jansen et al., 2010). In the beneficial experience, mice encountered female urine, where the pheromones are thought to induce positive affect (Aikey et al., 2002). In the neutral experience condition, mice were placed in a clean cage. After a baseline CJB measurement, they were exposed to one of the three experiences followed by a second CJB testing point. They found no significant differences between the two CJB testing points in the adverse or neutral experience conditions. Interestingly, in the beneficial experience condition, mice responded more pessimistically to the most

ambiguous cue and reduced responding at the negative cue. This suggests that the results were not fully attributable to CJB, and other factors may be contributing.

Further, Bračić et al. (2022) used the Krakenberg task to investigate the role of genetic and environmental factors on CJB in female C57BL/6J and B6D2F1N mice in either scarce (regular housing) or complex (daily access to enriched environment playgrounds) environments. Neither genotype nor environment significantly affected CJB responding. In Krakenberg et al. (2019b), Krakenberg et al. (2020) and Bračić et al. (2022), they failed to demonstrate changes in affective state with their chosen manipulations. Therefore, this task has yet to demonstrate sufficient construct validity. Viktorov (2022) further adapted the Krakenberg et al. (2019a) touchscreen-based task with modified training stages and new cues (squares with vertical and horizontal lines) and investigated potential pharmacological-induced changes in CJB. Contrary to expectations, they were unable to alter CJB after treatment with amphetamine or ketamine compared to controls and therefore failed to demonstrate predictive validity. Collectively, these findings suggest that although the task uses translational methods and has internal validity, it has insufficient construct and predictive validity.

1.5 Pharmacological Validation

Given the importance of adequate task validation, previous research has focused on pharmacologically validating judgement bias tasks as a way of demonstrating predictive validity. Predictive validity has also been considered a gold standard for validating behavioural tests that measure affective state (De Vry et al., 1997; McArthur & Borsini, 2006). Pharmacological validation involves administering drugs that are known to alter affect either positively or negatively in humans to induce predicted shifts in judgement bias (Neville et al., 2020). In animals, it is presumed that a positive CJB reflects a putative positive affective state and negative CJB reflects a putative negative affective state (Gencturk & Unal, 2024). One important note when referring to animal studies is that one cannot conclude that affect changes induced by pharmacological agents *directly* influenced judgement bias. There are many ways that the drugs may have acted to alter judgement bias, and they may not be acting in the same way in animals and humans (Neville et al., 2020). Pharmacological validation of CJB tasks has been conducted in

various species. While many studies have been conducted in rats (Enkel et al., 2010; Golebiowska & Rygula, 2017; Hales et al., 2016; Hales et al., 2017; Hales et al., 2020; Hales et al., 2022; Neville et al., 2020; Rygula et al., 2014a; Rygula et al., 2014b; Rygula et al., 2015), other studies have included species such as dogs (Kis et al., 2015), chickens (Iyasere et al., 2017), sheep (Doyle et al., 2011; Verbeek et al., 2014a, Verbeek et al., 2014b) and pigs (Stracke et al., 2017a; Stracke et al., 2017b).

1.5.1 Positive CJB

In attempts to find pharmacological treatments for affective disorders, previous studies have sought to demonstrate the efficacy of various pharmacological agents to induce positive and negative affect. In rats, scopolamine (acetylcholine muscarinic receptor antagonist), higher dose CP-101,606 (NMDA receptor antagonist, selective to Glu N2B subunit) and ketamine (NMDA receptor antagonist), agents previously shown to have rapid antidepressant effects in humans, have been used to induce positive shifts in CJB via acute administration (Hales et al., 2020). Other agents used to induce an optimistic bias include lower dose citalopram (selective serotonin reuptake inhibitor), higher dose *d*-amphetamine (dopamine releaser), amphetamine (psychostimulant) and medium dose of lithium (mood stabilizer) (Hales et al., 2017; Rygula et al., 2014a; Rygula et al., 2015). One notable treatment is bupropion (BUP). Preliminary results found that acute administration of BUP induced a positive shift in CJB in a previously used touchscreen-based task in mice (Lopez-Cruz et al., in prep).

1.5.2 Bupropion (BUP)

BUP is classified as an atypical antidepressant that acts as a noradrenaline-dopamine reuptake inhibitor (NDRI) (Patel et al., 2016). It blocks the presynaptic reuptake of norepinephrine and dopamine from the synaptic cleft (Bardal et al., 2011; Stahl et al., 2004). Work suggests that BUP works primarily in the nucleus accumbens and prefrontal cortex (Stahl et al., 2004). Clinically, BUP reduced anhedonic symptoms compared to placebo in depressed outpatients (Tomarken et al., 2004). Many randomized controlled trials suggest that BUP is superior to placebo when treating depression (Patel et al., 2016; Clark et al., 2023). Additionally, Walsh et al. (2018) found that acute BUP treatment on

healthy volunteers was able to increase positive emotional processing and decrease negative emotional processing. BUP biased participants towards recognizing ambiguous faces as happy and reduced attention for fearful faces in comparison to placebo.

Previous research using BUP in mice has found that it decreases immobility time in the FST and TST (Dhir & Kulkarni, 2007). Also, mice encountering chronic unpredictable mild stress that received BUP had decreased immobility time in the TST, increased sucrose preference in the SPT and increased time spent in the open arms in the EPM (Gavzan et al., 2023). BUP injections have also been shown to reverse depression-like (TST) and anhedonia-like behaviour (SPT) in mice that was induced by housing in large cages (Kurogi et al., 2023).

1.5.3 Negative CJB

In rats, acute administration of FG-7142 (anxiogenic drug), co-administration of corticosterone and reboxetine (to pharmacologically mimic neurobiological stress) and corticosterone have been used to induce negative shifts in CJB (Enkel et al., 2010; Hales et al., 2016; Hales et al., 2022). Another notable treatment is tetrabenazine (TBZ). Stuart et al. (2017) found that acute treatment of TBZ induced a negative bias in their affective bias test in rats. Additionally, preliminary results found that acute administration of TBZ induced a negative shift in CJB in a previously used touchscreen-based task in mice (Lopez-Cruz et al., in prep).

1.5.4 Tetrabenazine (TBZ)

TBZ inhibits the vesicular monoamine transporter 2 (VMAT), which prevents monoamines from being loaded into vesicles in presynaptic neurons (Pettibone et al., 1984; Scherman, 1986). This therefore increases degradation and depletion of monoamines such as dopamine, serotonin, and norepinephrine (Lane et al., 1976; Pettibone et al., 1984). TBZ has been prescribed in humans for hyperkinetic movement disorders such as Huntington’s disease (de Tommaso et al. 2011, Kenney & Jankovic, 2006). TBZ has previously induced fatigue, apathy, and depressed mood in humans (Caroff et al., 2018; Frank, 2010).

TBZ has also been used in animal models of depression (Kent et al., 1986; Preskorn et al., 1984; Wang et al., 2010). Carratalá et al. (2023) conducted a study in mice and found that TBZ increased immobility in the forced swim test but did not affect sucrose consumption or anxiety (elevated plus maze, light-dark box) in either sex. Additionally, BUP reversed these effects as demonstrated by decreased immobility time and increased time swimming and climbing in the forced swim test.

1.6 Rationale and Objectives

For this project, we aimed to use translational and non-aversive methods to assess CJB. The use of touchscreen-based technology is a compelling way to investigate CJB. We therefore sought to eliminate stressful confounds from the task itself and have a task that could be applicable to other non-human animal species and potentially humans in the clinic. The use of preclinical research using mouse models is essential for better understanding of the underlying neurobiological basis contributing to affect-related disorders to develop targeted therapeutics (Lopez-Cruz et al., 2021).

We also aimed to develop a task with both construct and predictive validity, which could detect both positive and negative shifts in CJB. Previous tasks used to assess affective state have used aversive paradigms such as the FST or TST (Gencturk & Unal, 2024). Of the existing CJB tasks, some use inherently aversive methods including footshock in their paradigms (Enkel et al., 2010; Hodges et al., 2022; Rygula et al., 2012). Therefore, it makes it difficult to determine if the behavioural measurement reflects the genuine measurement or if it is due to the aversive nature of the task itself. Also, tactile, and olfactory-based CJB tasks may not be applicable for translation in humans and spatial and auditory-based CJB tasks may not be easily comparable between animals and humans. Additionally, validated tasks assessing CJB must demonstrate construct validity via changes from affect manipulations and animal behavioural outputs should produce the typical graded curve (internal validity) (Hintze et al., 2018; Resasco et al., 2021). However, many previous CJB tasks did not attempt to demonstrate construct validity (Hintze et al., 2018; Krakenberg et al., 2019) or were unable demonstrate a change in affective state with their chosen manipulations (Bailoo et al., 2018; Boleij et al., 2012; Kloke et al., 2014; Krakenberg et al., 2020; Novak et al., 2015; Novak et al., 2016).

Surprisingly, a low number of studies using pharmacological manipulations have been conducted in mice and further research has been suggested (Neville et al., 2020).

Therefore, there were three main objectives for this thesis:

1. Design and test three different CJB touchscreen tasks using the same visual stimuli
2. Pharmacologically validate the best task
3. Ecologically validate the best task

For objective 1, we adapted the touchscreen tasks from Krakenberg et al. (2019a), and Lopez-Cruz et al. (in prep) and designed a novel touchscreen paradigm combining the training of two pre-existing touchscreen tasks. For objective 2, we validated the most successful task using the pharmacological agents, BUP and TBZ, which have been previously shown to induce positive and negative affect, respectively, in humans and previously been shown to induce positive and negative CJB in rodents (Lopez-Cruz et al., in prep). For objective 3, we further validated this task using a mild, ecologically relevant stressor (injection and handling). In sum, this thesis presents a novel adaptation of CJB testing using touchscreen operant chambers that can assess positive and negative shifts in CJB and is sensitive enough to detect changes in CJB bought on by a mild stressor.

2 Methods and Materials

2.1 Animals

In this study, Cohort 1 consisted of male (n = 24) and female (n = 23) C57BL/6J mice (Jackson Laboratory). One female was euthanized prior to training due to malocclusion. Cohort 2 consisted of male (n = 8) and female (n = 8) C57BL/6J mice (Jackson Laboratory). Mice were maintained on a 12-hour reverse light-dark cycle with regular lights off at 9:00 AM and on at 9:00 PM. They were trained and tested during the dark phase between the hours of 12:00 PM and 7:00 PM. Room temperature was set at 22-25℃ and the humidity was maintained between 40%-60%. Mice were aged approximately three months at the onset of pretraining. All procedures abided by the ethical guidelines of the Canadian Council of Animal Care (CCAC) at Western University and followed the approved animal use protocol (2021-082).

2.2 Housing and Food Restriction

Animals were housed in shoebox cages (19.56 cm x 30.91 cm x 13.34 cm) in same-sex groups of 2-4. Some male mice were separated from cage mates when fighting was observed, resulting in six single-housed animals (n = 3 for Task 1, n = 1 for Task 2, n = 2 for Task 3). Environmental enrichment provided in the cages included a diamond twist, wooden chew block, cardboard tunnel, Enviro-Dri, Biofresh bedding, nestlets and twist bits (see Figure 2).

To increase reinforcement value of the reward and motivation to respond to stimuli in the touchscreen operant chamber, mice were food restricted to and maintained at 85-90% of their baseline free-feeding weight throughout training and testing. Baseline was calculated as the average weight over the three days preceding food restriction. Each day, each mouse was given 1.5-4 grams of food, which contained 54% carbohydrates, 21.3% protein, 3.8% fat, and 20.9% micronutrients/other (Bio-Serv, Flemington, New Jersey). Water was provided *ad libitum*.

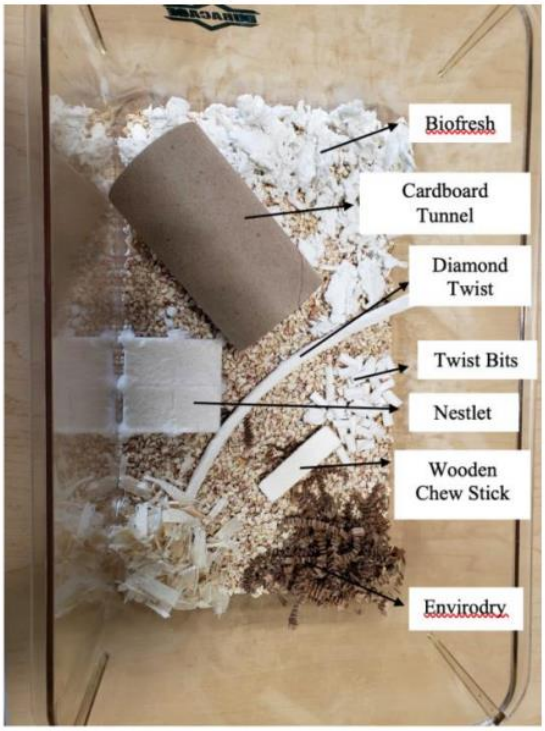


Figure 2. Environmental enrichment included in the mouse home cages.

2.3 Touchscreen Apparatus

To conduct behavioural experiments, we used sixteen Bussey-Saksida touchscreen operant chambers (see Figures 3 and 4; Lafayette Instrument Company, Lafayette IN). The chambers were trapezoid shaped with three black walls opening onto a touchscreen on one side and a reward magazine on the opposite side, with perforated stainless-steel flooring (dimensions: 17 cm length depth from screen to magazine, 23.8 cm width at the screen and 4.6 cm width at the reward magazine). The reward magazine delivered Neilson strawberry milkshake reward (Saputo Dairy Products, Montreal, QC) which was pumped through peristaltic tubing directly into the metal tray opening. The reward magazine also contained a tray light which turned on when reward was delivered. The apparatus contained a speaker for sound delivery, an overhead house light which activated during incorrect responses and an overhead video camera which permitted the observation of animals during training and testing. Chambers were housed in blue boxes which were constructed from fibreboard and contained a fan for ventilation and

attenuating sound. A three-window black Perspex mask was inserted in front of the touchscreen for Task 1, Task 2 and the visuomotor conditional learning (VMCL) portion of Task 3. A two-window mask was used for the pairwise visual discrimination (PVD) portion of Task 3. Behavioural task programs were run with Animal Behaviour Environment Test (ABET) II Touch software (Campden Instruments Ltd, Lafayette IN) and Whisker Server (Whisker Standard Software, Lafayette Instrument, Lafayette IN) to record behavioural responses.

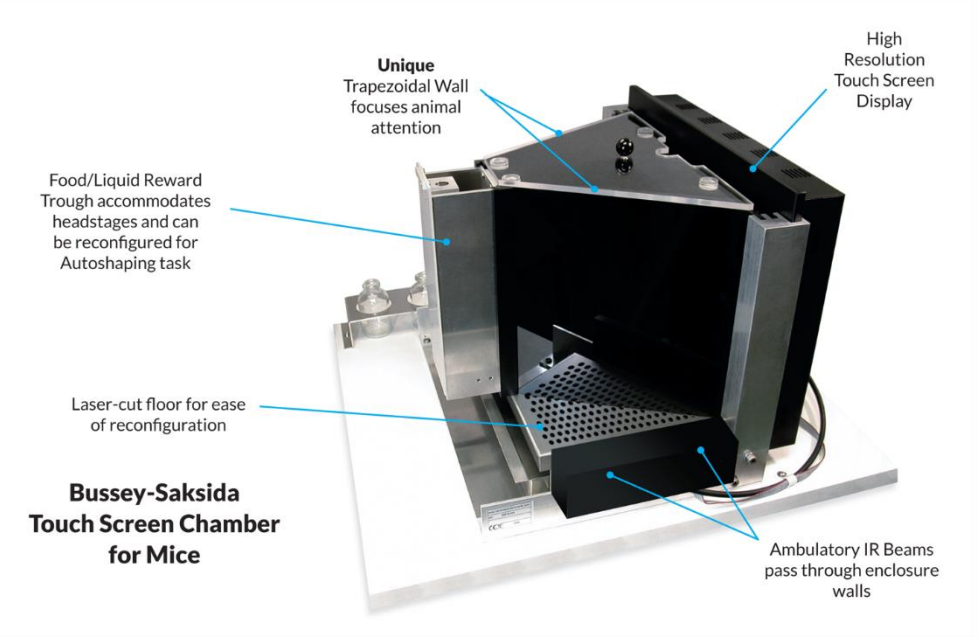


Figure 3. The Bussey-Saksida mouse touchscreen operant chamber (Lafayette Instrument Company & Campden Instruments Ltd, 2021).

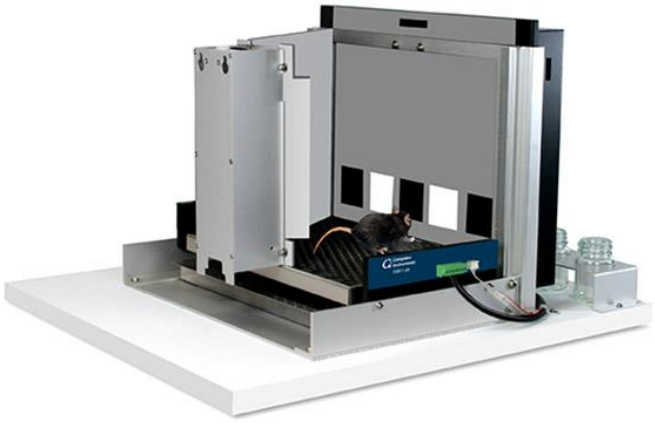


Figure 4. A mouse completing a task in the touchscreen chamber (Lafayette, 2024).

2.4 Pre-training

Prior to CJB testing, completion of standard pre-training was required in which mice learned how to use the touchscreen system. Prior to touchscreen pre-training, mice were given a small dish with strawberry milkshake reward in their home cage for three consecutive days to allow them to habituate to the taste and to prevent food neophobia. Mice completed pre-training comprising several stages (outlined below). The first three stages were the same for all three tasks. Beyond that, the stages varied for each of the CJB tasks. Mice completed one session (training/probe) per day. Chambers were cleaned with 10% ethanol after completing each session. More specific details on the pre-training stages can be found in the standard operating procedures (SOPs) for Visuomotor Conditional Learning (VMCL) Task (Touchscreen Cognition, VMCL SOP), 2-Choice Pairwise Visual Discrimination (PVD) Task (Touchscreen Cognition, PVD SOP) and Image Continuous Performance Task (iCPT) (Touchscreen Cognition, CPT SOP).

2.5 Task Design and Pharmacological Validation Experiment

2.5.1 CJB Task 1 (Lopez-Cruz Variant)

The first task was based on the rodent continuous performance task (rCPT) Go/No-Go design and was adapted from a previously used touchscreen task developed by Lopez-Cruz et al. (in prep). In this Go/No-Go paradigm, animals were required to respond to receive a reward (go) or not respond to avoid a mild punishment (no-go) (Gencturk & Unal, 2024). The main changes included the visual cues, stimulus duration, limited-hold times (i.e. the amount of time a mouse has to respond to the screen following image presentation; this usually extends 0.5 seconds beyond removal of the image from the screen) and the implementation of an additional training stage. This set of cues (same cue rotated varying amounts) was used to eliminate novel visual features introduced in the ambiguous cues in the original design. Animals completed pre-training stages “Habituation 1” to “Habituation 2b” and continued with discrimination training stages as described below.

2.5.1.1 Habituation 1

During the first stage, animals explored the touchscreen chamber for 10 minutes. The touchscreens were turned on and the three-window mask was used. No stimulus or reward was presented. This stage enabled animals to become familiar with the chamber.

2.5.1.2 Habituation 2a

Mice then completed two sessions of “Habituation 2a”, where they explored the touchscreen chamber for 20 minutes. During this stage, animals became acquainted with the strawberry milkshake reward system: the milkshake tray light turned on, a tone played (3 kHz, 1000 ms), and milkshake (800 ms, ~20 µl) was delivered into the reward magazine. Exiting from the reward magazine turned the light off. Animals were required to consume the milkshake to proceed to the next stage.

2.5.1.3 Habituation 2b

Next, mice completed “Habituation 2b” which was the same as “Habituation 2a” except the session time increased to 40 minutes. During Habituation stages, each mouse was removed from the chamber as soon as the session terminated.

2.5.1.4 Stimulus Touch

In this stage, animals learned to touch a white square stimulus presented in the centre window. The white square remained illuminated for 10 seconds. If the mouse nose-poked the square within the limited-hold period (10.5 seconds, i.e. during the 10 seconds while illuminated or 0.5 seconds afterward), the stimulus was removed, a tone was played (1000 ms, 3 kHz), the milkshake tray light turned on and milkshake reward (800 ms, ~20 µl) was delivered. The light turned off once the mouse entered the tray to collect the reward, the after reward pause timer was initiated, followed by the inter-trial interval (ITI; 2 seconds). If no response was made during the limited-hold period, the stimulus was removed, and the ITI was initiated. Animals were required to achieve 60 or more rewards (hits) in 45 minutes to proceed to the next stage.

2.5.1.5 Target Stimulus Touch

In this stage, animals learned to touch a specific stimulus (circles with lines instead of the white square) presented in the centre window. The target cue was synonymous with the designated positive cue, which was maintained throughout the stages. The orientation of the positive cue was counterbalanced such that for half of the animals, the positive cue was rotated 45° left from vertical and for the other half, the cue was rotated 45° right from vertical. The framework was the same as "Stimulus Touch"; however, the stimulus duration and limited-hold period decreased to 5 and 5.5 seconds respectively. Animals were required to achieve 60 or more rewards (hits) in 45 minutes to proceed to the next stage.

2.5.1.6 1 Target (Positive Cue) and 1 Non-Target (Snowflake Cue)

Initially, many animals were struggling to acquire the original 1 Target and 1 Non-Target stage (target: positive cue, non-target: negative cue). Since the positive and negative cues are visually similar, it is likely that the mice struggled to discriminate between the two cues initially. Therefore, an intermediate stage with a non-target cue that was significantly more visually distinct than the target cue was introduced (snowflake cue).

In this stage, animals were introduced to a non-target cue (see Figure 5). Both the target (positive cue) and non-target cue (snowflake cue) were presented in a pseudo-random order with equal probability. When the target cue was displayed there were 2 possible outcomes: 1) the mouse correctly nose-poked the cue within the limited-hold period and received a reward (hit) or 2) the mouse incorrectly withheld responding (miss). Following a hit, the cue was removed, a tone was played (1000 ms, 1.6 KHz), the milkshake tray light turned on and milkshake reward (800 ms or 20 µl) was delivered. Following a miss, the cue was removed, and the ITI began. When the non-target cue was displayed there were 2 possible outcomes: 1) the mouse correctly withheld responding and the cue was removed (correct rejection), or 2) the mouse incorrectly nose-poked the cue (false alarm). Following a correct rejection, the ITI began. Following a false alarm, the cue was immediately removed, a noise was played (1000 ms, 0.1 KHz), the house light flashed (1000 ms with 30 ms on time and 20 ms off time), no reward was given, and the ITI

began. In this stage, the stimulus duration and limited-hold period decreased to 3 and 3.5 seconds, respectively. Animals were required to achieve a d' score greater than 0.6 over two consecutive sessions with a minimum of 7 sessions on this stage.

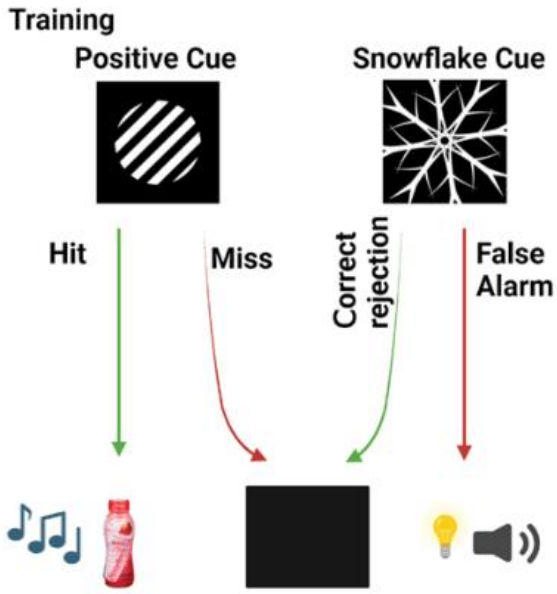


Figure 5. The "1 Target (Positive Cue) and 1 Non-Target (Snowflake Cue)" stage in which animals learned how to discriminate between the positive cue and snowflake cue. In this example, the positive cue was rotated 45° right from vertical.

2.5.1.7 1 Target (Positive Cue) and 1 Non-Target (Negative Cue)

The procedure for this stage (Figure 6) was the same as the previous stage, with the exception that the non-target cue was changed to the negative cue (e.g. cue rotated 45° left from vertical) instead of the snowflake cue and the stimulus duration and limited-hold period were 2 and 2.5 seconds, respectively. Animals were required to achieve a d' score greater than 0.6 over two consecutive sessions with a minimum of 7 sessions on this stage before proceeding to testing probes.

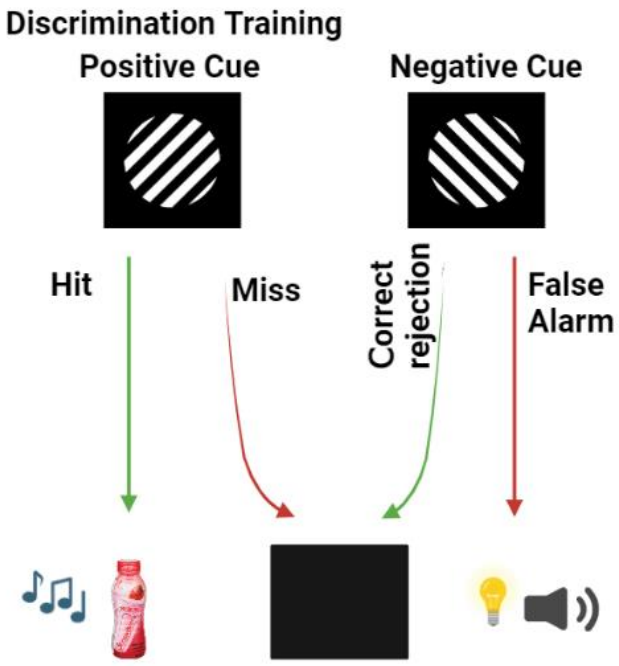


Figure 6. The "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)" stage in which animals learned how to discriminate between positive and negative cues. In this example, the positive cue was rotated 45° right from vertical and the negative cue was rotated 45° left from vertical.

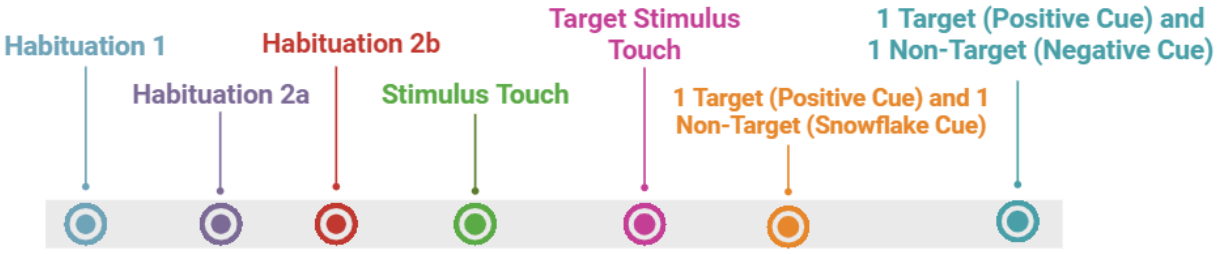


Figure 7. Order of pre-training and discrimination stages in CJB Task 1 (Lopez-Cruz Variant).

2.5.1.8 1 CJB Task 1 (Lopez-Cruz Variant) Testing Probes

Testing probes were run similarly to the previous training stage with a limited-hold of 2.5 seconds and stimulus duration of 2 seconds. During a testing probe week, animals were tested for five consecutive sessions and an average for the week was calculated for each measure.

During testing probes, mice were presented with 130 trials in 45 minutes. These trials consisted of 100 trained cues (50 positive cues and 50 negative cues) with 30 ambiguous cues (10 near-positive, 10 true ambiguous, 10 near-negative) interspersed amongst the trained cues. The near-positive and near-negative cues were rotated 22.5° from vertical, and the true ambiguous cue was vertical (see Figure 8). The order of cue presentation was pseudo-randomized. Additionally, no trained cue was presented more than three consecutive times, and an ambiguous cue was not presented more than two consecutive times. Responses to the ambiguous cues were used as indicators of cognitive judgement bias; touching the true ambiguous cue was considered an optimistic response due to the mouse interpreting the ambiguous cue positively (where they were previously rewarded). Conversely, rejecting the true ambiguous cue was considered a pessimistic response due to the mouse interpreting it negatively (to avoid the noise and light). Overall, touching a greater number of true ambiguous cues would indicate a positive CJB whereas rejecting a greater number of true ambiguous cues would indicate a negative CJB (see Figure 9).

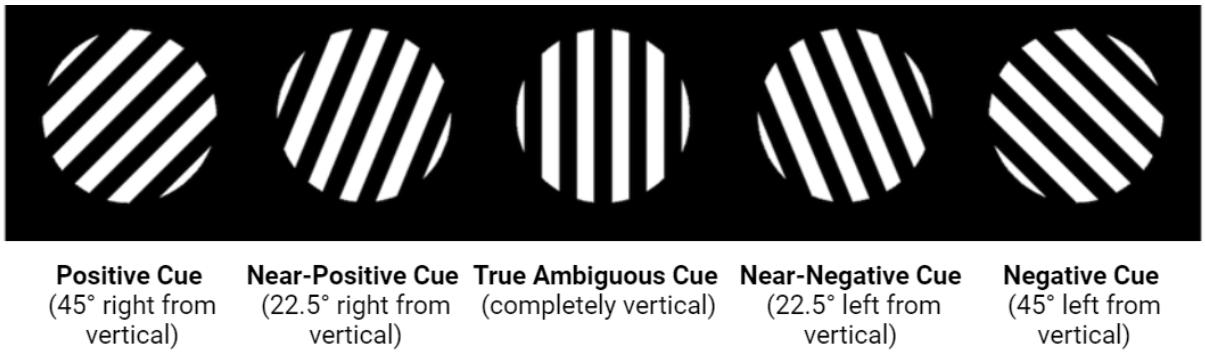


Figure 8. The five cues used during testing probes.

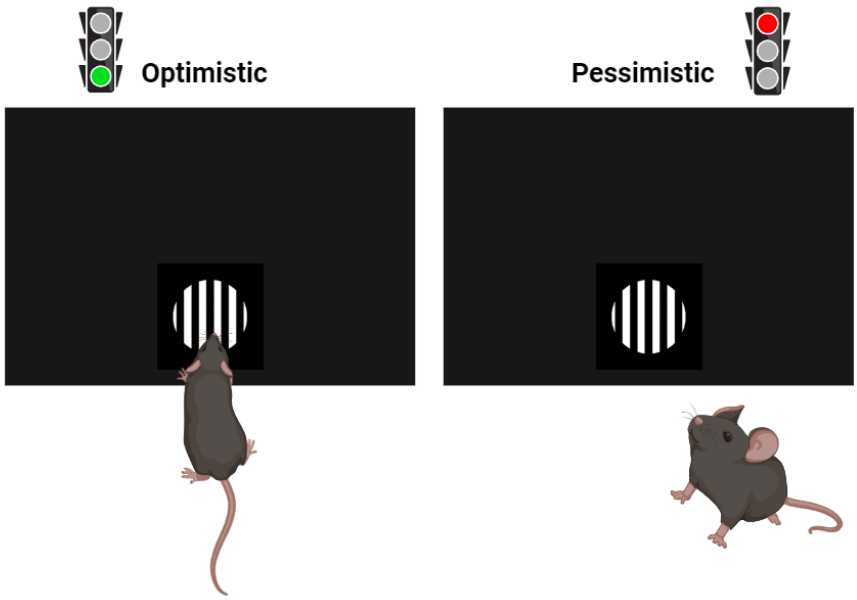


Figure 9. Depiction of optimistic and pessimistic responding to the true ambiguous cue. The mouse will have previously learned to touch the positive cue to receive reward and to not touch the negative cue to avoid a noise and light. Therefore, touching the true ambiguous cue was considered an optimistic response due to the mouse interpreting the ambiguous cue positively (where they were previously rewarded). Conversely, rejecting the true ambiguous cue was considered a pessimistic response due to the mouse interpreting it negatively (to avoid the noise and light).

2.5.2 CJB Task 2 (Krakenberg Variant)

The second task was adapted from a previously published touchscreen based CJB task developed by Krakenberg et al. (2019). This task was a Go/Go paradigm (active choice), where animals were required to perform two different types of behaviours to either receive reward or avoid mild punishment. The main changes were the visual cues and parameters of some of the training stages. The same five circular cues were used for this task as in Task 1 (Lopez-Cruz Variant). Animals completed pre-training stages "Habituation 1" to "Habituation 2b" as previously described and continued with the additional pre-training and discrimination training stages as described below.

2.5.2.1 Initial Touch

Animals then completed "Initial Touch" during which they began to associate touching the touchscreen with a reward. A white square stimulus was presented for 30 seconds. The square was displayed in either the left, centre, or right window. The position was selected pseudo-randomly, and the square was not displayed in the same position more than three times consecutively. When no response was made during that period, a tone was played (3 kHz, 1000 ms), the milkshake tray light turned on and a small reward was provided (800 ms, ~20 µl). Conversely, when a response was made, a tone was played, the milkshake tray light turned on, and a larger reward was provided (2400 ms, ~60 µl). The light turned off once the mouse entered the tray to collect the reward and the ITI (20 seconds) began. After the ITI, the next stimulus was presented. The necessary criterion for this stage was completing 30 trials (stimulus touches) in 60 minutes.

2.5.2.2 Must Touch

Next, mice completed “Must Touch”. This stage was almost identical to the previous stage, except this time mice learned to touch the illuminated stimulus to receive reward. The stimulus remained on the screen until a touch was made which resulted in a tone being played (3 kHz, 1000 ms), the milkshake tray light turning on and milkshake being delivered (800 ms, ~20 µl). The light turned off once the mouse entered the tray to collect the reward, and the ITI was re-initiated, followed by another stimulus presentation. No reward was given if the mouse touched a blank area of the screen. The criterion for advancement was completing 30 trials (stimulus touches) in 60 minutes.

2.5.2.3 Must Initiate

The next stage was “Must Initiate” in which animals learned to initiate subsequent trials. The stimulus remained on the screen until a response was made which resulted in a tone being played (3 kHz, 1000 ms), the milkshake tray light turning on and milkshake being delivered (800 ms, ~ 20 µl). The light turned off once the mouse entered the tray to collect the reward, and the ITI reinitiated. After the ITI, the tray light reilluminated, and the mouse was required to enter and exit the reward magazine before the next stimulus was presented. The criterion for advancement from this stage was completing 30 trials in 60 minutes.

2.5.2.4 Punish Incorrect 1

Afterwards, animals proceeded to “Punish Incorrect 1” in which they were trained not to select an incorrect location. The training was the same as “Must Initiate”, however if they chose the incorrect location (blank, non-illuminated window), the house light turned on (5 second time out) with no reward provided. After the time out period, the house light turned off and the ITI (20 seconds) began. Animals must have completed two consecutive sessions of a minimum of 23/30 correct trials in 60 minutes to proceed to “Punish Incorrect 2”.

2.5.2.5 Punish Incorrect 2

Next, mice proceeded to “Punish Incorrect 2” in which they were trained to first nose-poke a central location and then nose-poke a stimulus in one of the flanker locations (left or right sides). The training was the same as “Punish Incorrect 1”; however, the mouse had to make two nose-pokes (centre followed by flanker) before receiving a reward. After initiating the trial, a random image from the Pairwise Visual Discrimination Task training image set (Campden Instruments Ltd) was presented in the central location. The mice were required to nose-poke this image which resulted in the additional presentation of a white square on either the left or right flanking window. Correctly touching the white square resulted in a tone being played (3 kHz, 1000 ms), the milkshake tray light turning on and milkshake being delivered (800 ms, ~20 µl). After consuming the reward and exiting the reward magazine, the ITI (20 seconds) reinitiated. However, incorrectly touching the blank non-illuminated window caused the house light to turn on (5 second time out) with no reward provided. After the time out period, the house light turned off and the ITI began. Animals were required to complete two consecutive sessions of a minimum of 23/30 correct trials in 60 minutes. The timeline for “Pre-training” is outlined in Figure 10.

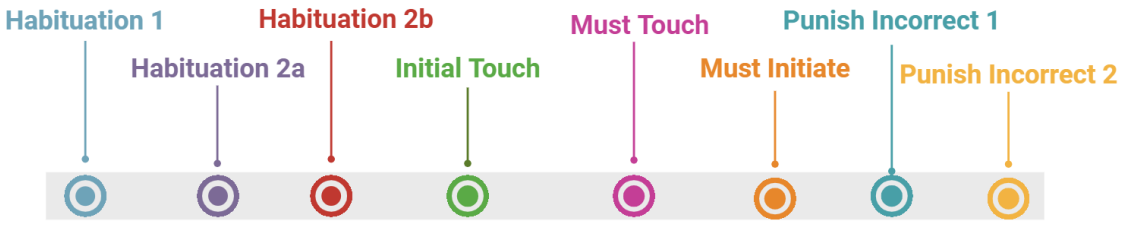


Figure 10. Order of pretraining stages in CJB Task 2 (Krakenberg Variant).

2.5.2.6 Discrimination Training

After completing pre-training, animals were subject to five discrimination stages. These discrimination stages were adapted from Krakenberg et al. (2019) and were presented with a novel stimulus set: white circular cues with black lines (see Figure 8). There were two training conditions, the “Positive Condition”, and the “Negative Condition” whereby the “Positive Condition” required a response to one side and the “Negative Condition”

required a response to the opposite side. The positive and negative cues were rotated 45° left or right from vertical. The cues corresponding to the positive and negative conditions were counterbalanced and correct side was counterbalanced.

2.5.2.6.1 Discrimination 1

Animals were presented with one cue (Positive or Negative) in the centre window and had unlimited time (within session limits) to make a response. Once touched, two white squares in the left and right flanking windows were presented immediately, requiring the mouse to choose the correct side. In the "Positive Condition", animals received a large milkshake reward (800 ms, ~20 µl, two tones separated by 0.5 seconds) for the correct side and a neutral outcome (no reward or punishment) for the incorrect side. In the "Negative Condition", animals received a small milkshake reward (400 ms, ~10 µl, one tone) for touching the correct side and no milkshake reward, house light activation and time out period for touching the incorrect side. Cues were presented an equal number of times in a pseudo random order. The ITI was 10 seconds. Correction trials, where the same cue was presented in successive trials until a correct response was made, were implemented for the negative condition. Correction trials were rewarded/punished the same as regular trials but did not contribute to the total trial counter. To move to "Discrimination 2", animals were required to complete 50 trials in 20 min and with a minimum of 80% correct for two consecutive days.

Throughout discrimination training, animals were struggling to meet the 80% correct criteria and were consistently performing approximately at 50%. Therefore, the following changes were implemented for all discrimination phases: 1) the positive/negative cue with the two flanking white squares were illuminated simultaneously requiring the mouse to touch once (flanker) instead of twice, 2) correction trials were added to the "Positive Condition", 3) the time out period was reduced from five seconds to two seconds, and 4) the delay between the tones was increased from 0.5 seconds to 0.75 seconds.

2.5.2.6.2 Discrimination 2

The training was the same as "Discrimination 1", except in the "Positive Condition" where a response to the incorrect side resulted in a small milkshake reward (400 ms, ~10

µl, one tone). This was done to reduce the risk of the mice developing a side bias. Therefore, if they repeatedly chose the same side, it would result in the same amount of milkshake awarded regardless of the side chosen. The necessary criterion was that animals needed to complete a total of 100 trials at 80% correct or higher for two consecutive days. In “Discrimination 2-5”, animals were moved back to the previous stage if they failed to meet criterion for four consecutive days.

2.5.2.6.3 Discrimination 3

Training was the same as “Discrimination 2”, except correction trials were present only for the first 25 trials. “Pseudo-probes”, where the trials would yield a neutral outcome (no reward but no houselight/time out period), were added during trials 26-100. Pseudo-probes were implemented so that mice became accustomed to experiencing neutral outcomes (which would occur for ambiguous cues during testing probes). This session included 2 pseudo-probes. Animals must have completed a total of 100 trials at 80% correct or higher for two consecutive days to proceed to “Discrimination 4”.

2.5.2.6.4 Discrimination 4

Training was the same as “Discrimination 3”, except correction trials were present for trials 1-15. This stage included 4 pseudo-probes during trials 16-100. Animals must have completed a total of 100 trials at 80% correct or higher for two consecutive days to proceed to “Discrimination 5”.

2.5.2.6.5 Discrimination 5

Training was the same as “Discrimination 4”, except correction trials were present for trials 1-5. This stage included 6 pseudo-probes during trials 6-100. Animals must have completed a total of 100 trials at 80% correct or higher for two consecutive days to proceed to testing probes.

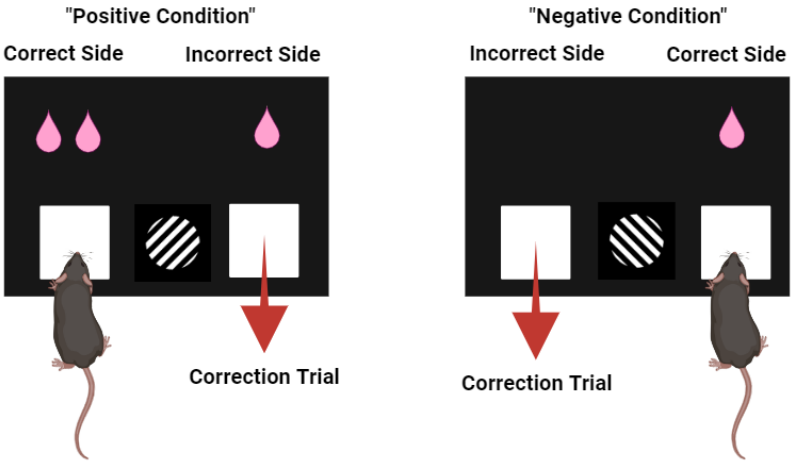


Figure 11. An example trial in "Discrimination 2". The positive cue is rotated 45° right from vertical and the negative cue is rotated 45° left from vertical. In this example, when the positive cue is presented, the mouse must respond to the left side to receive a large milkshake reward and when the negative cue is presented, the mouse must respond to the right side to receive a small milkshake reward. Responding to the incorrect side in either condition results in a correction trial.

2.5.2.6.6 CJB Task 2 (Krakenberg Variant) Testing Probes

Once animals completed "Discrimination Training", they moved onto experimental testing probes. For testing, animals were required to complete 100 trials in 60 minutes. During each session, animals were presented with trained cues (positive and negative) with intermittent exposure to ambiguous cues which were untrained, unrewarded, and unpunished. They received 88 trained cues (44 positive, 44 negative) and 12 ambiguous cues (4 near-positive, 4 true ambiguous, 4 near-negative). The order of cue presentation was randomized, no trained cue was presented more than three consecutive times, and no ambiguous cue was presented more than two consecutive times.

Animal responses to the ambiguous cues were used as indicators of their CJB. The true ambiguous cue was of particular interest because it is visually the most ambiguous (directly in the middle of positive and negative). Animals viewing the true ambiguous cue and responding more to the left side would indicate optimistic responding (interpreting the true ambiguous cue positively), reflecting a positive CJB. Conversely, animals responding more to the right side would indicate pessimistic responding (interpreting the

true ambiguous cue negatively), reflecting a negative CJB. Finally, an animal responding equally to both sides would indicate a neutral CJB. Figure 12 depicts these options.

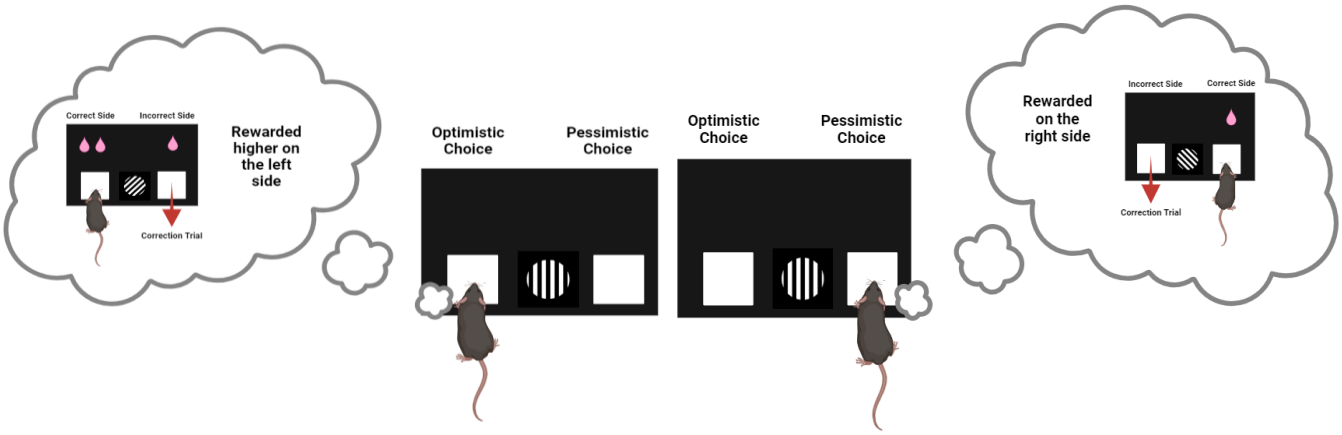


Figure 12. Depiction of optimistic and pessimistic responding to the true ambiguous cue. The mouse will have previously learned that when they were presented with the cue rotated 45° right from vertical, they were given more milkshake reward on the left side than right side. Therefore, responding to the left side when encountering the true ambiguous cue would be an optimistic response because the mouse would interpret it positively and respond to the corresponding side (where they were rewarded higher). They would have also learned that when they were presented with the cue rotated 45° left from vertical, they were given milkshake reward on the right side and not the left. Therefore, responding to the right side when encountering the true ambiguous cue would be a pessimistic response because the mouse would interpret it negatively and respond to the corresponding side (where they were rewarded).

2.5.3 CJB Task 3 (2-Stage Novel Variant)

The third task was a novel task combining designs from visuomotor conditional learning (VMCL) and pairwise visual discrimination (PVD) paradigms. The rationale behind training two tasks was that animals would learn the valence of the trained cue in the PVD portion and apply that in the VMCL testing format. This task is also a Go/Go design, making it less vulnerable to motivation effects. Animals completed pre-training stages “Habituation 1” to “Punish Incorrect 2”, as previously described (Task 2) and continued with the following stages. Due to the nature of this task design, animals were trained on the VMCL task followed by the PVD task. The same five circular cues were used for this task as in Tasks 1 and 2.

2.5.3.1 Visuomotor Conditional Learning (VMCL) Task

In this task, animals were required to learn that the positive cue required responding to one side whereas the negative cue required responding to the opposite side (the valence of these cues was learned subsequently during PVD training, described below). Both positive and negative cues were presented an equal number of times during a session (15 trials each). They were also presented pseudo-randomly, and each cue was not presented more than three consecutive times. When a cue was presented in the centre window, the mouse had unlimited time to touch it. Once touched, two white squares in the left and right flanking windows appeared simultaneously. Touching the correct side resulted in a tone being played (3 kHz, 1000 ms), the milkshake tray light turning on and milkshake being delivered (800 ms, ~20 µl). After consuming the reward and exiting the reward magazine, the ITI (20 seconds) reinitiated. However, touching the incorrect window caused the house light to turn on (5 second time out) with no reward provided. After the time out period, the house light turned off and the ITI began. Additionally, a correction trial loop began where the same cue was repeated until a correct choice was made. The animals were required to complete two consecutive sessions of 24/30 (80%) to move onto the next stage. Correct side was counterbalanced.

Throughout VMCL training, some animals were struggling to meet 80% correct criteria and were consistently performing approximately at chance (50%). Therefore, the following change was implemented for those animals: they were retrained on more visually distinct cues (e.g. “icicle” versus “equal sign”) before retrying the original cues. The rationale for this was for animals to learn the rules with easier cues, before moving them back to the more visually similar trained cues.

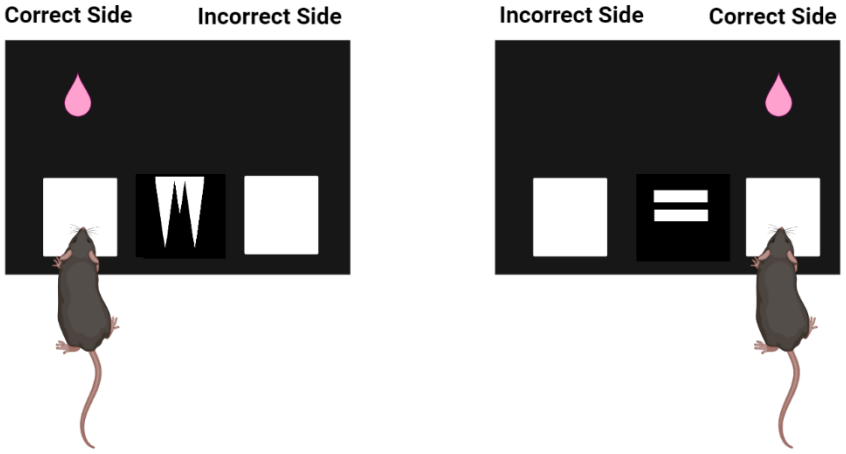


Figure 13. Easy version of VMCL. In this example, when the icicle is presented, the mouse must respond to the left side (rewarded) and when the equal sign is presented, the mouse must respond to the right side (rewarded). Correct side was counterbalanced between animals.

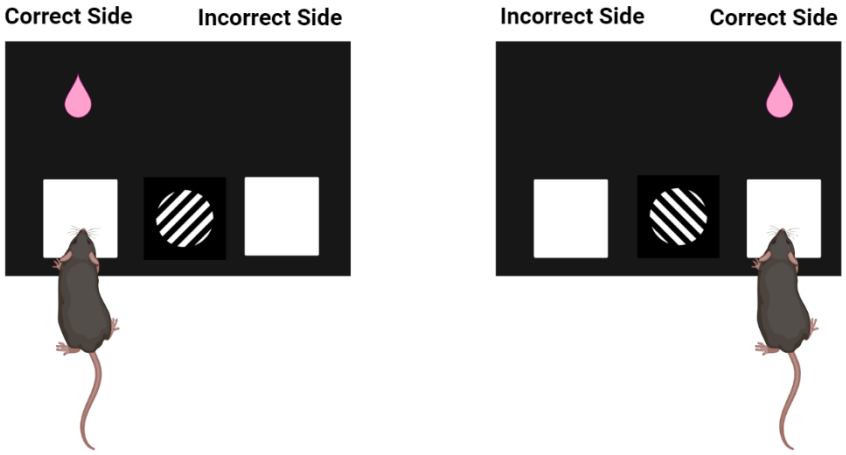


Figure 14. Hard version of VMCL. In this example, when the cue rotated 45° right from vertical is presented, the mouse must respond to the left side (rewarded) and when the cue rotated 45° left from vertical is presented, the mouse must respond to the right side (rewarded). Correct side was counterbalanced between animals.

2.5.3.2 Pairwise Visual Discrimination (PVD) Task

Animals learned the acquisition phase of the PVD task using the two-window mask. In this task, animals were required to learn that touching the positive cue resulted in reward and that touching the negative cue was unrewarded. Both the positive and negative cues were presented simultaneously, and the mice learned to touch the positive cue regardless of the spatial location. The ordering of the positive and negative cues was pseudo-random, and no pairing order was repeated more than three consecutive times. Touching the correct cue resulted in a tone being played (3 kHz, 1000 ms), the milkshake tray light turning on and milkshake being delivered (800 ms, ~20 µl). After consuming the reward and exiting the reward magazine, the ITI (20 seconds) reinitiated. However, touching the incorrect cue caused the house light to turn on (5 second time out) with no reward provided. After the time out period, the house light turned off and the ITI began. Additionally, a correction trial loop began in which the same trial (same stimulus locations) was repeated until a correct choice was made. The animals were required to

complete two consecutive sessions of 24/30 correct responses (80%) to move onto the next stage. Correct/incorrect images were counterbalanced.

Throughout PVD training, some animals were struggling to meet 80% correct criteria and were consistently performing approximately at chance (50%). Therefore, the following changes were implemented for those animals: they were retrained on more visually distinct cues (e.g. “fan” versus “marbles” in PVD) before retrying the original cues. The rationale for this was for animals to learn the rules on easier cues, before moving them back to the more visually similar trained cues.

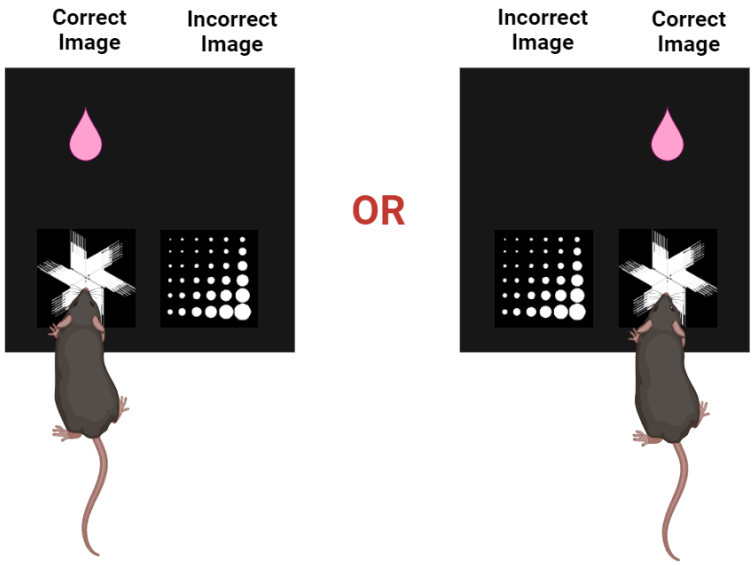


Figure 15. Easy version of PVD. In this example, the fan cue is correct (rewarded) and the marbles cue is incorrect (house light on, time out).

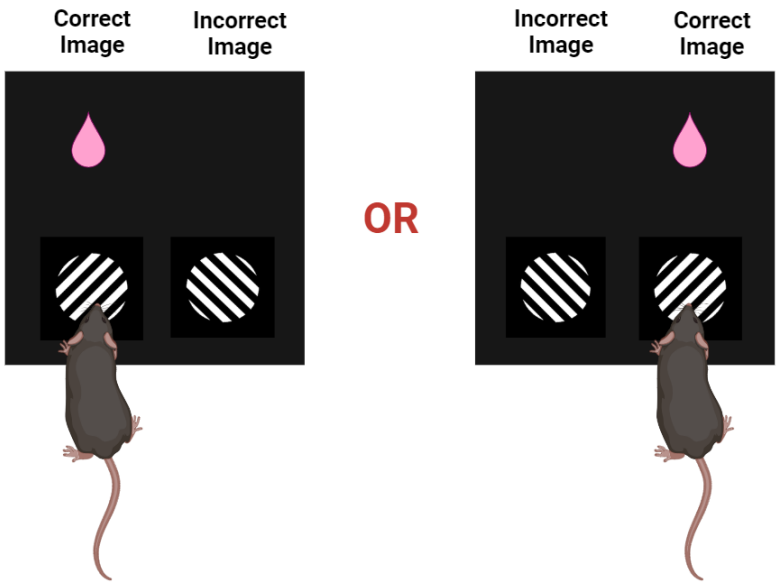


Figure 16. Hard version of PVD. In this example, the cue rotated 45° right from vertical is correct (rewarded) and the cue rotated 45° left from vertical is incorrect (house light on, time out).

Overall, animals were required to meet criteria for the stages in the following order: Easy VMCL, Hard VMCL, Easy PVD and Hard PVD, then a VMCL Check to ensure the VMCL rule was remembered prior to probing. Once animals successfully met criteria for both VMCL, PVD and performed a VMCL check (meeting 80% for at least one session), they moved onto experimental testing probes. Figure 17 depicts the order of training for animals.

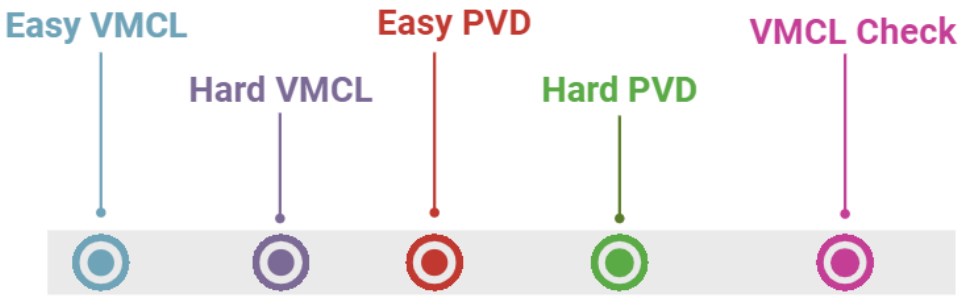


Figure 17. The order of training stages for CJB Task 3 (2-Stage Novel Variant).

2.5.3.3 CJB Task 3 (2-Stage Novel Variant) Testing Probes

During testing probes, animals were required to complete 72 trials in 90 minutes with the ITI reduced to 10 seconds. In each session, they received 60 trained cues (30 positive, 30 negative) with 12 ambiguous cues interspersed amongst the trained cues (4 near-positive, 4 true ambiguous, 4 near-negative). The order of cue presentation was pseudo-randomized, trained cues were not presented more than three consecutive times, and ambiguous cues were not presented more than two consecutive times. Testing probes did not include correction trials. During a testing probe week, animals were tested over five daily sessions and an average for the week was calculated for each measure.

Animal responses to the ambiguous cues were used as indicators of their CJB. In reference to the previous hypothetical example (positive cue: cue rotated 45° right from vertical, rewarded on left side in VMCL), animals viewing the true ambiguous cue and responding more to the left side would indicate optimistic responding (interpreting the true ambiguous cue positively), reflecting a positive CJB. Conversely, animals responding more to the right side would indicate pessimistic responding (interpreting the true ambiguous cue negatively), reflecting a negative CJB. Finally, an animal responding equally to both sides would indicate a neutral CJB. In alignment with the previous example, Figure 18 depicts these options.

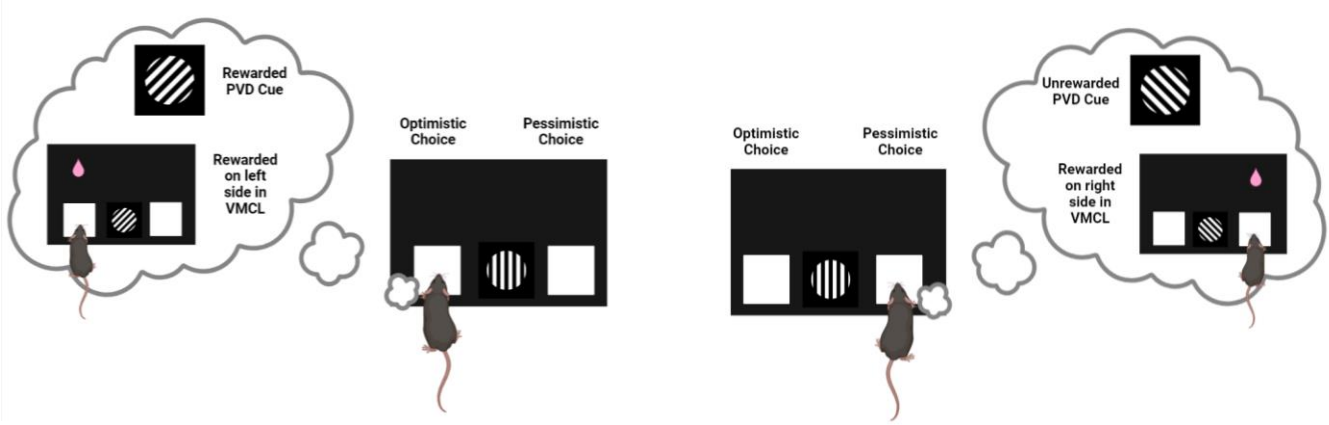


Figure 18. Depiction of optimistic and pessimistic responding to the true ambiguous cue. The mouse will have previously learned that when they were presented with the cue rotated 45° right from vertical, they were rewarded in PVD, and needed to respond to the left side if they encountered this cue in VMCL. Therefore, responding to the left side when encountering the true ambiguous cue would be an optimistic response because the

mouse would interpret it positively (where they were previously rewarded in PVD) and respond to the corresponding side. They would have also learned that when they were presented with the cue rotated 45° left from vertical, they were not rewarded in PVD, and needed to respond to the right side if they encountered this cue in VMCL. Therefore, responding to the right side when encountering the true ambiguous cue would be a pessimistic response because the mouse would interpret it negatively and respond to the corresponding side (where they were unrewarded in PVD).

2.5.4. Pharmacological Agents

2.5.4.1 Bupropion (BUP)

Bupropion hydrochloride was purchased from Sigma Aldrich. BUP powder was dissolved in 0.9% NaCl (saline) solution (vehicle) to a concentration of 0.5, 0.75, and 1 mg/mL such that when injected intraperitoneally (IP) at a volume of 0.01 mL/g of bodyweight 30 minutes prior to touchscreen testing, mice received doses of 5, 7.5, or 10 mg/kg (or vehicle). Doses and administration time were chosen based on previous work conducted by Lopez-Cruz et al. (in prep). Solutions were stored at 4^{o}C.

2.5.4.2 Tetrabenazine (TBZ)

TBZ was purchased from Sigma Aldrich. Tetrabenazine powder was dissolved in dimethyl sulfoxide (DMSO) and then diluted in 0.9% NaCl to 20% DMSO in 80% saline to 0.6 mg/mL. 2N Hydrogen chloride (HCl) was slowly added to adjust the solution to a final pH of 4.5 to dissolve remaining precipitate. Animals were injected intraperitoneally (IP) at a volume of 0.01 mL/g of bodyweight, resulting in a dose of 6 mg/kg. They were injected with either TBZ or vehicle (80% saline, 20% DMSO) 2 hours prior to touchscreen testing. Doses and administration time were chosen based on previous work conducted by Lopez-Cruz et al. (in prep). Solutions were stored at 4^{o}C.

2.5.5 Timeline and Design

The three separate touchscreen tasks were conducted at the same time. The same set of five cues were used on separate cohorts of mice (n = 15 for Task 1, n = 16 for Task 2, n = 16 for Task 3) using a within-subjects design for each cohort.

Following touchscreen pre-training and task training, mice performed baseline probes consisting of testing probes of their respective task without any treatment. The week following this they began testing probes with pharmacological interventions. BUP and TBZ were administered on separate weeks to induce positive and negative affective states, respectively. During the probe weeks, BUP (5 mg/kg, IP) and TBZ (6 mg/kg, IP) were administered for five consecutive sessions with a subsequent washout period. BUP and TBZ and their respective vehicles were counterbalanced through an experimenter-blinded Latin-square design (to counterbalance order of treatments for all animals) (by testing week). Upon seeing no effect at the lower dose of BUP, another Latin-square design was followed which included two higher doses (BUP (7.5 mg/kg, IP), BUP (10 mg/kg, IP), and vehicle). Figure 19 reflects an experimental timeline for Task 1 (Lopez-Cruz Variant). Testing probe days with technical issues (e.g. milkshake tubing clogs) or animal health-related issues were not included in analysis. Some animals in Tasks 2 and 3 had not received BUP treatment at the time the 5 mg/kg data were generated. They therefore received the 10 mg/kg dose only (no 5 or 7.5 mg/kg) to condense the timeline and reduce the number of injections received.

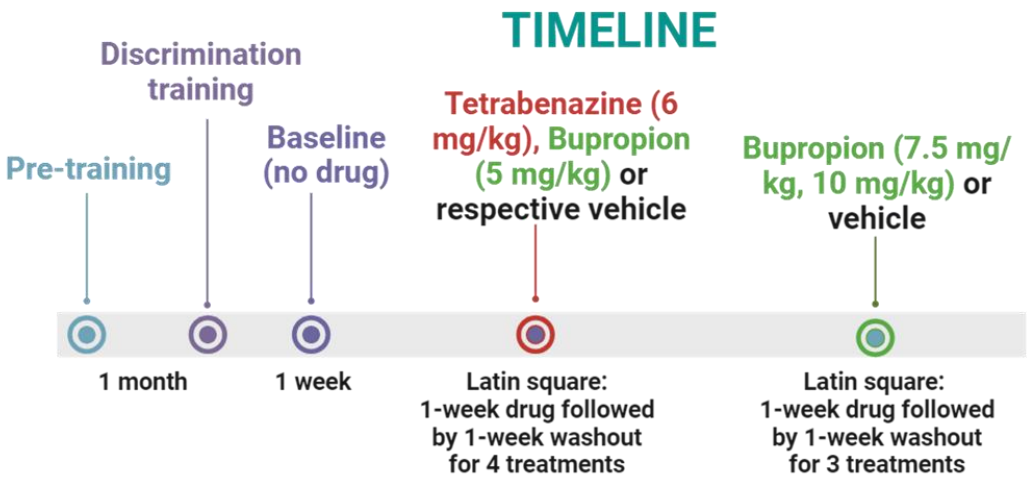


Figure 19. Experimental timeline for CJB Task 1 (Lopez-Cruz Variant) animals.

2.5.6 Testing and Maintenance Training Washout Period

Following testing, animals received a 9-day washout/maintenance period (weekends and weekdays) during which they completed maintenance training. For Task 1 maintenance, animals completed "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)" and were required to achieve a d' score greater than 0.6 over two consecutive sessions. Maintenance training for Task 1 was conducted a minimum of two to three times per week to ensure they were meeting criteria for the trained cues. For Task 2 maintenance, animals completed "Discrimination 5" and were required to reach two consecutive sessions of 80% or higher. For Task 3 maintenance, animals were required to meet criteria once for each PVD and VMCL (at least 80% or 24/30 correct for each task). Overall, animals continued maintenance sessions until they met the appropriate criteria before moving on to the next treatment. After the last treatment session in the Latin square, animals were required to meet criteria for maintenance one time to verify that the treatment had not impaired the animal's ability to perform the task.

2.6 Handling, Transport, and Injection Stress Experiment

Based on previous data, animals appeared to have a more negative CJB when comparing baseline (no injection) to any of the injection conditions (vehicle or drug treatment). It was unclear whether this was simply due to the animals learning about the ambiguous cues, potential injection stress, or both. Therefore, a subsequent objective was to determine if the experience of receiving an injection induced a negative shift in CJB. Additionally, stricter training criteria assisted with teasing apart learning effects and injection effects.

Animals (Cohort 2: 8 males, 8 females) completed CJB Task 1 (Lopez-Cruz Variant), as described previously. During the discrimination training of this task, some of the mice were struggling to meet criteria for "1 Target (Positive Cue) and 1 Non-Target (Snowflake Cue)" and "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)" stages. Therefore, some changes were made to the training protocols. First, correction trials were implemented in these stages to help with the acquisition of the task rules. During correction trials, if animals made false alarms (incorrectly touching the negative

cue), they repeated the same trial until correct rejection was achieved. Second, more stringent criteria were implemented to ensure high discrimination between cues during testing probes; that is, the previous criteria which consisted of a d' score of greater than 0.6 was changed so that animals were required to meet both a hit rate of 0.8 or higher and a false alarm rate of 0.4 or lower.

2.6.1 Timeline and Design

Similar to the pharmacological experiment, mice completed pre-training, discrimination training and then testing probes. They completed a baseline CJB measurement where they completed testing probes under regular conditions with no drug administration. Following baseline, mice completed testing probes following no injection, saline (0.9% NaCl) injection 30 minutes prior to touchscreen testing and saline + DMSO injection (80% saline, 20% DMSO) 2 hours prior to touchscreen testing. During the probe weeks, treatments were administered for five consecutive sessions with a subsequent maintenance period (9 days, as in previous experiment). Saline injection, saline + DMSO injection and no-injection conditions were counterbalanced through a Latin-square design (by testing week). Figure 20 demonstrates the timeline.

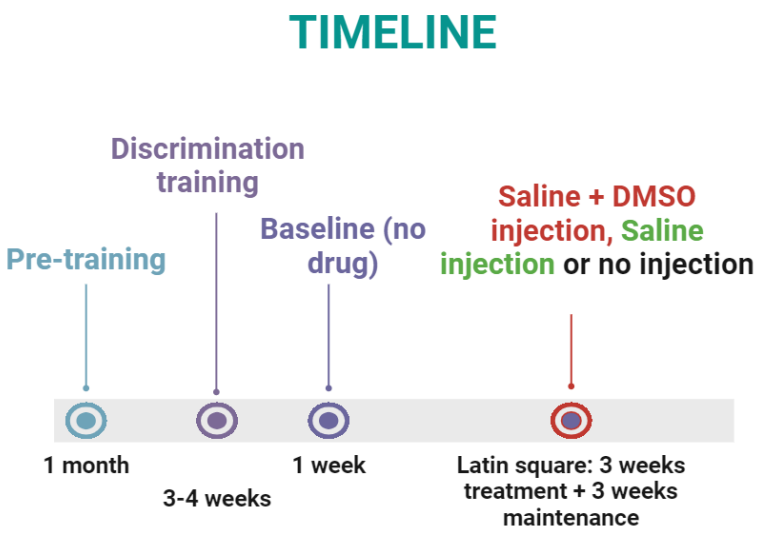


Figure 20. Experimental timeline for handling, transport, and injection stress experiment.

For both injection conditions, animals were taken into the injection room, injected, and returned to their home cage prior to testing. For the no-injection condition, animals were handled normally and placed in the chamber at the testing time. Testing probe days with technical issues or animal health-related issues were not included in analysis. During maintenance, animals completed "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)" and were required to achieve a hit rate of 0.8 or higher and a false alarm rate of 0.4 or less over two consecutive sessions before proceeding to the next condition. Maintenance was conducted a minimum of two to three times per week to ensure they were meeting criteria for the trained cues. After the last set of sessions in the Latin square, animals were required to meet criteria for maintenance one time to verify that the treatment did not impair the animal's ability to perform the task.

2.7 Data Collection and Statistical Analyses

Data were organized and animal testing probe week averages were calculated in Microsoft Excel. All statistical analyses and graphing were performed using GraphPad PRISM version 9.5.1 (GraphPad Software, Inc., San Diego, California) or JASP version 0.18.3 (JASP, Amsterdam, the Netherlands).

2.7.1 CJB Task 1 (Lopez-Cruz Variant)

During discrimination training, three response measures were used to calculate different performance metrics: response rate, discrimination sensitivity and response bias (see formulas below). Response Rate measured the proportion of touches to the cue relative to the total number of cues presented. The formula is depicted below such that X would be one of the five cues. Discrimination Sensitivity measured the animal's ability to discriminate between cues where a higher value indicated a greater ability to discriminate between positive and negative cues. Response Bias measured the animal's decision-making criterion for whether to respond or not. A higher score indicated a positive bias and more liberal responding (more likely to respond). A lower score indicated a negative bias, and more conservative responding (less likely to respond).

- ***Response Rate*** = *Number of touches to cue X/number of total X cues presented*

- ***Discrimination Sensitvity*** **(d')** = $z(Hit\ Rate) - z\ (False\ Alarm\ Rate)$

- ***Response Bias*** **(c)** = $0.5 * z(Hit\ Rate) + z(False\ Alarm\ Rate)$

During testing probes, responses were quantified using a response rate ranging from 0 to +1. Response rates were calculated for all five cues. A high response rate was expected for the positive cue, a low response rate was expected for the negative cue and intermediate response rates were expected for the ambiguous cues. For the true ambiguous cue, 0 would be the most pessimistic, 0.5 would be neutral and +1 would be the most optimistic.

2.7.2 CJB Task 2 (Krakenberg Variant)

Behaviour was quantified using a choice score ranging from -1 to +1. Choice scores were calculated for all five cues. A high choice score was expected for the positive cue, a low choice score was expected for the negative cue and intermediate choice scores were expected for the ambiguous cues. For the true ambiguous cue, the higher the choice score indicated an optimistic choice, the lower the choice score indicated a pessimistic choice and a choice score of 0 would be a neutral choice. Optimistic choices were indicated by the animal responding to the side where they previously received a larger milkshake reward for the positive cue, whereas pessimistic choices were indicated by the animal responding to the side where they were previously rewarded for the negative cue. This was the formula used:

- ***Choice Score*** *= N Choices ("optimistic) – N Choices ("pessimistic")/ N Choices ("optimistic + "pessimistic")*

2.7.3 CJB Task 3 (2-Stage Novel Variant)

Behaviour was quantified using a choice score ranging from 0 to +1. Choice scores were calculated for all five cues. A high choice score was expected for the positive cue, a low choice score was expected for the negative cue and intermediate choice scores were

expected for the ambiguous cues. For the true ambiguous cue, a higher choice score indicated more optimistic choices, a lower the choice score indicated more pessimistic choices and a choice score around 0 indicated neutral choices. Optimistic choices would refer to the animal responding to the side where they were previously rewarded for the positive cue whereas pessimistic choices would refer to the animal responding to the side where they were previously rewarded for the negative cue. This was the formula used:

- ***Choice Score*** *= N Choices ("optimistic) – N Choices ("pessimistic")/ N Choices ("optimistic + "pessimistic")*

The main statistical model used was a two-way repeated measures analysis of variance (ANOVAs) with cue and treatment/condition as within-subjects factors and significance set to $p < 0.05$. Šidák post hoc tests were completed if interactions were found in the ANOVA. Additionally, Holm post hoc analyses were conducted for main effects of treatment/condition. *A priori* planned comparisons using paired-t tests (for TBZ or 5 mg/kg BUP versus vehicle) or one-way repeated measures ANOVAs with Tukey post hoc analyses (7.5 mg/kg, 10 mg/kg BUP versus vehicle or saline injection, saline + DMSO injection versus no injection) were conducted for the true ambiguous cue (the most ambiguous cue), where differences were expected to occur based on pharmacological affective manipulations. Greenhouse-Geisser corrections were made when sphericity was violated. Both sexes were combined in the main graphs with exploratory sex-based analyses performed separately and represented separately in the Appendix A (due to lack of statistical power). Cohen's d and η^2 effect sizes were calculated where appropriate.

Other extra measures were included such as response latency, response rate for the positive cue, response rate for the negative cue, discrimination sensitivity (d') and response bias (c). Response latency measured the length of time taken to touch a cue and was measured for all five cues. For latencies where animals did not touch a particular cue, those sessions were removed from that particular analysis. Response latencies were statistically compared using a 2-way repeated measures ANOVA with cue and treatment/condition as within-subject factors with Šidák post hoc tests where appropriate.

Response rate for the positive cue, response rate for the negative cue, d' and c were calculated *a priori* using the above formulas. For each of these five metrics, treatment/condition and vehicle were statistically compared using a paired t-test or one-way repeated measures ANOVA (as above, based on the number of treatment/condition groups in the analysis) with Tukey post hoc tests. Error bars on graphs reflected mean ± standard error of the mean (SEM). Significances were designated as * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

3 Results

In all CJB graphs displayed, cues are depicted as follows: the positive cue (rotated 45° right from vertical), near-positive (rotated 22.5° right from vertical), near-negative (rotated 22.5° left from vertical) and the negative cue (rotated 45° left from vertical). However, in practice, the valences of these cues were counterbalanced between animals and thus the stimulus valences in the figures represent one arm of the counterbalanced design.

3.1 CJB Task Comparison – Training Progression and Duration

3.1.1 CJB Task 1 (Lopez-Cruz Variant)

All animals (n = 15) successfully completed pre-training, discrimination training stages and testing probes. One female mouse completed all testing probes, although was removed from the analyses for taking 44 sessions to acquire the task (greater than two standard deviations above the average). Therefore, 14 animals completed all pre-training and discrimination training before or within 35 sessions with an average of 28 sessions (27.786 ± 2.486). Additionally, three single-housed male animals were removed from analyses. Previous research has associated social isolation with depressive-like behaviour (Berry et al., 2012; Grigoryan et al., 2022; Ieraci et al., 2016; N. Liu et al., 2020), which is relevant to CJB. Therefore, analysis of these three animals appears in Appendix A. Overall, for the combined analyses, there were 5 males and 6 females (n = 11 total). The furthest stage that animals successfully completed is depicted in Figure 24a.

3.1.1.1 Baseline

After meeting criteria for “1 Target (Positive Cue) and 1 Non-Target (Negative Cue)”, animals (n = 11) underwent a baseline CJB measurement where they completed five consecutive days of testing probes with no pharmacological manipulation. As expected, animals displayed a high response rate for the positive cue (0.862 ± 0.05), low response rate for the negative cue (0.356 ± 0.105) and intermediate responding for the ambiguous cues. They also appeared to respond optimistically at baseline with a high response rate

(0.722 ± 0.127) in response to the true ambiguous cue. On the first probe day for three of the animals, there was an error in the code resulting in the presentation of a higher proportion of positive than negative cues (e.g. 60/40 instead of the normal 50/50). However, unpaired t-tests were conducted between the true ambiguous response rates for the animals with the error compared to the animals without the error on probe day 1, and no significant differences were found ($p = 0.511$). This suggests that the unequal proportions on probe day 1 did not significantly impact the results. The error was corrected for all subsequent probe sessions. Additionally for baseline responding, unpaired t-tests were conducted between average response rates for positive and negative cues for animals that had the cue rotated 45° left from vertical as their positive cue versus animals with the cue rotated 45° right from vertical as their positive cue. Overall, no significant differences were found for the response rate for the positive cue ($p = 0.319$), or for the response rate for the negative cue ($p = 0.646$) suggesting that which image was presented as the positive cue did not affect overall performance. Figure 21 depicts the overall baseline CJB curve.

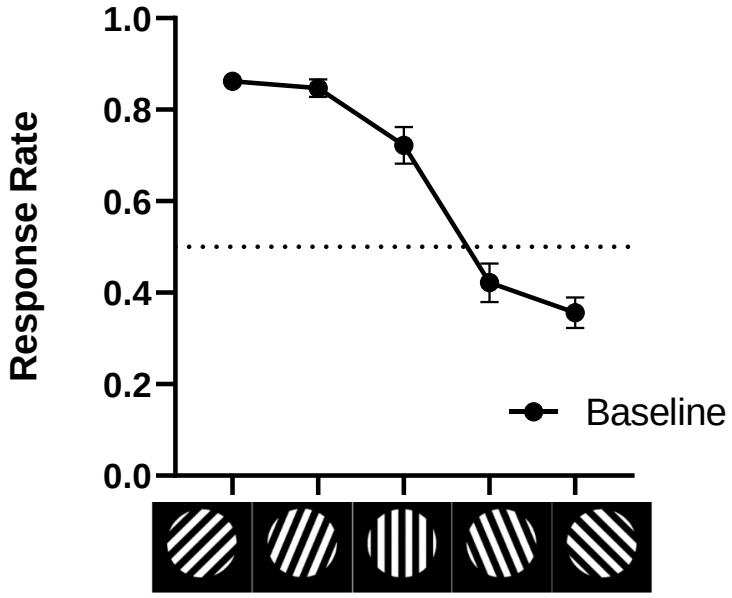


Figure 21. Baseline CJB responding for n = 11 animals on the CJB Task 1 (Lopez-Cruz Variant)**.**

3.1.2 CJB Task 2 (Krakenberg Variant)

All animals (n = 16) successfully completed pre-training stages before or within 32 sessions with an average of 21 sessions (21.176 ± 5.626). 14 animals were unable to complete all discrimination training phases and were therefore discontinued. Of these animals, 12 completed "Punish Incorrect 2", one completed "Discrimination 3" and one completed "Discrimination 4". Two animals (12.5%) were able to complete all pre-training and discrimination training phases and took an average of 102 sessions total. Of these two animals, one completed some of the testing probes and the other completed all testing probes. The furthest stage that animals successfully completed is depicted in Figure 24b.

3.1.2.1 Baseline

As expected, animals displayed a high choice score for the positive cue (0.891 ± 0.0900), low choice score for the negative cue (-0.659 ± 0.00643) and intermediate choice scores for the ambiguous cues. They also appeared to respond optimistically at baseline with a choice score (0.25 ± 0.0707) above chance (i.e. 0). Figure 22 demonstrates the baseline curve. Due to the low number of animals able to complete the task (n = 2), it is not possible to draw any definitive conclusions from these data.

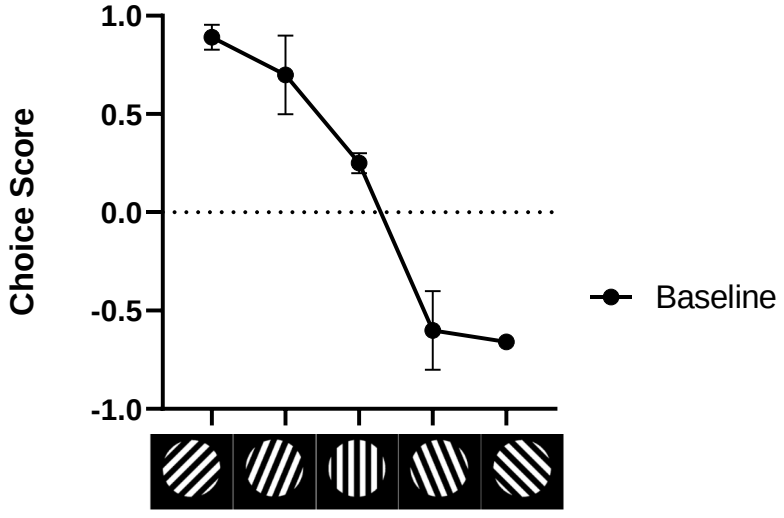


Figure 22. Baseline CJB Responding for n = 2 animals on the CJB Task 2 (Krakenberg Variant).

3.1.3 CJB Task 3 (2-Stage Novel Variant)

All animals (n = 16) successfully completed pre-training stages before or within 33 sessions with an approximate average of 21 sessions (21.25 ± 6.361). Two animals completed as far as “Easy PVD”, one animal completed “Hard PVD” and five animals completed “Easy VMCL”. Therefore, these eight animals were discontinued due to inabilities to meet criteria for one or both VMCL and PVD. Half of the animals (n = 8) (50%) completed pre-training and discrimination training and took an average of 134 sessions. Of those eight animals, seven animals completed all testing sessions, and one animal successfully completed training, the baseline session and one probe session; however, this mouse was discontinued due to inability to meet criteria between probe sessions. Additionally, two animals (males) successfully completed baseline and all probe sessions. However, because they were single-housed, their data are not shown. Animals (n = 4) that did not require the “Easy” versions of PVD and VMCL, took an average of 78 days. In comparison, animals that required the “Easy” versions of PVD and VMCL (n = 4) took an average of 190 days. The furthest stage that animals successfully completed is depicted in Figure 24c.

3.1.3.1 Baseline

As expected, animals displayed a high choice score for the positive cue (0.519 ± 0.119), low choice score for the negative cue (-0.667 ± 0.0967) and intermediate choice scores for the ambiguous cues. They also appeared to respond optimistically at baseline with a choice score (0.240 ± 0.242) above chance (i.e. 0). Figure 23 demonstrates the baseline curve. Due to the low number of animals able to complete the task (n = 5), it is not possible to draw any definitive conclusions from these data.

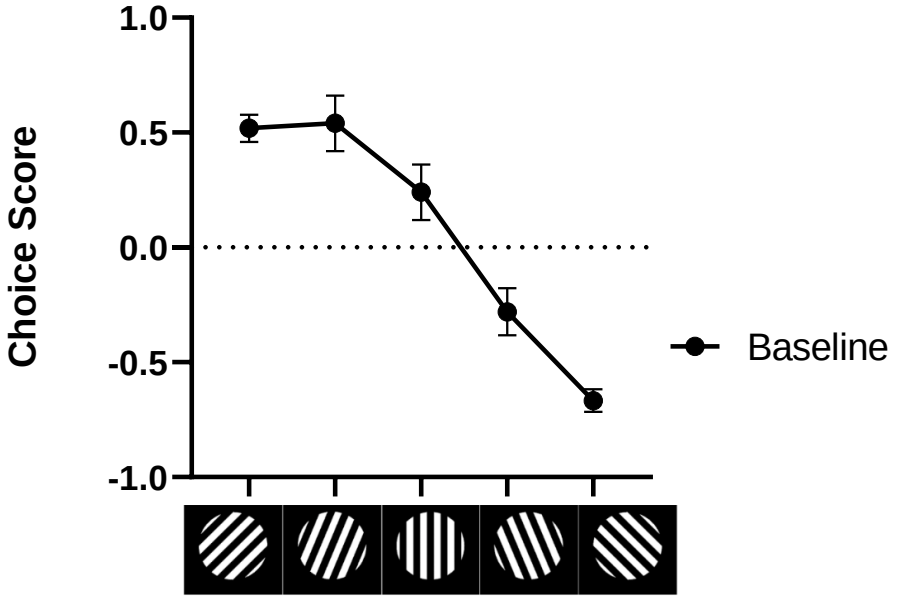


Figure 23. Baseline CJB Responding for n = 5 animals (females) on the CJB Task 3 (2-Stage Novel Variant).

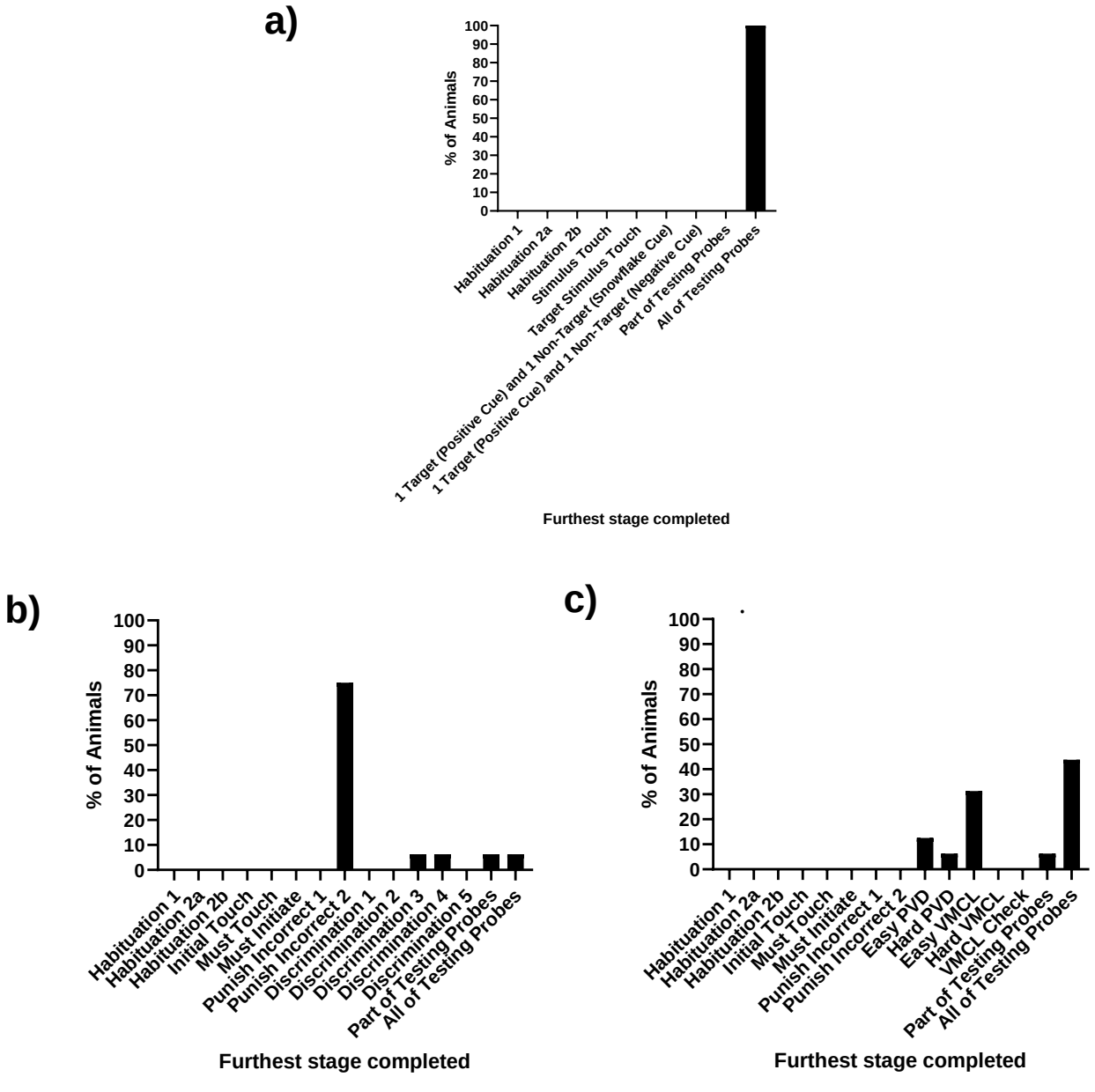


Figure 24. Furthest Stage Completed for Each CJB Task. a) CJB Task 1 (Lopez-Cruz Variant): 100% of animals completed all stages (pre-training, discrimination training and testing probes). B) CJB Task 2 (Krakenberg Variant): 6.25% of animals completed all stages. c) CJB Task 3 (Novel 2-Stage Variant): 43.75% of animals completed all stages.

3.1.4 Comparison of the Three CJB Tasks

Figure 25 compares training duration for all three tasks. Although each task produced the expected baseline response curves seen in CJB, a larger proportion of animals were able to complete CJB Task 1 (Lopez-Cruz Variant) and in a shorter time frame. Therefore, CJB Task 1 (Lopez-Cruz Variant) was used for subsequent experiments; however, all data from the other two tasks are found in Appendix A.

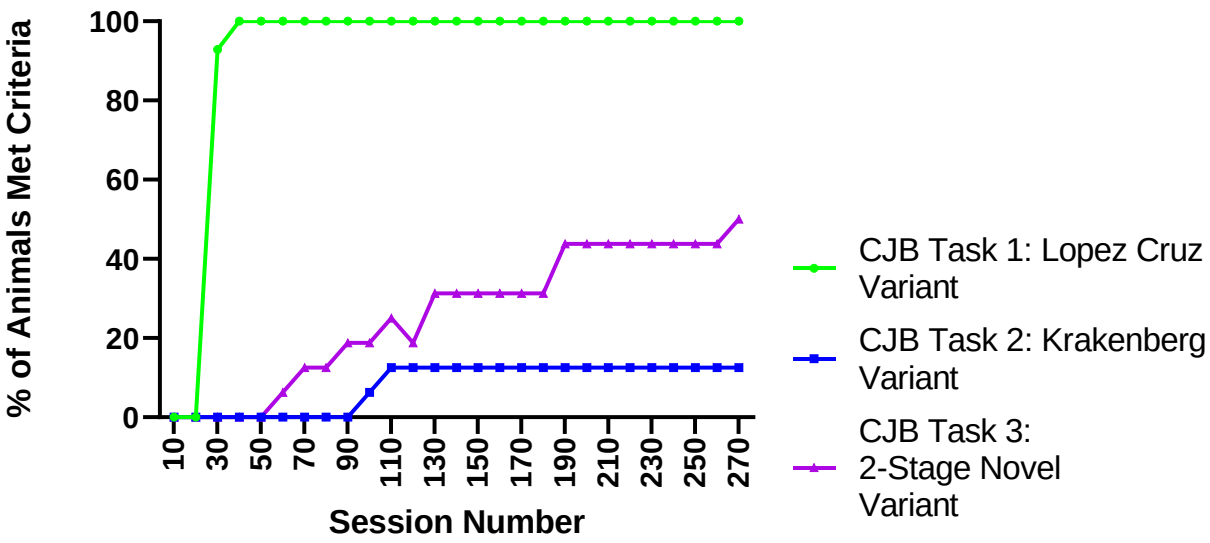


Figure 25. Survival Curve for Each CJB Task (Task 1 n = 14, Task 2 n = 16, Task 3 n = 16). CJB Task 1 (Lopez-Cruz Variant) had the shortest training time of all 3 tasks and all animals met criteria to proceed to testing probes.

3.2 Pharmacological Manipulations

One female mouse showed notable changes in locomotor behaviour (reduced locomotion and increased latency to respond to the touchscreen) following TBZ treatment. As behaviour was normal with BUP treatment, the animal was removed from TBZ analyses only.

3.2.1 Bupropion (BUP) (5 mg/kg) Produced a Trend Towards a Positive Shift in CJB

There was a main effect of cue ($F_{(1.479, 14.79)} = 118.5$, $\eta^2 = 0.8212$, $p < 0.0001$; Figure 26), a trend towards an effect of drug treatment ($p = 0.0511$; Figure 26) and no interaction of drug x cue ($p = 0.592$; Figure 26). *A priori* comparison of treatment groups at the true ambiguous cue found a non-significant trend towards an effect ($p = 0.0766$; Figure 26 inset). These data suggest that bupropion (BUP) at this dose did not increase response rate for the true ambiguous cue compared to vehicle, although there was a trend towards significance.

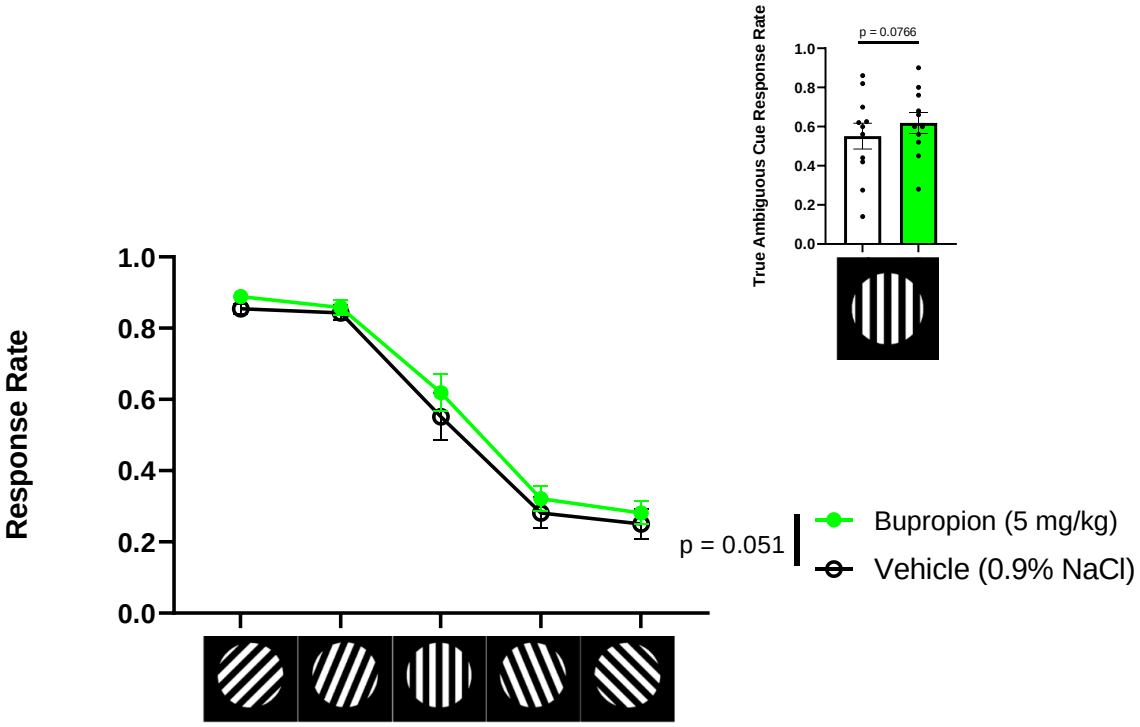


Figure 26. Effect of BUP (5 mg/kg) on CJB Responding (n = 11). Lower dose BUP trended towards increasing response rate at the true ambiguous cue when compared to vehicle.

3.2.2 Bupropion (BUP) (7.5 and 10 mg/kg) Induced a Positive Shift in CJB

As the initial low dose of BUP suggested that there may be an effect on CJB, higher doses were used, and mice were tested again. There was a main effect of cue ($F_{(1.772, 17.72)} = 184.6$, $\eta^2 = 0.8367$, $p < 0.0001$; Figure 27) and a trend towards an interaction of drug

treatment x cue (p = 0.0565; Figure 27). There was also a main effect of drug treatment ($F_{(1.801, 18.01)}$ = 8.683, η^2 = 0.01348, p < 0.01; Figure 27) with both 7.5 and 10 mg/kg BUP significantly higher than vehicle: 7.5 mg/kg (p < 0.01) and 10 mg/kg (p < 0.01). *A priori* planned comparisons of the effects of drug on the true ambiguous cue revealed a significant effect of drug treatment ($F_{(1.436, 14.36)}$ = 12.05, η^2 = 0.5465, p < 0.01) at the true ambiguous cue with higher response rates with both 7.5 mg/kg (Cohen's d = 0.911, p < 0.001; Figure 27 inset) and 10 mg/kg doses (Cohen's d = 0.970, p < 0.05; Figure 27 inset), and no differences between 7.5 and 10 mg/kg (p = 0.834). Overall, these results suggest a significantly increased response rate between BUP and vehicle at the true ambiguous cue.

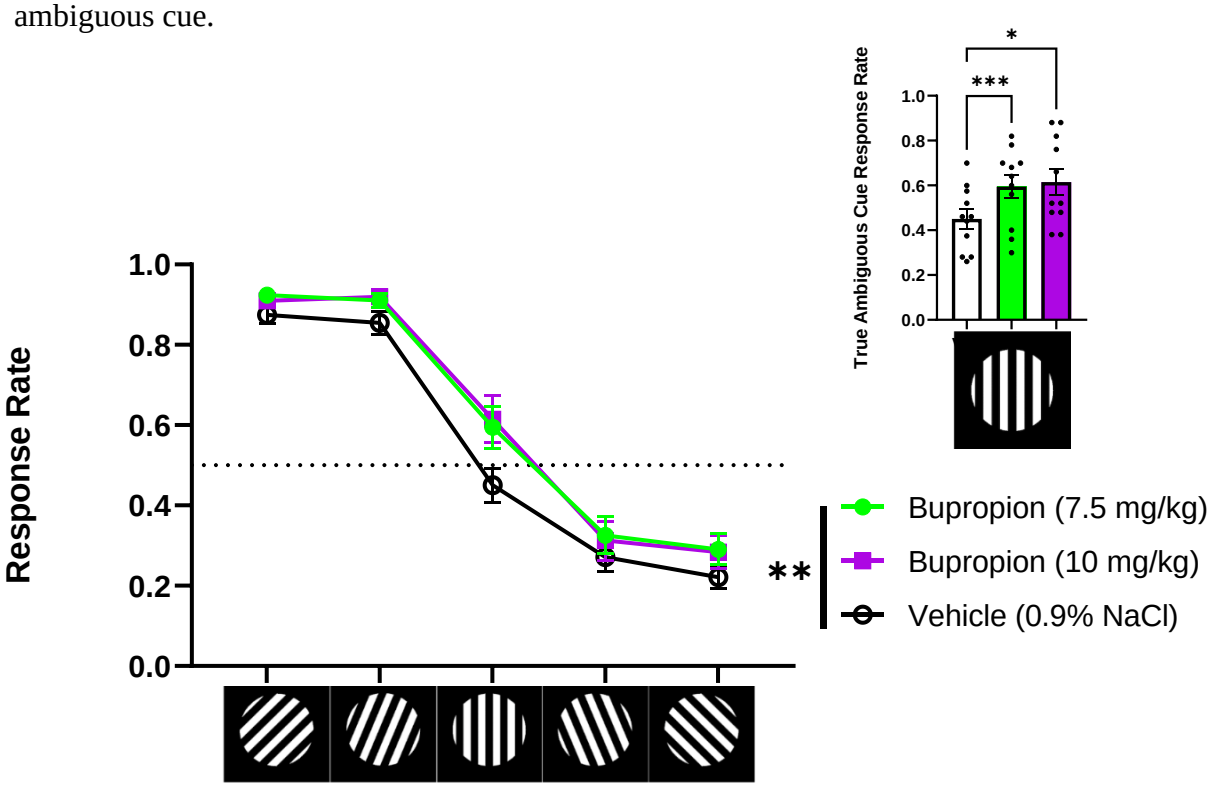
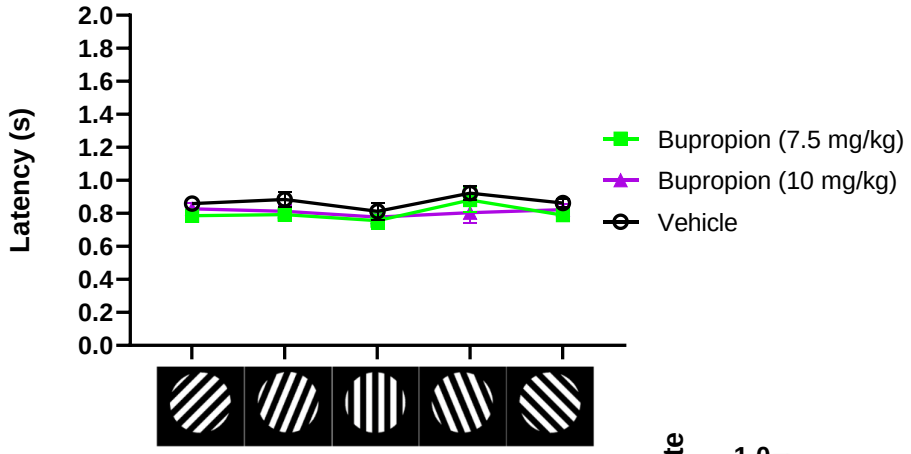


Figure 27. Effect of BUP (7.5 and 10 mg/kg) on CJB Responding (n =11). The 2-way repeated measures ANOVA found a main effect of drug treatment. *A priori* tests found that both 7.5 and 10 mg/kg BUP significantly increased response rate for the true ambiguous cue versus vehicle.

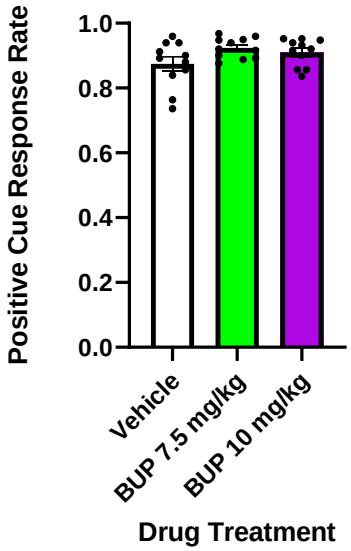
There were no effects of either cue or treatment on response latency (all, p > 0.0848; Figure 28a) suggesting that animals responded to all cues with similar response times regardless of treatment. Response rates at the positive cue and the negative cue as well as

discrimination sensitivity and response bias were assessed. When treated with BUP, mice had a trending increase in response rate for the positive cue ($p = 0.0537$; Figure 28b) and for the negative cue ($p = 0.0569$; Figure 28c). These results suggest that animals had non-significant increases in response rates for the trained cues when treated with BUP or vehicle. These effects produced a significant change towards a more liberal response bias ($F_{(1.875,\ 18.75)} = 6.103$, $R^2 = 0.3790$, $p < 0.05$) with BUP treatment at only the 7.5 mg/kg dose ($p < 0.05$; Cohen's $d = 0.901$; Figure 28d) with a trend in the 10 mg/kg dose ($p = 0.0872$; Cohen's $d = 0.581$; Figure 28d). Notably, mice showed no changes in their ability to discriminate between the positive and negative cues ($p = 0.8683$; Figure 28e). Overall, these data suggest that while BUP may produce small, non-significant in overall responding, it induces a clear and robust positive shift in CJB shown by its large and significant effects seen at the true ambiguous cue.

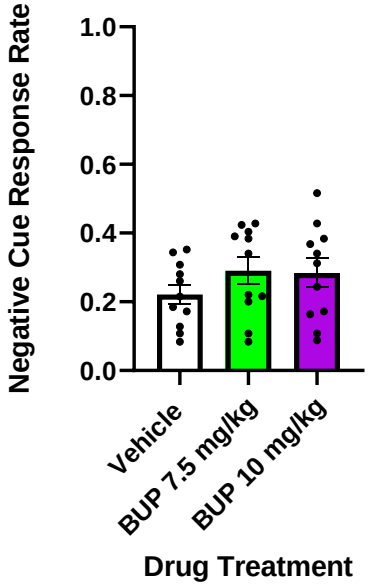
a)


Latency (s)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Bupropion (7.5 mg/kg)
Bupropion (10 mg/kg)
Vehicle

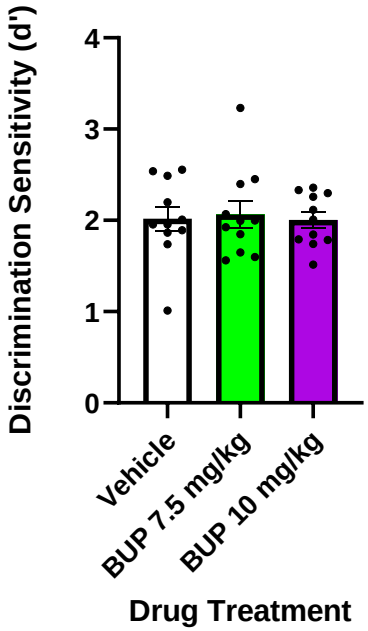
b)


Positive Cue Response Rate
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
BUP 7.5 mg/kg
BUP 10 mg/kg
Drug Treatment

c)


Negative Cue Response Rate
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
BUP 7.5 mg/kg
BUP 10 mg/kg
Drug Treatment

d)


Discrimination Sensitivity (d')
4
3
2
1
0
Vehicle
BUP 7.5 mg/kg
BUP 10 mg/kg
Drug Treatment

e)

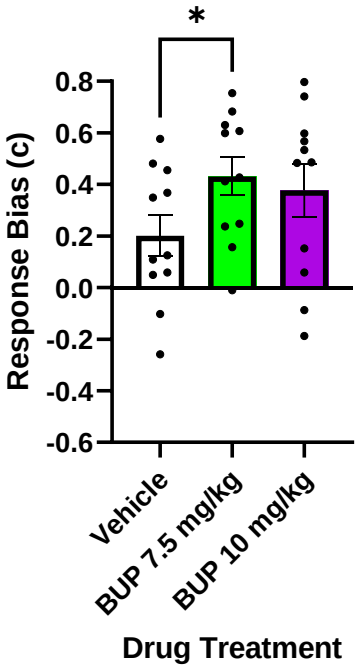

*
Response Bias (c)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
Vehicle
BUP 7.5 mg/kg
BUP 10 mg/kg
Drug Treatment

Figure 28. Effects of BUP (7.5 and 10 mg/kg) on CJB responding. a) BUP did not affect response latencies for the five cues b) BUP non-significantly increased response rate for positive cue c) BUP non-significantly increased response rate for the negative cue d) BUP did not affect discrimination sensitivity (d') e) BUP induced a more liberal response bias (c) at 7.5 mg/kg only.

3.2.3 Tetrabenazine (TBZ) (6 mg/kg) Induced a Negative Shift in CJB

There was an cue x drug treatment interaction ($F_{(4, 36)} = 3.188$, $\eta^2 = 0.00970$, $p < 0.05$; Figure 29), a main effect of cue ($F_{(4, 36)} = 120.6$, $\eta^2 = 0.8062$, $p < 0.0001$; Figure 29) and a main effect of drug treatment ($F_{(1, 9)} = 18.25$, $\eta^2 = 0.3909$, $p < 0.01$; Figure 29). Post hoc analyses revealed significant differences at all ambiguous cues – near-positive ($p < 0.05$), true ambiguous ($p < 0.0001$) and near-negative cues ($p < 0.05$; Figure 29) – but no trained cues (both, $p > 0.236$; Figure 29). An *a priori* test found that when treated with TBZ, mice had a lower response rate when presented with the true ambiguous cue ($t_{9)} = 4.148$, Cohen's d = 1.286145, $p < 0.01$; Figure 29 inset). These data suggest that TBZ significantly reduced response rate for the ambiguous cues in the task compared to vehicle.

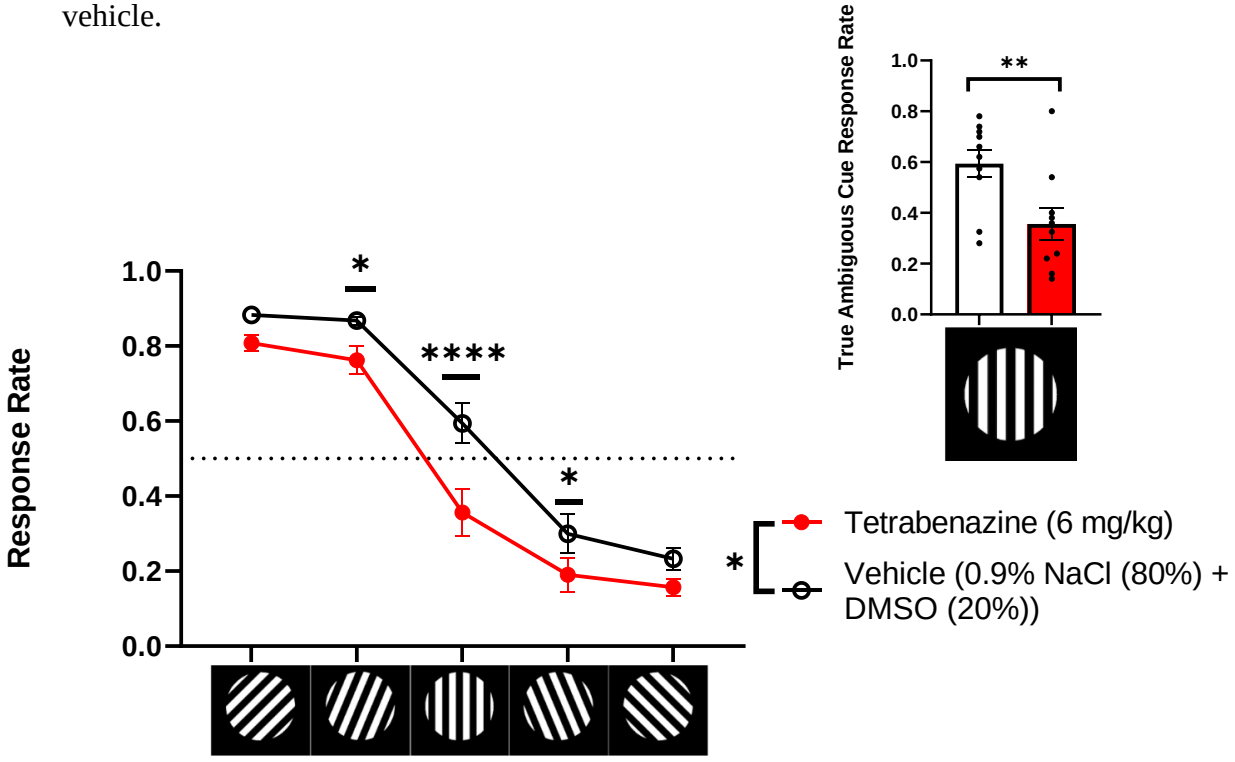


Figure 29. Effect of TBZ on CJB Responding (n =10). The 2-way repeated measures ANOVA found a significant drug treatment x cue interaction. Post hoc tests reflected differences between TBZ and vehicle at the near-positive, true, and near-negative

ambiguous cues. *A priori* tests discovered that TBZ significantly reduced response rates for the true ambiguous cue when compared to vehicle.

There were no effects of either cue or treatment on response latency (all, $p > 0.0693$; Figure 30a) suggesting that animals responded to all cues with similar response times regardless of treatment. Despite post hoc analyses suggesting non-significant effects of treatment at the trained cues (Figure 30), response rate at these cues, as well as discrimination sensitivity and response bias were assessed. When treated with TBZ, mice had significantly lower response rates when presented with the positive cue ($t_{9)} = 3.071$, Cohen's $d = 1.48378$, $p < 0.05$; Figure 30b) and significantly lower response rates when presented with the negative cue ($t_{9)} = 2.723$, Cohen's $d = 0.936972$, $p < 0.05$; Figure 30c). These findings suggest that TBZ reduced responding at both trained cues, in addition to the ambiguous cues. This produced a significant change towards a more conservative response bias with TBZ treatment ($t_{9)} = 4.240$, Cohen's $d = 1.4598$, $p < 0.01$; Figure 30e). Notably, however, mice showed no changes in their ability to discriminate between the positive and negative cues ($p = 0.7957$; Figure 30d). These data collectively suggest that TBZ induces a negative shift in CJB but also may affect motivation, leading to a decrease in overall responding during the task, despite the decreases in response rate being greater at the ambiguous compared to the trained cues.

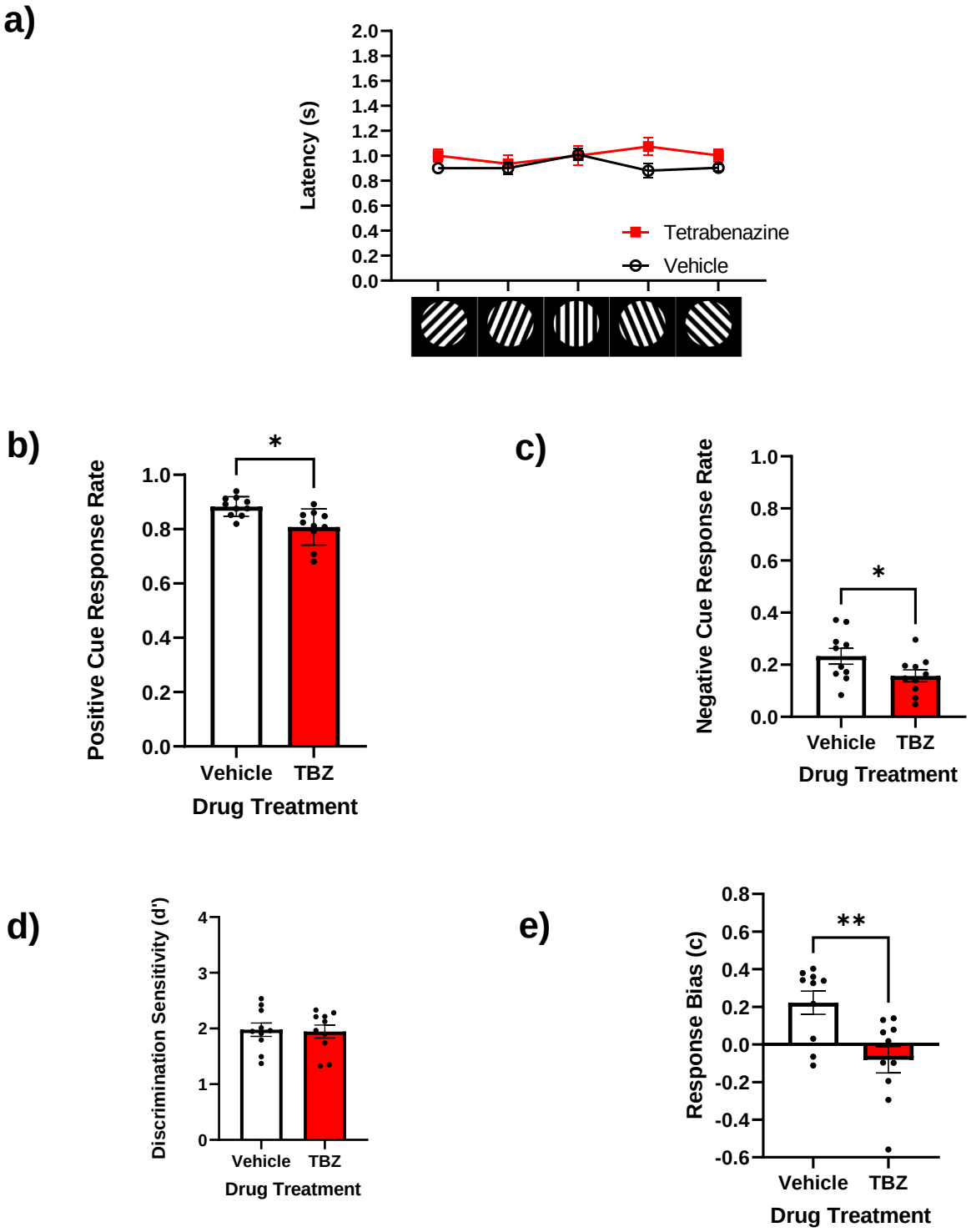


Figure 30. Effects of TBZ on CJB responding. a) TBZ did not affect response latencies for the five cues b) TBZ significantly reduced response rate for the positive cue c) TBZ significantly reduced response rate for the negative cue d) TBZ did not affect discrimination sensitivity (d') e) TBZ induced a more conservative response bias (c).

3.3 Handling, Transport, and Injection Stress Experiment

As TBZ treatment may have led to off-target effects on motivation, a secondary intervention was sought to validate whether the Lopez-Cruz Variant CJB task could measure negative shifts in cognitive bias. It was observed during testing with pharmacological interventions that there was a decrease in response rate for the ambiguous cues between baseline measurement and any treatment, including both vehicle treatments. To determine whether this was solely due to reduced responding following learning that these cues never resulted in reward or whether the task is sensitive enough to measure changes in cognitive bias from the stress of handling, transport and injection, an experiment was carried out in which mice were left undisturbed in the home cage or injected with the previous vehicle treatments prior to CJB testing.

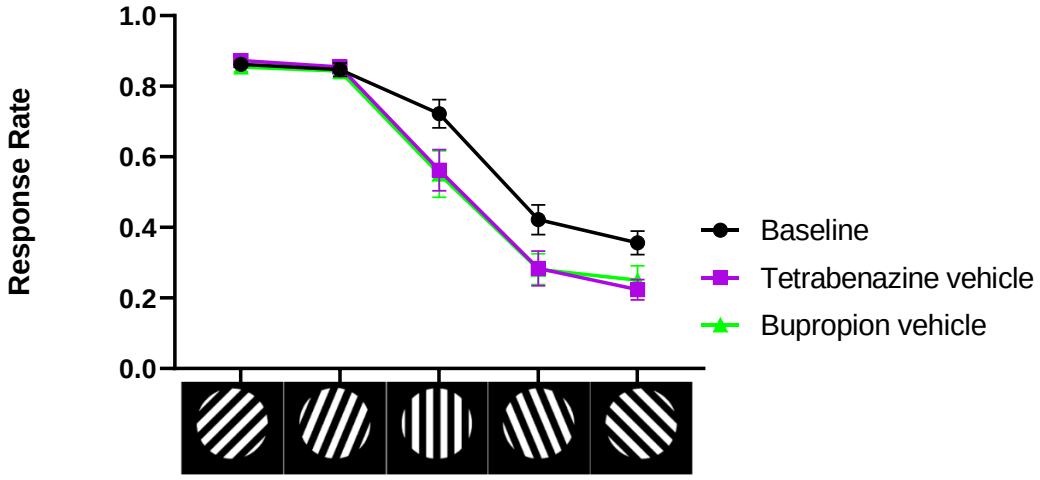


Figure 31. Comparing CJB Baseline to TBZ and BUP Vehicle Injections. Both types of vehicle injections appeared to decrease the response rate at the near-negative, negative, and true ambiguous cues.

3.3.1 Training Progression

All animals (n = 16) successfully completed pre-training and discrimination training (stricter criteria) stages before or within 66 sessions with an average of 44 sessions (44.313 ± 13.470). One female mouse died before completing all three conditions. Therefore, 8 males and 7 female (n = 15 total) completed testing. Progress for all animals is depicted below (Figure 32).

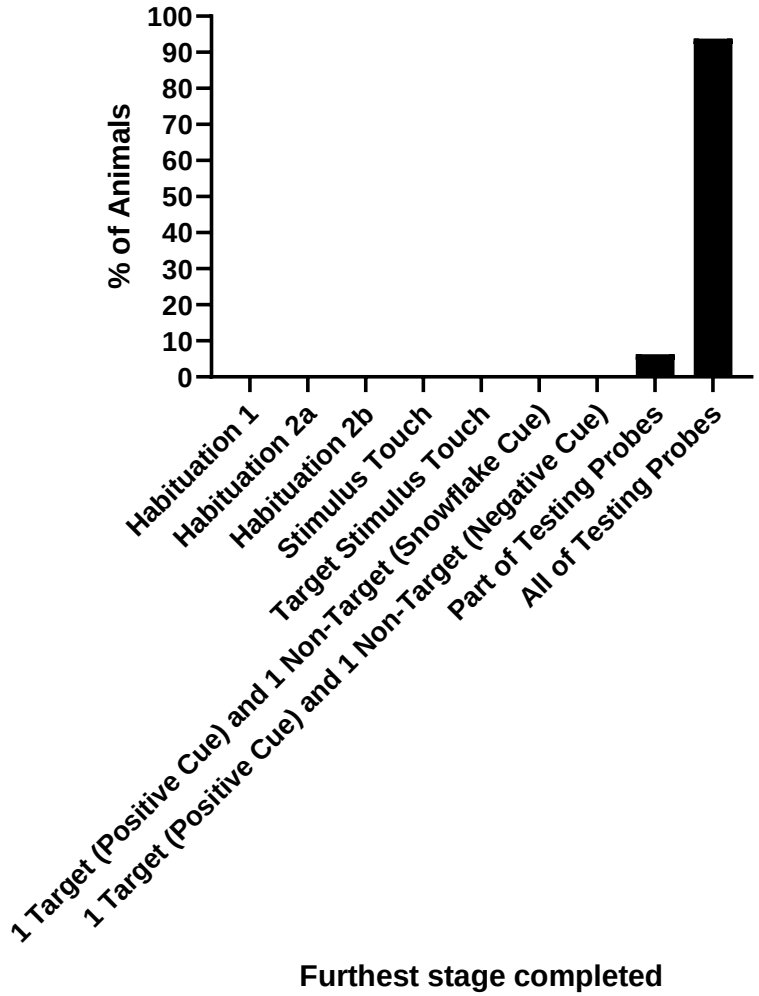


Figure 32. Furthest Stage Completed. n =15 animals completed all discrimination training and probe testing sessions and n = 1 animal completed discrimination and part of probe testing sessions and was removed from further analyses.

3.3.2 Baseline

After meeting criteria for "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)", animals (n = 15) all completed a baseline CJB measurement. Four mice quickly reached criterion, but it was observed that they had high false alarm rates. As such, they returned to "1 Target (Positive Cue) and 1 Non-Target (Negative Cue)" with stricter criteria (two consecutive sessions of 0.8 or higher hit rate, 0.4 or lower false alarm rate) before reperforming the entirety of their baseline sessions.

The animals' baseline produced the typical curve seen in CJB. As expected, animals displayed a high response rate for the positive cue (0.859± 0.0577), low response rate for the negative cue (0.326± 0.0963) and intermediate responding at the ambiguous cues.

They also appeared to respond optimistically to the true ambiguous cue at baseline (0.733± 0.147). Figure 33 depicts the baseline CJB curve.

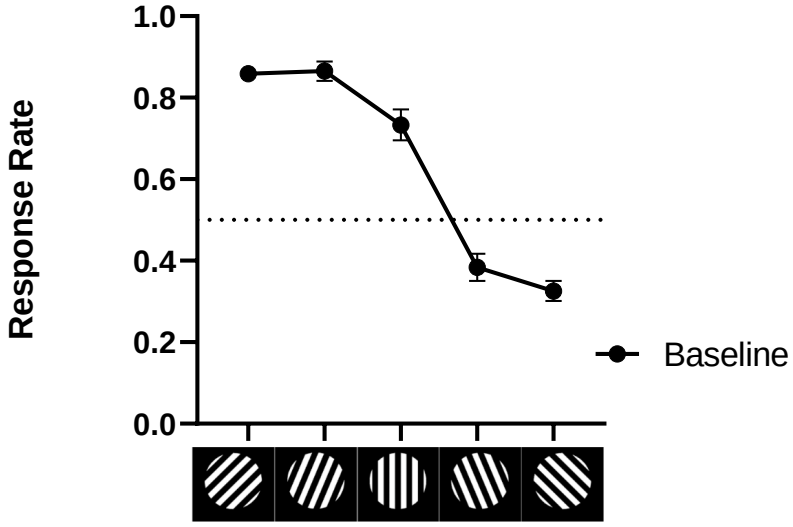


Figure 33. Baseline CJB responding (n = 15 Animals) for handling, transport, and injection stress experiment.

3.3.3 Handling, Transport, and Injection Alone Were Sufficient to Induce a Negative Shift in CJB

To investigate the effects of handling and injection on CJB, animals (n = 15) were exposed to three conditions: the "Saline Injection" condition (administered 30 minutes prior to testing), the "Saline + DMSO Injection" condition (administered 2 hours prior to testing) and the "No-Injection" condition (handled in home cage). One male mouse was rerun in the "No-Injection" condition due to being misplaced in the wrong cage during the probe week. There was a main effect of cue ($F_{(2.122,\ 29.71)} = 321.1$, $\eta^2 = 0.8623$, $p < 0.0001$; Figure 34), but no interaction of condition x cue ($p = 0.221$; Figure 34). There was also a main effect of condition ($F_{(1.952,\ 27.33)} = 9.751$, $\eta^2 = 0.01065$, $p < 0.001$; Figure 34) with both injection groups significantly lower than no-injection: saline ($t_{(13)} = -4.111$, $p < 0.001$) and saline + DMSO ($t_{(13)} = -3.452$, $p < 0.01$). *A priori* planned comparisons revealed a significant effect of condition ($F_{(1.733,\ 24.26)} = 4.634$, $\eta^2 = 0.2487$, $p < 0.05$; Figure 34 inset) with a significant decrease in response rate for the "Saline Injection" condition when compared to the "No-Injection" condition ($p < 0.01$; Figure 34 inset). However, non-significant differences were found between "Saline Injection" versus

"Saline + DMSO Injection" (p = 0.7459; Figure 34 inset) and saline + DMSO injection versus no injection (p = 0.1536; Figure 34 inset). Overall, these results suggest that saline injection 30 minutes prior to testing was sufficient to significantly decrease response rate at the true ambiguous cue compared to the "No-Injection" condition.

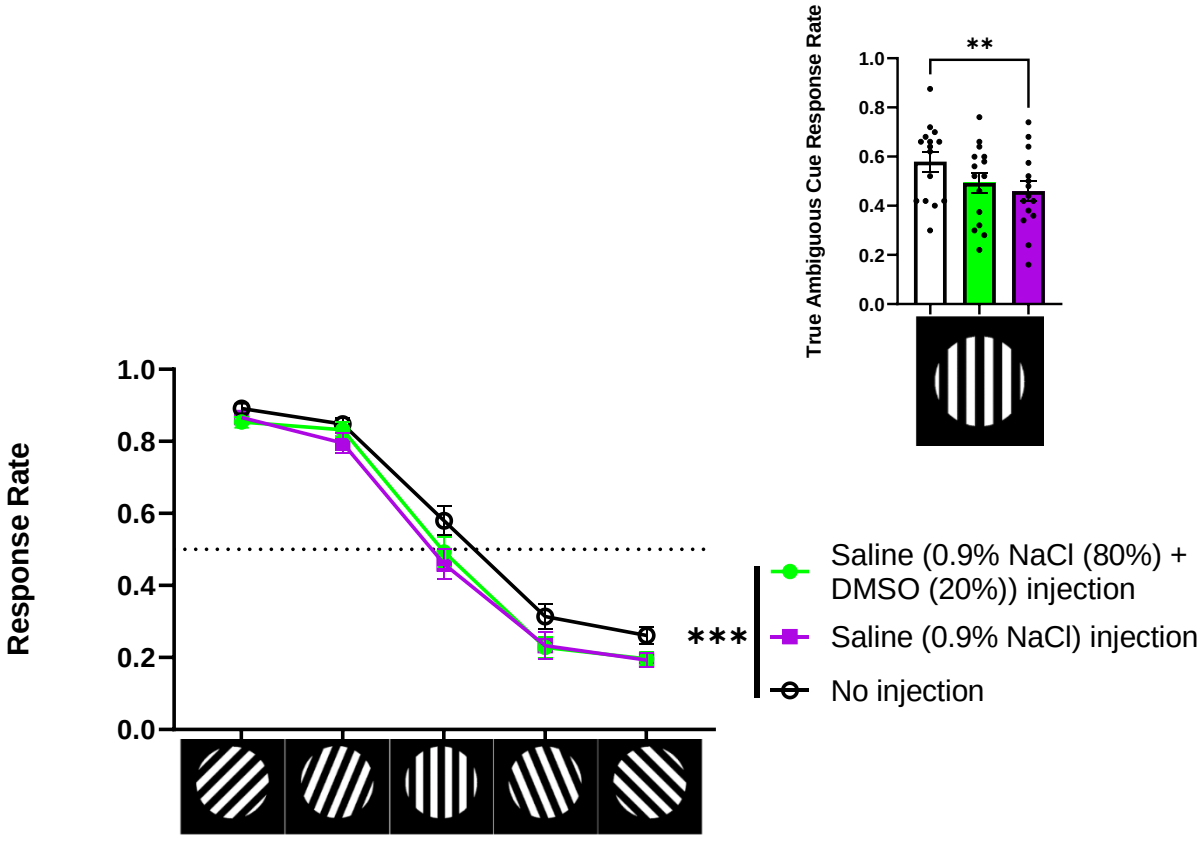


Figure 34. Effect of Saline Injection and Saline + DMSO Injection on CJB Responding (n = 15). 2-way repeated measures ANOVA found a main effect of condition. *A priori* tests found that "Saline Injection" significantly decreased response rate for the true ambiguous cue versus "No-Injection".

In terms of response latencies, there was an interaction of condition x cue ($F_{(3.087, 43.22)}$ = 4.237, η^2 = 0.05152, p < 0.01; Figure 35a), main effect of condition ($F_{(1.659, 23.22)}$ = 4.050, η^2 = 0.04392, p < 0.05; Figure 35a) and no effect of cue (p = 0.454; Figure 35a). Šidák post hoc tests revealed significant differences between saline + DMSO injection and no-injection at the positive cue (p < 0.05; Figure 35a) and negative cue (p < 0.05; Figure 35a). Additionally, there were significant differences between saline injection and no-injection at the positive cue (p < 0.01; Figure 35a), true ambiguous cue (p < 0.05; Figure

35a) and negative cue ($p < 0.01$; Figure 35a). There was also a significant difference between saline injection and saline + DMSO injection at the near-negative cue ($p < 0.05$; Figure 35a). These results suggest that animals responded faster when following no injection compared to receiving an injection at the respective cues listed above, but, critically, all groups fell well within the 2.5 second response window.

Response rates at the positive and negative cue were also assessed. Similar response rates for the positive cues were found regardless of condition ($p = 0.0593$; Figure 35b). However, response rates for the negative cue were significantly decreased in the "Saline Injection" ($p < 0.05$; Figure 35c) and "Saline + DMSO Injection" conditions ($p < 0.05$; Figure 35c) compared to the "No-Injection" condition. These findings suggest that animals had similar response rates to the positive cue; however, animals had higher response rates for the negative cue when receiving no injection compared to receiving either type of injection. These effects produced significant changes towards more conservative response biases in the "Saline Injection" ($p < 0.05$; Figure 35e) and "Saline + DMSO Injection" conditions ($p < 0.01$; Figure 35e). This suggests that when receiving either type of injection, animals responded more conservatively to cues than animals receiving no injection. Notably, this effect was driven by an increase in correct rejections (i.e. a decrease in the response rate for the negative cue) in the injection groups whereas there was no difference in response rate for the positive cue. As such, the changes in trained cue responses does not appear to be driven by overall changes in motivation. This stands in contrast to TBZ which decreased responding to both trained cues. Importantly, mice showed no changes in their ability to discriminate between the positive and negative cues ($p = 0.642$; Figure 35d). Overall, this demonstrates that saline injection 30 minutes prior to testing is sufficient to induce a negative shift in CJB.

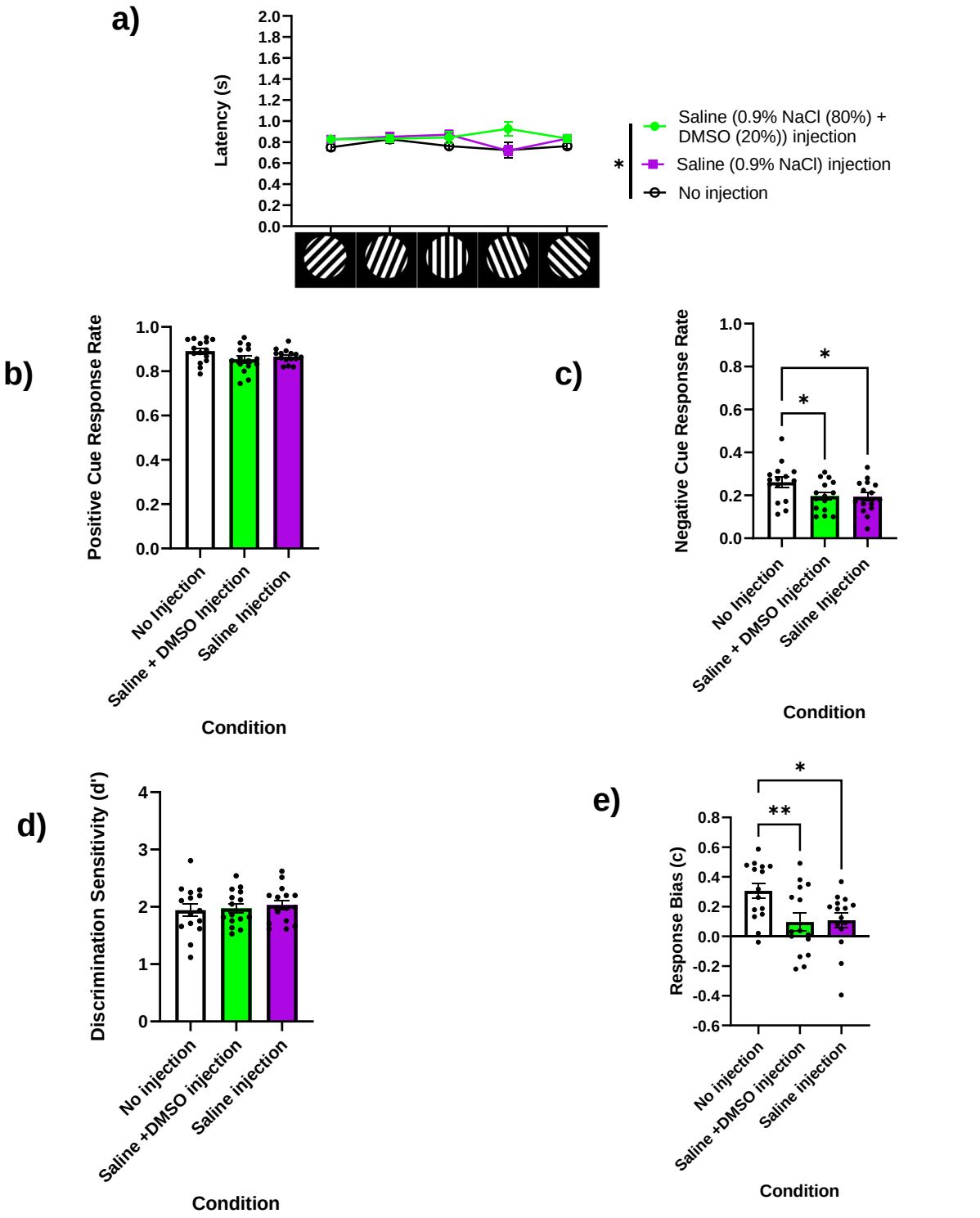


Figure 35. Effects of Saline Injection and Saline + DMSO Injection on CJB responding. a) Response latencies were higher with injection, but values fell within response window b) Condition did not affect response rate for positive cue c) Both injection conditions decreased response rate for the negative cue d) Condition did not affect discrimination sensitivity (d') e) Both injection conditions induced more conservative response biases (c).

3.3.4 Comparing Baseline and No-Injection Condition

To assess the effect of repeated testing on CJB responding (n = 15), a two-way repeated measures ANOVA was performed with cue and phase (baseline versus "No-Injection" probes) as within subject's factors. There was an interaction of phase x cue ($F_{(2.177, 30.48)}$ = 8.716, η^2 = 0.01308, $p < 0.001$; Figure 36), a main effect of cue ($F_{(2.577, 36.08)}$ = 222.4, η^2 = 0.8276, $p < 0.0001$; Figure 36) and main effect of condition ($F_{(1.000, 14.00)}$ = 4.920, η^2 = 0.01013, $p < 0.05$; Figure 36). Šidák post hoc tests revealed significant differences between "No-Injection" and baseline at the true ambiguous cue only ($p < 0.05$; Figure 36). Overall, these results suggest that there was a significant decrease in response rate at the true ambiguous cue when comparing baseline to the "No-Injection" condition. This indicates a decrease in response to the ambiguous and unrewarded cues following initial baseline measurement. However, as the saline and saline + DMSO injection conditions showed significant decreases in ambiguous cue response rate compared to the "No-Injection" condition, with these conditions counterbalanced via Latin square design, the changes to responding in the initial pharmacological experiment following baseline are likely due to a combination of learning that the ambiguous cues are unrewarded and further negative shifts in cognitive bias.

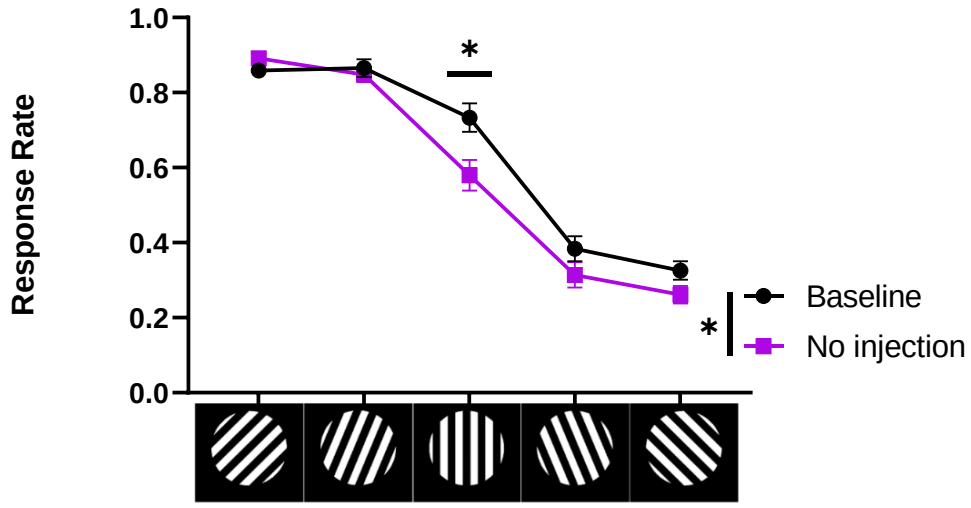


Figure 36. Baseline vs "No-Injection" CJB Responding (n = 15). There was a significant decrease in response rate at the true ambiguous cue for the no injection condition compared to baseline.

4 Discussion

This thesis investigated the development and validation of a touchscreen-based task assessing CJB in mice. We attempted three separate task designs, and successfully validated one of three tasks, CJB Task 1 (Lopez-Cruz Variant). We first pharmacologically validated the task using two agents, tetrabenazine (TBZ) and bupropion (BUP), known to manipulate affective state. After completing within-subjects Latin square designs, we found that BUP administration (7.5 mg/kg and 10 mg/kg) produced a positive shift in CJB and TBZ administration (6 mg/kg) produced a negative shift in CJB. We also investigated the effects of handling, transport, and injection on CJB as a validation using an ecologically relevant stressor. We found that an injection of saline 30 minutes prior to testing induced a negative shift in CJB. Collectively, these results support the validity of a non-aversive and translatable CJB task that can be used to understand and develop treatments for affective disorders in mouse models.

4.1 CJB Task Comparison – Training Progression and Duration

We aimed to develop a task that is non-aversive, contains good construct and predictive validity and that can detect positive and negative shifts in CJB. We trained animals simultaneously on CJB Task 1 (Lopez-Cruz Variant), CJB Task 2 (Krakenberg Variant) and CJB Task 3 (2-Stage Novel Variant). Overall, animals completing CJB Task 1 acquired the task and met the appropriate criteria to proceed to testing probes in the shortest time (approximately 1 month to complete pre-training and discrimination training) (Figure 24 and 25). In both CJB Task 2 and CJB Task 3, animals took approximately 21 sessions for pre-training (Figure 24 and 25). The two animals from CJB Task 2 that made criteria for probe tests took an average of 2.75 months to complete discrimination training (Figure 24 and 25). Half of the animals (n = 8) from CJB Task 3 that made criteria for probes took an average of 3.75 months to complete PVD/VMCL training (Figure 24 and 25). In comparison, in the study by Krakenberg et al. (2020), animals took approximately one month for pre-training and approximately four months for discrimination training. Therefore, the training duration times from Krakenberg et al. (2020) are comparable to CJB Tasks 2 and 3, but longer than CJB Task 1.

In all three CJB tasks, the typical graded response curve (baseline) that is seen in CJB tests was produced (Hintze et al., 2018). A smooth, monotonic curve is key in CJB tasks to ensure that the ambiguous cues are interpreted with respect to the previously learned reference cues and not interpreted as irrelevant or novel cues (Hintze et al., 2018; Mendl et al., 2009). Indeed, some CJB tasks have failed to produce this curve and have produced curves that are flat or erratic (Bateson & Nettle, 2015; Burman et al., 2009; Hintze et al., 2017). Although all three tasks generated the expected CJB response curves, CJB Task 1 was superior due to the short training time and because all animals were able to acquire the task.

4.2 Advantages and Disadvantages of the CJB Task Designs

One advantage of CJB Task 2 (Krakenberg Variant) and CJB Task 3 (2-Stage Novel Variant) is that they are Go/Go paradigms in which animals are required to make a response in both positive and negative conditions. Go/Go paradigms have been thought to be more reliable than Go/No-Go (Gencturk & Unal, 2024). Researchers have previously criticized Go/No-Go task designs due to the difficulties related to interpreting immobility or lack of response (Nguyen et al., 2020). Additionally, when presented with an ambiguous cue during testing probes, the lack of response could be intentional or the animal could not be attending (Roelofs et al., 2016). In CJB Task 1 (Lopez-Cruz Variant), animals were trained so that the positive cue response rate was sufficiently high (> 80%) to minimize the number of stimuli missed due to a lack of attention. Additionally, the response rate at the negative cue and response latencies for all cues were used as control measures to monitor changes in motivation.

For CJB Task 3 (2-Stage Novel Variant), a disadvantage of this task was the long training time. A majority of the animals struggled with training and therefore were moved back to easier versions of the task with more visually distinct cues (“Easy VMCL” and “Easy PVD”) before moving onto harder versions of the task with the circular line cues (“Hard VMCL” and “Hard PVD”). If we were to reattempt training for this task, we would introduce the easier versions of the tasks first in hopes of condensing the training time to streamline the process. Another limitation of this task was that animals were required to

learn and remember how to perform two separate touchscreen tasks (VMCL and PVD). It is possible that the animals did not transfer knowledge between the two tasks (cue valence from PVD onto the VMCL testing format) and simply treated them as separate tasks. This may be possible considering the apparent lack of drug effects (particularly TBZ) in the animals that did complete testing probes.

For CJB Task 2 (Krakenberg Variant), the majority of animals trained on this task could not meet criteria to move onto testing probes. This may likely be due to the combination of the complexity of the task (left vs right side responding with different reward contingencies) combined with the more visually similar stimuli (circular line cues). Although the paradigm was successful in Krakenberg et al. (2020), it is possible that their use of horizontal line cues were simpler cues for the mice to discriminate. Because some animals were successful in completing pre-training and discrimination training in our adapted version of the task, it appears that it is possible for animals to learn the task with our cues. Due to lack of time, animals were excluded after failing to meet criteria and performing at approximately chance (50% correct) after several months. However, if the task was attempted again, we would introduce more visually distinct cues at the beginning for animals to learn the rules before introducing more visually similar cues (as done in the CJB Task 3 (2-Stage Novel Variant)). It is possible that if the mice had relearned the task with easier cues first that they would have been able to acquire the task.

4.3 Experiments: CJB Task 1 (Lopez-Cruz Variant)

4.3.1 Bupropion (BUP) (5 mg/kg) Induced a Positive Shift in CJB

In this study, we used the antidepressant, bupropion (BUP) to induce an optimistic-like judgement bias. In our first Latin square which included TBZ, BUP (5 mg/kg) and their respective vehicles, we found that the lower dose of BUP (5 mg/kg) produced a statistical trend ($p = 0.0766$) towards a positive shift in CJB (Figure 26). We therefore completed a second Latin-square including two higher doses of BUP and vehicle. We found that both higher doses of BUP (7.5 and 10 mg/kg) induced positive shifts in CJB as seen by the significant increases in responding to the true ambiguous cue compared to vehicle (Figure

27). Additionally, BUP did not significantly change response latencies or responding to the trained cues, indicating that it did not generally increase responding to cues (Figure 28). Preliminary results from the laboratory found that 10 mg/kg BUP induced a positive CJB (Lopez-Cruz et al., in prep). The effects of BUP have been investigated outside the context of affective disorders in naïve rodents and healthy volunteers. In mice, BUP decreased immobility time in the FST and TST, suggesting it exerted antidepressant effects to reverse behavioural despair (Dhir & Kulkarni, 2007). Also, BUP biased participants towards recognizing ambiguous faces as happy and reduced attention for fearful faces in healthy volunteers (Walsh et al., 2018). Overall, this suggests that BUP was able to produce an optimistic-like judgement bias, which is likely attributable to changes in affect.

4.3.2 Tetrabenazine (TBZ) Induced a Negative Shift in CJB

We also used tetrabenazine (TBZ) to induce a pessimistic-like judgement bias. Overall, we found that TBZ (6 mg/kg) induced a negative shift in CJB as seen by the significant decrease in responding to the true ambiguous cue compared to vehicle (Figure 29). This aligns with preliminary results from the laboratory which found that TBZ (6 mg/kg) induced a negative CJB (Lopez-Cruz et al., in prep). TBZ has also been shown to induce a negative bias in the rat affective bias test (Stuart et al., 2017). However, our results must be interpreted with caution. Although response latencies were not affected by TBZ, the mice decreased their responding at all other cues, both trained and ambiguous (Figure 30). Additionally, following TBZ administration, mice shifted their responding to be more conservative (low c score) (Figure 30). Although the magnitude of the decrease comparing TBZ and vehicle was the largest at the true ambiguous cue, it is likely that the effects seen are not solely attributable to changes in affect. Other work using TBZ in the touchscreen task assessing effort-based choice (concurrent fixed ratio 1/choice task) found that TBZ administration in mice produced deficits in effort-related motivation. Animals treated with TBZ reduced their responding for high effort/high value reward and increased their responding for low effort/low value reward (Yang et al., 2020). Human work has also reported TBZ-induced depression and effort-related symptoms including fatigue (Caroff et al., 2018; Frank, 2010). Given those findings, the effects of TBZ seen

in our study are likely due to a combination of both decreased motivation and overall responding as well as negative affect.

4.3.3 Handling, Transport and Injection Alone were Sufficient to Induce a Negative Shift in CJB

An additional objective of this study was to investigate the effects of handling, transport, and injection, an ecologically relevant stressor, on CJB. Previous data from the pharmacological experiments revealed significant decreases in responding to the true ambiguous cue when comparing baseline (no manipulation) to both vehicle injection conditions. It was therefore unclear whether this decreased responding was due to the animals learning that responding to the ambiguous cues was unrewarded, a potential injection experience-related stress or a combination of both. When comparing baseline to the "No-Injection" condition, there was a significant decrease in responding to the true ambiguous cue, suggesting that animals had learned that the true ambiguous cue was unrewarded (Figure 36). However, we found that a saline injection administered 30 minutes prior to testing induced a further negative shift in CJB as seen by the significant decrease in responding to the true ambiguous cue compared to the "No-Injection" condition (Figure 34). Overall, this suggests that part of the change in CJB responding was attributable to learning the cues and the rest of the effect was attributable to the handling, transport, and injection-related stress.

There is limited research on injection-based stress. Du Preez et al. (2020) investigated the effects of 6 weeks of repeated injection of saline on anxiety and depressive-like behaviours in male mice. They found increased anxiety-like behaviour via the open field test but no evidence of depressive-like behaviour in the forced swim test and sucrose preference test. Additionally, they found lower levels of tumour necrosis factor alpha (TNFα) and interleukin-4 levels and increased corticosterone reactivity, suggesting a hyperactive hypothalamic-pituitary-adrenal axis (HPA) axis, commonly found in anxiety and depression-related conditions (Chen et al., 2015; Du Preez et al., 2020). This suggests that repeated injections could contribute to a hyperactive HPA axis. Our findings also align with work from Drude et al. (2011) who compared plasma corticosterone changes in mice after either zero, one or two injections of saline. They found that mice that

received two saline injections had significantly higher average plasma corticosterone concentrations than mice that received no injection. Corticosterone levels also peaked 30 minutes after the second injection and returned back to normal levels within two hours. This may explain why in the current study, negative CJB was detected 30 minutes after saline injection but not two hours after saline + DMSO injection. Future studies using this task should consider investigating any injection-induced changes from baseline, especially if probes will be completed over multiple testing weeks.

Animals responded faster to the cues in the no injection condition compared to both injection conditions (saline injection and saline + DMSO injection). Although the mice responded similarly to the positive cue, they also decreased their responding to the negative cue and responded more conservatively to cues in both injection conditions compared to the "No-Injection" condition. Previous research has suggested that pharmacological manipulations of affect can affect responding not only at the ambiguous cues, but also at the negative trained cue (Neville et al., 2020). Additionally, research has shown that individuals that had induction of negative affect or naturally self-reported negative affect showed greater response to negatively-valenced stimuli through greater attention to negative words (Bradley et al., 1997; Trevarthen et al., 2019). Therefore, in the present study, it is possible that after injection with saline or saline + DMSO, the mice interpreted the negative cue even more negatively and were more likely to withhold responding from it. Additionally, since there was no difference in response at the positive cue, handling, transport, and injection, does not appear to decrease motivation.

4.3.4 Sex Differences

While both male and female mice were used throughout, these experiments were not sufficiently powered to detect sex differences. Therefore, the main figures contained both sexes and exploratory sex-based analyses for each sex are represented in Appendix A. In these analyses, we found that in both males and females, 7.5 mg/kg BUP induced positive shifts in CJB as seen by the significant increase in responding to the true ambiguous cue compared to vehicle (Appendix A Figures A3 and A4). In males, there was a trend towards a negative shift in CJB after TBZ administration (Appendix Figure A6). This failure to reach significance is likely due to the low n (n = 5). In females, TBZ induced a

negative shift in CJB as seen by the significant decrease in responding to the true ambiguous cue compared to vehicle (Appendix A Figure A7). In terms of handling, transport and injection in males, there did not appear to be any differences between the conditions (Appendix A Figure A10). However, in females, it appeared that both the "Saline Injection" and "Saline + DMSO Injection" conditions induced negative shifts in CJB (Appendix A Figure A11). Specifically, saline injection significantly decreased responding to the true ambiguous cue and near-negative cue compared to "No-Injection", and "Saline + DMSO Injection" significantly decreased responding for the negative and near-negative cue compared to "No-Injection". Collectively, this suggests that females but not males displayed pessimistic-like judgement biases from experiencing handling, transport, and injection. This finding aligns with work from Ryabinin et al. (1999) who found that male C57 mice were able to habituate to repeated handling and injection of saline as seen by the blunted immediate early gene response in stress-related brain regions with protein levels similar to control animals. Therefore, in our study, it is possible that the male mice habituated to the repeated injections over the multiple weeks of testing.

4.3.5 Single-Housed Animals

In our initial pharmacological experiment (prior to the first Latin square), we single-housed three male mice due to fighting. Throughout their testing, it appeared that these mice responded pessimistically regardless of treatment received as shown by their low response rates to the true ambiguous cue for all treatment groups. Although there appeared to be a slight increase in response rate at the true ambiguous cue for BUP (10 mg/kg) in these three animals, suggesting that it may be possible to induce a positive shift in CJB, a larger sample size would be necessary to investigate this further. Some work found that 4-8 weeks of social isolation during adulthood induced hyperactivity in the open field (OF) test, increased immobility time in the forced swim test (FST) and tail suspension test and decreased sucrose preference (Hu et al., 2023; Ieraci et al., 2016; N. Liu et al., 2020; Mileva & Bielajew, 2015), suggesting increased anxiety and depression-like behaviours. However, other researchers have found contradictory findings. Gorlova et al. (2018) found that three weeks of social isolation in adult male rats did not differ

from group-housed animals for the FST or sucrose preference test (SPT) and did not induce a depressive-like phenotype. Additionally, Alshammari et al. (2020) did not find any differences between isolated rats and control rats for OF, SPT or FST. Given the mix of findings, further investigation is needed to better understand the impact of social isolation, notably, no research has yet explored the effects of social isolation on CJB in male and female mice.

4.4 Limitations

One of the limitations of this study was the reduction in overall responding to cues and conservative response bias from TBZ administration, making it difficult to separate changes in CJB attributed to changes in motivation versus changes in affect. Future studies could use a lower dose of TBZ to reduce motivational impairments while still inducing a negative shift in CJB. An alternative could be to use a different pharmacological agent such as corticosterone to induce a negative shift in CJB, which has previously been done in a rat CJB test by Hales et al. (2022).

Another limitation was the low numbers per sex. Therefore, it is unclear at this point whether sex differences exist, and data must be interpreted with caution. In humans, there is minimal research that has investigated sex differences in interpretation bias. Major depressive disorder (MDD) is two times more prevalent in women than in men (Bekker & van Mens-Verhulst, 2007). Previous research on individuals with chronic depression found that women tended to report greater functional impairment and greater illness severity than men in self report measures (Kornstein et al., 2000). Additionally, the interpretation of ambiguous entities as negative, a cognitive symptom of MDD, has been found to be more prevalent in females than males (Mansour et al., 2006). In animal work, there is also limited research that has explored sex differences in CJB. A systematic review and meta-analysis by Neville et al. (2020) investigated pharmacological manipulations. They found that sex did not appear to moderate the effects of pharmacological manipulations of affective state on CJB. Future studies should be sufficiently powered and include both males and females and analyze using sex as a biological factor. This could also be used to better understand sex-specific differences in

response to various stressors or pharmacological agents to develop more targeted therapies.

An additional limitation of the study design was that it appeared that the mice learned that the true ambiguous cue was not associated with a reward or punishment and therefore decreased their responding as shown by the drop when comparing baseline versus “No-Injection”. Some previous studies using judgement bias tests found that with repeated testing, the ambiguous cues lost their ambiguity as the animals learned that the cues were unrewarded and altered their responding (Roelofs et al., 2016). Some methods have been used to avoid learning which include using a partial reinforcement schedule during training where some trials for the trained cues are unreinforced, and by including unreinforced trials for the trained cues during testing (Bateson et al., 2015; Richter et al., 2012). Another solution could be to use a secondary reinforcer during training and testing. For example, Keen et al. (2014) used a clicker as a secondary reinforcer to their primary reinforcer (food reward) whereby during ambiguous trials, the clicker continued but not the food reward. Other ways to circumvent this problem could be to increase the time between testing conditions (greater than one week) to reduce the likelihood of the mice remembering the cues or to minimize the number of repeated testing sessions.

4.5 Future Directions

There is a lot that remains unknown about the neurobiological mechanisms underlying CJB. Some cognitive mechanisms thought to be involved in judgement biases include prior expectation of and sensitivity to reward and punishment (Mendl et al., 2009; Neville et al., 2020). There is evidence suggesting that serotonin, opioid, GABA, and dopamine activity in regions including the nucleus accumbens, orbitofrontal cortex, mesolimbic dopamine projections and amygdala play a role in encoding the probability and value of reward and punishment (Berridge et al., 2009; Boureau & Dayan, 2011; Mendl et al., 2009; Neville et al., 2020; Owens & Nemeroff, 1994). Specifically, when an individual is faced with an ambiguous situation, it would be interesting to understand if there are specific neurobiological changes occurring when they make an optimistic or pessimistic choice. Therefore, future work could look into neurotransmitter activity during task

performance using tools such as fiber photometry to record activity in neuronal populations.

Other applications of this CJB task include exercise and diet interventions. Previous work has associated high-fat diets with reduced hippocampal volume and increased vulnerability for anxiety and depression (Anderson et al., 2001; Jacka et al., 2015). Research has also found that high fat diets produced anxiety-like and anhedonic behaviour in rats (Dutheil et al., 2016) and a high-fat high-sugar diet was associated with depressed mood and depressive symptoms (Vermeulen et al., 2017). Additionally, research has found that acute exercise predicted higher positive affect in daily life in humans (Liao et al., 2015). It would be worthy of investigating whether a high fat/high sugar or sedentary lifestyle would reflect a negative CJB and whether these effects could be reversed using pharmacological or lifestyle interventions. Overall, this could allow us to investigate obesity-related changes in affect to improve quality of life.

Future studies could investigate CJB in mouse models of disease. Negative interpretation biases have been linked to depression (Everaert et al., 2017; Lee et al., 2016) and anxiety (Mathews & MacLeod, 2005), therefore future work could use the CJB task to measure CJB in mouse models of anxiety and depression and investigate the potential efficacy of future therapeutics. Additionally depressive symptoms and changes in mood are frequently reported in those with Alzheimer's disease (Zhao et al., 2016). Therefore, additional research could be done to investigate the potential of negative CJB in mouse models of neurodegenerative disease and to develop and test treatments that target disruptions in affect. Although this study provides evidence for a validated touchscreen CJB task in mice, a future goal of this translational research is to have an identical or highly similar test available for use in humans. Future work could look to non-pharmacologically validate this task in mice and humans with aims of applying this task in clinical populations to evaluate early changes in cognitive-emotional symptoms.

4.6 General Conclusions

Overall, in this work we sought to develop a non-aversive and translatable task with good construct and predictive validity that can detect positive and negative shifts in CJB. We attempted three different CJB task designs, and all tasks produced the typical CJB response curve for baseline responding. We proceeded to use CJB Task 1 (Lopez-Cruz Variant) due to the highest proportion of animals acquiring the task and the shortest training time. By using this task, we were able to successfully induce both a positive shift in CJB using higher doses of BUP (7.5 and 10 mg/kg) and a negative shift in CJB using TBZ (6 mg/kg) and also through handling, transport, and injection-related stress. Collectively, this suggests that this touchscreen based CJB task demonstrated both construct validity and predictive validity. Future studies should use this task to investigate underlying mechanisms of CJB, diet/exercise-based interventions and testing therapeutics in mouse models of affective disorders.

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*Main figures were created using BioRender. Graphs were created in GraphPad Prism.

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Appendices

Appendix A: Supplemental Results Figures

CJB Task 1 (Lopez-Cruz Variant)

Bupropion (5 mg/kg)

There was no effect of treatment or cue, nor any interaction between factors, on response latency (all $p > 0.105$; Figure A1a) suggesting that animals responded to all cues with similar response times regardless of treatment. Response rates at the positive and negative cues, as well as discrimination sensitivity and response bias were assessed. When treated with BUP, mice had significantly higher response rates when presented with the positive cue ($t_{(10)} = 2.697$, Cohen's $d = 0.770$, $p < 0.05$; Figure A1b), but similar response rates when presented with the negative cue ($p = 0.319$; Figure A1c). These findings suggest that BUP increased responding at the positive cue. This effect was clearly demonstrated by a significant change towards a more liberal response bias with BUP treatment ($t_{(10)} = 2.866$, Cohen's $d = 0.599$, $p < 0.05$; Figure A1e). Notably, however, mice showed no changes in their ability to discriminate between the positive and negative cues ($p = 0.617$; Figure A1d). These data collectively suggest that BUP increased animal responding during the task.

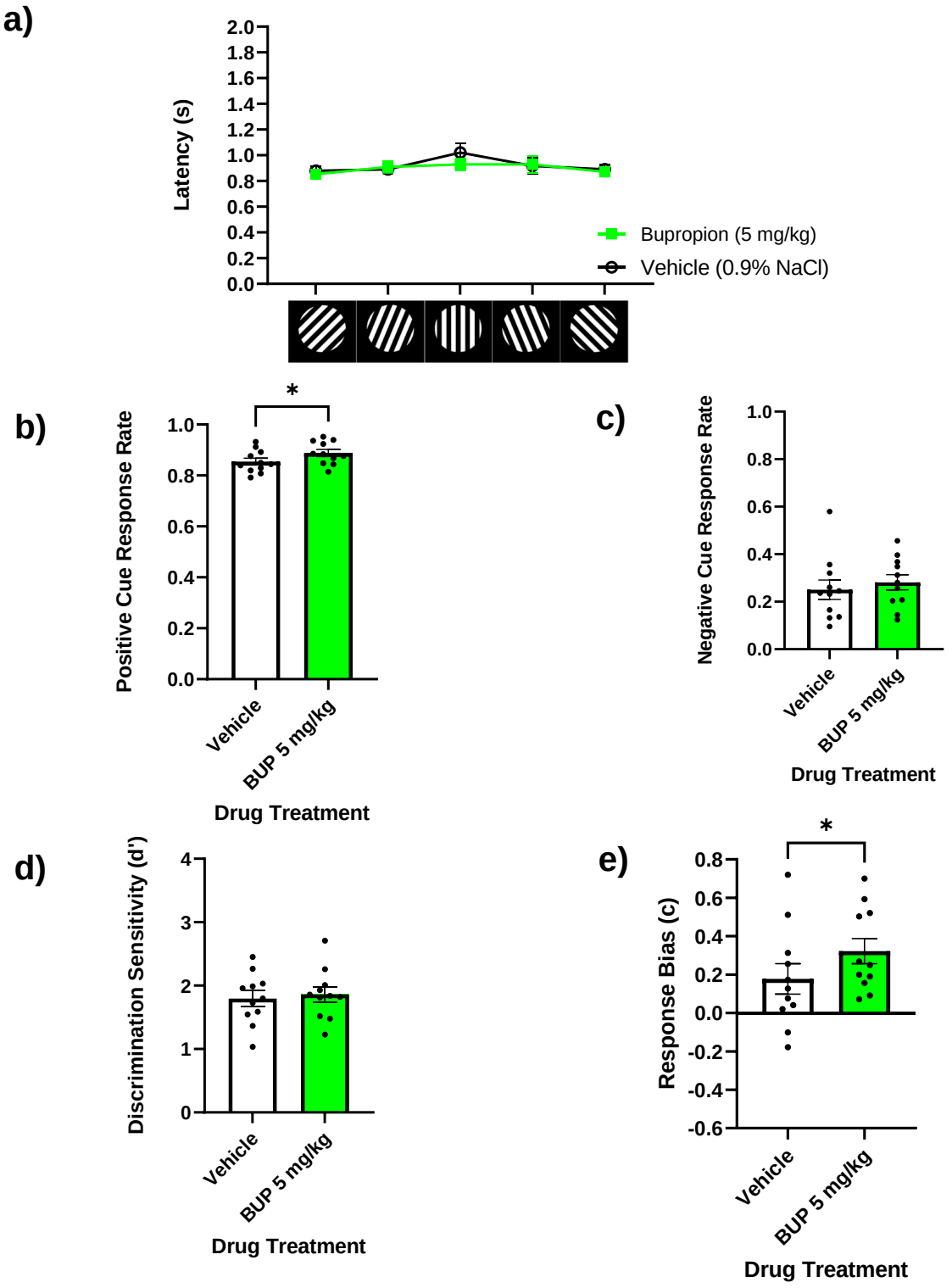

Figure A1. Effects of BUP (5 mg/kg) on CJB responding in CJB Task 1 (Lopez-Cruz Variant) a) BUP did not affect response latencies for the five cues b) BUP significantly increased response rate for positive cue c) BUP did not affect the response rate for the negative cue d) BUP did not affect discrimination sensitivity (d') e) BUP induced a more liberal response bias (c).

Single-Housed Males

To provide further insight into the single-housed animals, the graphs are presented below but they were not statistically analyzed due to the low n (n = 3). Based on the profiles for both drug treatments, single-housed animals appeared to respond similarly in the CJB task regardless of treatment received.

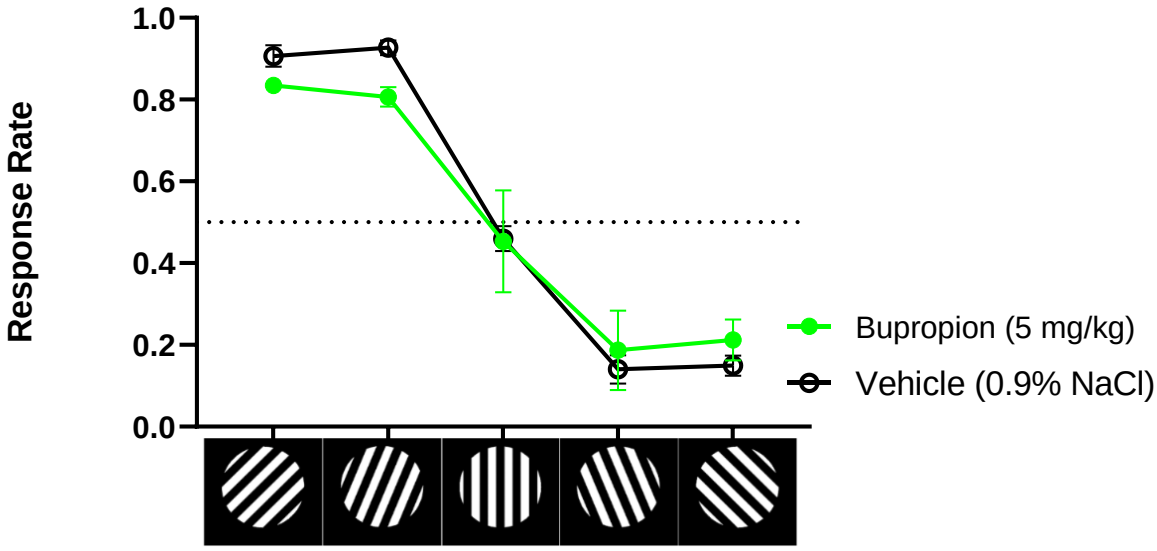

Figure A2. Effect of BUP (5 mg/kg) on CJB Responding in Single Housed Males (n =3) in CJB Task 1 (Lopez-Cruz Variant).

Bupropion (7.5 and 10 mg/kg)

Males

There were main effects of cue ($F_{(1.259, 5.036)}$ = 56.90, η^2 = 0.7804, p < 0.001; Figure A3) and drug treatment ($F_{(1.671, 6.684)}$ = 5.799, η^2 = 0.03088, p < 0.05; Figure A3) and no interaction of drug x cue (p = 0.240; Figure A3). When male mice were treated with BUP, they had a higher response rate at the true ambiguous cue for the 7.5 mg.kg dose (p < 0.05; Figure A3 inset). Overall, these data suggest a significantly increased response rate between BUP (7.5 mg/kg) and vehicle at the true ambiguous cue.

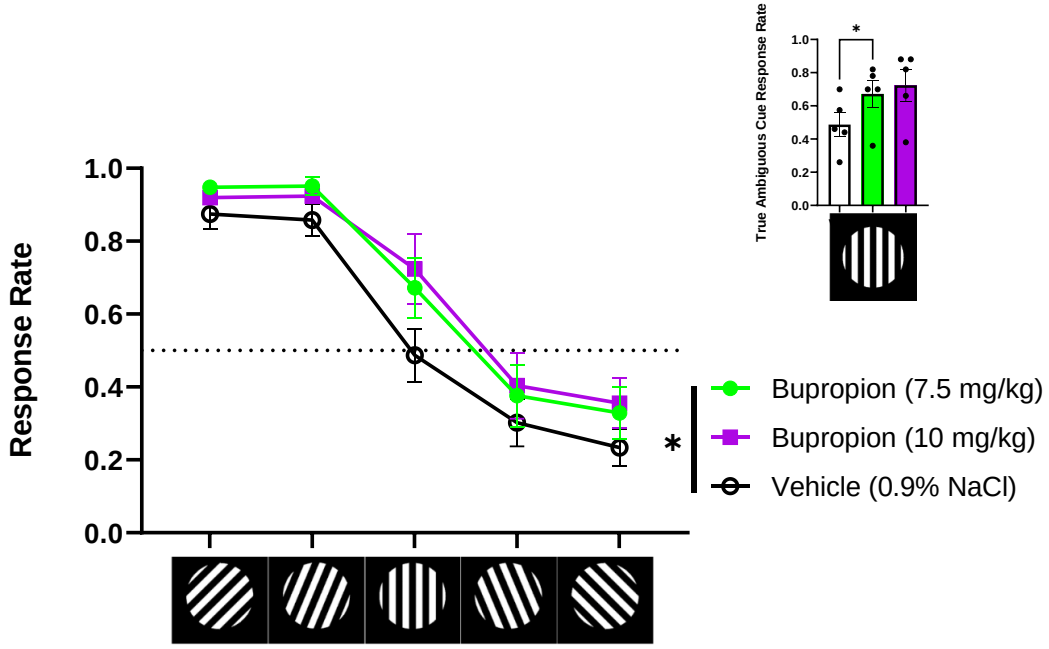

Figure A3. Effect of BUP (7.5 and 10 mg/kg) on CJB Responding in Males (n = 5). BUP (7.5 mg/kg) significantly increased response rate at the true ambiguous cue compared to vehicle in CJB Task 1 (Lopez-Cruz Variant).

Females

There were main effects of cue ($F_{(1.811, 9.056)}$ = 176.3, η^2 = 0.9194, $p < 0.001$; Figure A4) and drug treatment ($F_{(1, 5)}$ = 9.849, η^2 = 0.004225, $p < 0.05$; Figure A4) and no interaction of drug x cue (p = 0.114; Figure A4). When female mice were treated with BUP, they had a higher response rate at the true ambiguous cue for 7.5 mg/kg ($p < 0.05$; Figure A4 inset) and a trending increase for 10 mg/kg (p = 0.0675; Figure A4 inset). Overall, these results suggest a significantly increased response rate between BUP (7.5 mg/kg) and vehicle at the true ambiguous cue.

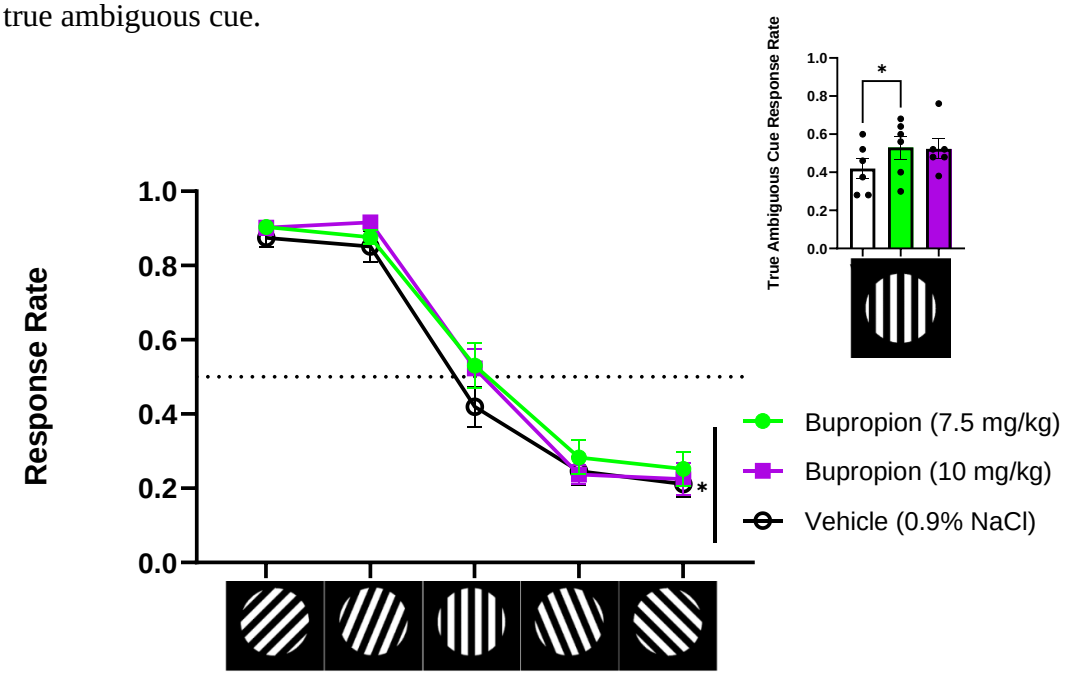

Figure A4. Effect of BUP (7.5 and 10 mg/kg) on CJB Responding in Females (n = 6). BUP (7.5 mg/kg) significantly increased response rate at the true ambiguous cue compared to vehicle in CJB Task 1 (Lopez-Cruz Variant).

Single-Housed Males

To provide further insight into the single-housed animals, the graphs are presented below but they were not statistically analyzed due to the low n (n = 3). Based on the drug treatment profiles, the 10 mg/kg BUP appeared to slightly increase response rate at the true ambiguous cue.

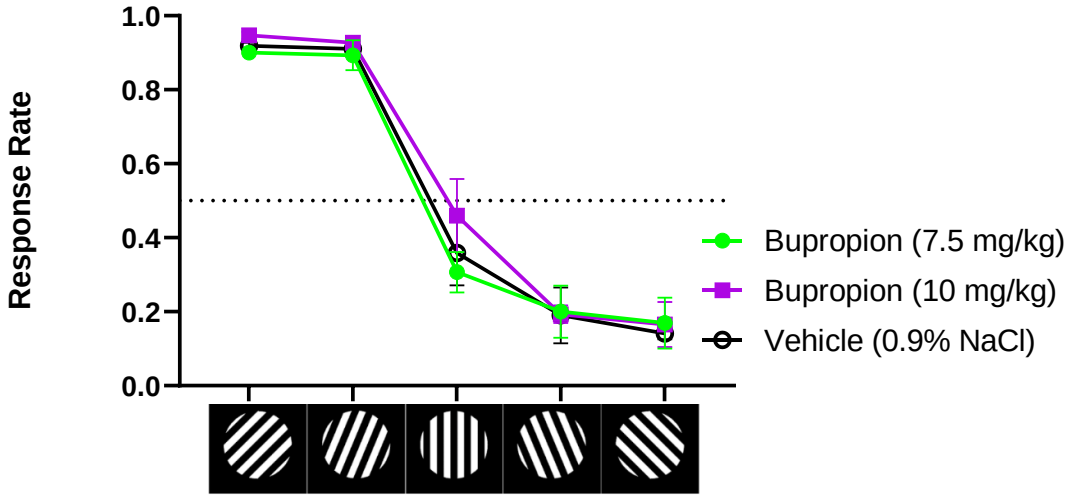

Figure A5. Effect of BUP (7.5 and 10 mg/kg) on CJB Responding in Single-Housed Males (n =3) in CJB Task 1 (Lopez-Cruz Variant).

Tetrabenazine

Males

There were main effects of cue ($F_{(2.374, 9.495)}$ =87.46, η^2 = 0.7656, p < 0.0001; Figure A6) and drug treatment ($F_{(1, 4)}$ = 9.733, η^2 = 0.05822, p < 0.05; Figure A6) but no cue x drug interaction (p = 0.416; Figure A6). When male mice were treated with TBZ, there was a trending decrease in response rate at the true ambiguous cue (p = 0.0890; Figure A6 inset). These data suggest that TBZ did not reduce response rate for ambiguous cues in the task compared to vehicle, although there was a trend towards significance.

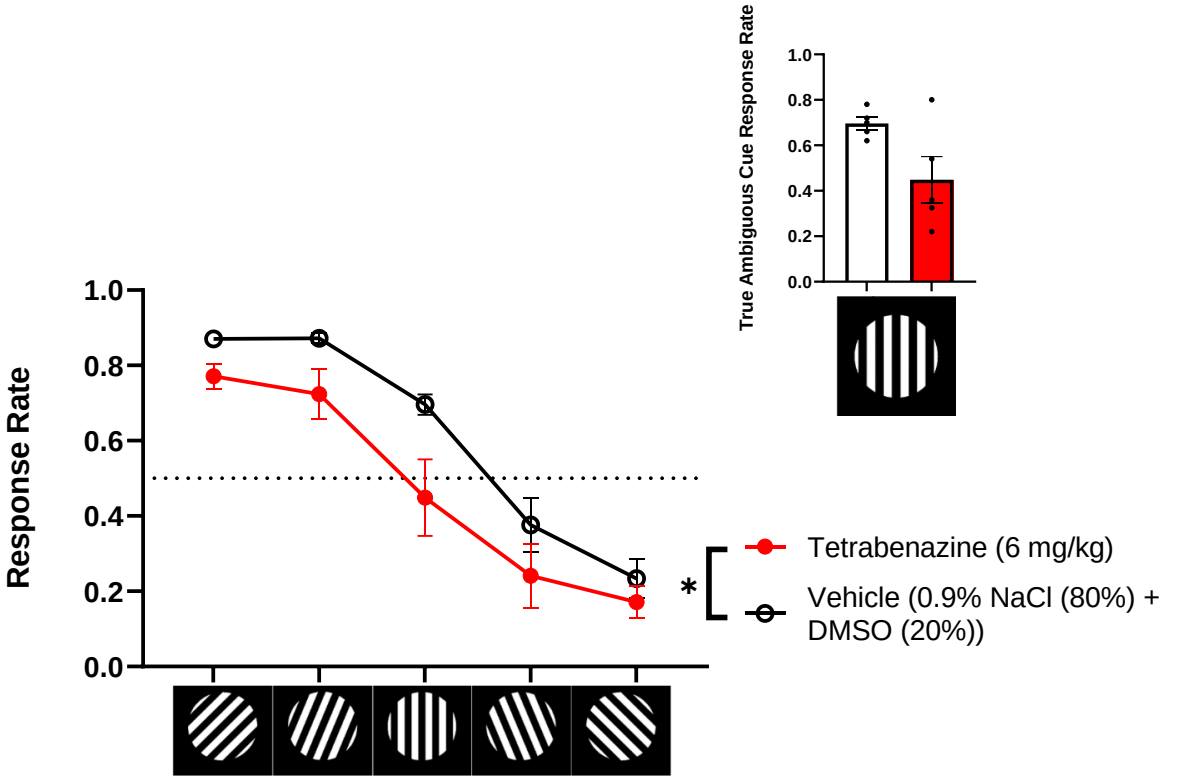

Figure A6. Effect of TBZ on CJB Responding in Males (n = 5). TBZ but did not signficantly reduce response rate at the true ambiguous cue compared to vehicle in CJB Task 1 (Lopez-Cruz Variant).

Females

There was a main effect of cue ($F_{(1.610, 6.440)} = 95.16$, $\eta^2 = 0.8919$, $p < 0.0001$; Figure A7), trend of drug treatment ($p = 0.0549$; Figure A7) and trend of cue x drug interaction ($p = 0.0819$; Figure A7). When female mice were treated with TBZ, they had a lower response rate at the true ambiguous cue ($t_{(4)} = 4.554$, Cohen's $d = 1.433959$, $p < 0.05$; Figure A7 inset). These data suggest that TBZ significantly reduced response rate for the true ambiguous cue in the task compared to vehicle.

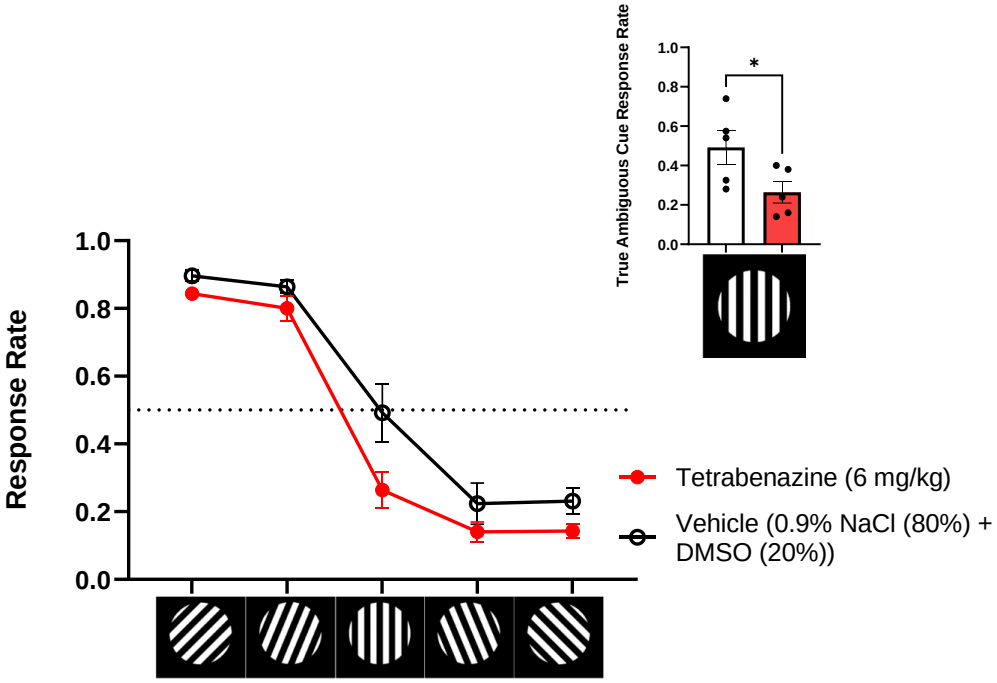

Figure A7. Effect of TBZ on CJB Responding in Females (n = 5). TBZ significantly reduced response rate at the true ambiguous cue compared to vehicle in CJB Task 1 (Lopez-Cruz Variant).

Single-Housed Males

To provide further insight into the single-housed animals, the graphs are presented below but they were not statistically analyzed due to the low n (n = 3). Based on the profiles for both drug treatments, single-housed animals appeared to respond similarly in the CJB task regardless of treatment received.

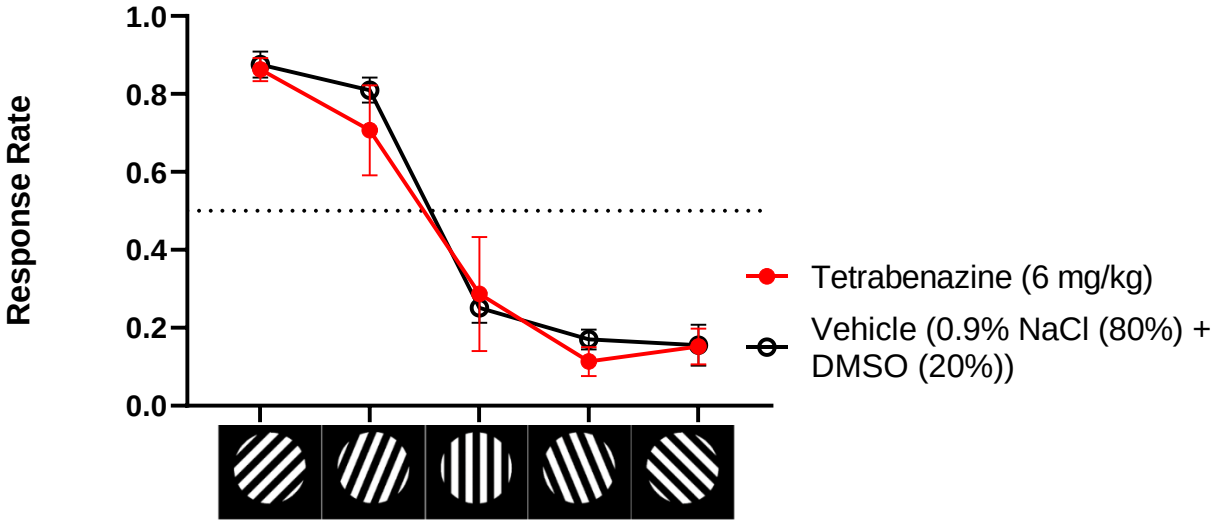

Figure A8. Effect of TBZ on CJB Responding in Single-Housed Males (n = 3) in CJB Task 1 (Lopez-Cruz Variant).

Handling, Transport, and Injection Stress Experiment

Males

There was a main effect of cue ($F_{(1.782, 12.47)}$ = 169.0 , η^2 = 0.8881, $p < 0.0001$; Figure A9) but no effect of condition ($p = 0.181$; Figure A9) and no interaction of condition x cue ($p= 0.695$; Figure A9). *A priori* planned comparisons also revealed no effect of condition ($p = 0.448$). Overall, these results suggest that response rate responding between "Saline Injection" and "No-Injection" was similar at the true ambiguous cue.

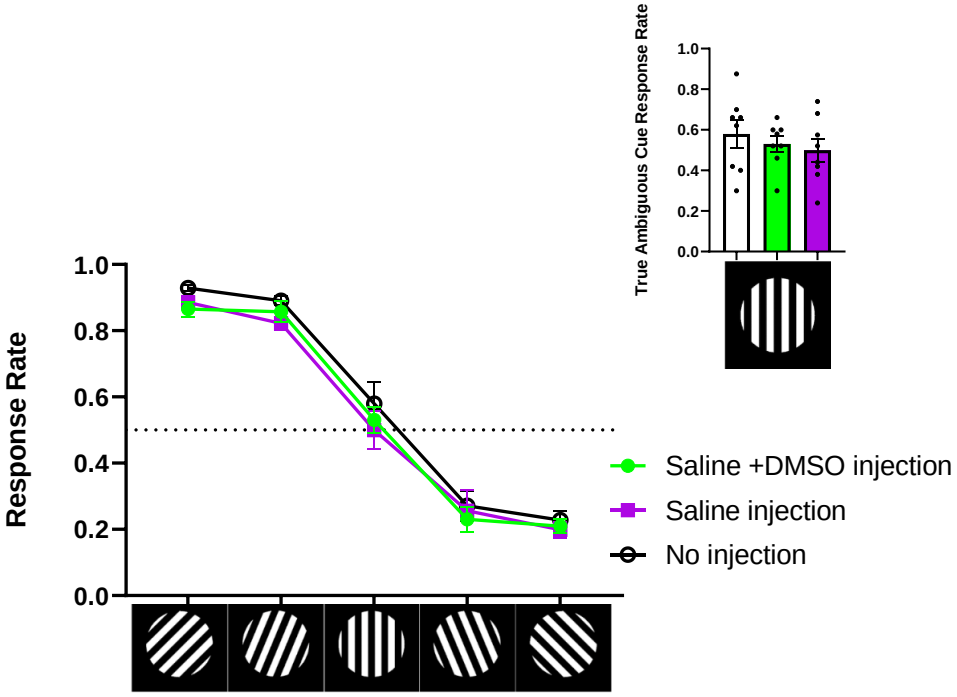

Figure A9. Effect of Saline Injection and Saline + DMSO Injection on CJB Responding in Males (n = 8). Neither type of injection significantly reduced response rate for the true ambiguous cue compared to "No-Injection" in CJB Task 1 (Lopez-Cruz Variant).

Females

There was an interaction of condition x cue ($F_{(2.861, 17.17)}$ = 3.649, η^2 = 0.01193, $p < 0.05$; Figure A10), a main effect of cue ($F_{(1.941, 11.65)}$ = 160.8, η^2 = 0.8402, $p < 0.0001$; Figure A10) and main effect of condition $F_{(1.454, 8.724)}$ = 11.14, η^2 = 0.02149, $p < 0.01$; Figure A10). Šidák post hoc tests revealed significant differences between "Saline Injection" and

"No-Injection" at the true ambiguous cue ($p < 0.05$; Figure A10) and near-negative cue ($p < 0.05$; Figure A10). Additionally, there were significant differences between "Saline + DMSO Injection" and "No-Injection" at the near-negative cue ($p < 0.001$; Figure A10) and negative cue ($p < 0.01$; Figure A10). *A priori* planned comparisons revealed a significant effect of condition ($F_{(1.898,\ 11.39)} = 6.528$, $\eta^2 = 0.5211$, $p < 0.05$; Figure A10 inset) at the true ambiguous cue with a significant decrease in response rate in the "Saline Injection" condition ($p < 0.05$; Figure A10 inset). Overall, these results suggest a significantly decreased response rate between "Saline Injection" and "No-Injection" at the true ambiguous and near-negative cues and also a significantly decreased response rate between "Saline + DMSO Injection" and "No-Injection" at the near-negative and negative cues.

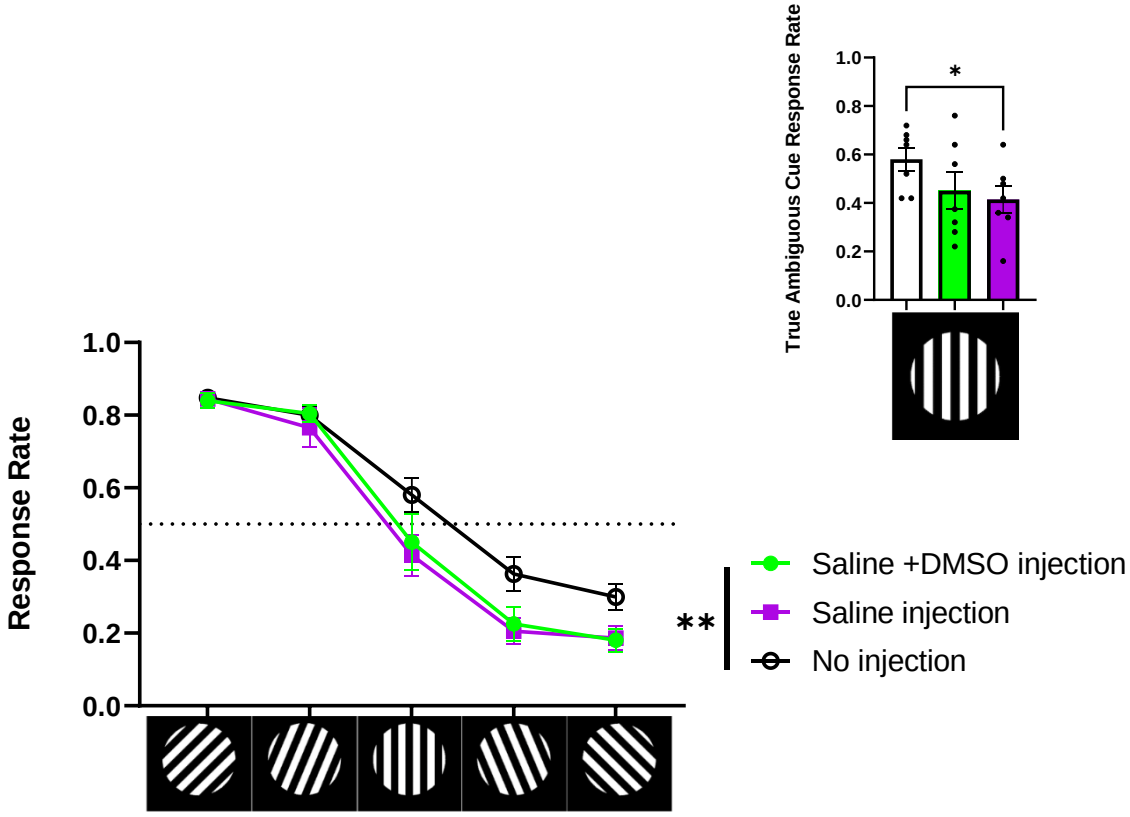

Figure A10. Effect of Saline Injection and Saline + DMSO Injection on CJB Responding in Females (n = 8). "Saline Injection" significantly reduced response rate for the true ambiguous and near-negative cues compared to "No-Injection". "Saline + DMSO Injection" significantly reduced response rate for the near-negative and negative cue compared to "No-Injection".

CJB Task 2 (Krakenberg Variant)

Testing Probes

One animal (male) successfully completed the baseline session and one probe session (Figure A11). Another animal (female) successfully completed the baseline session and all four probe sessions (Figure A12). Due to each graph being n = 1, data were not analyzed, and it is not possible to draw any definitive conclusions from these data.

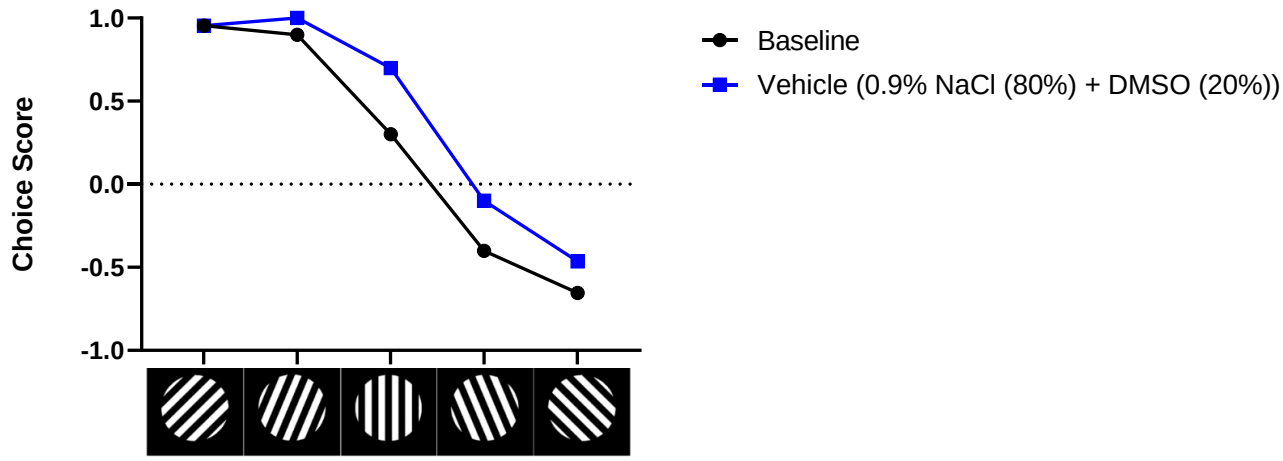

Figure A11. CJB Responding for n = 1 animal. The animal completed baseline and one testing probe session where it received vehicle (saline + DMSO) in CJB Task 2 (Krakenberg Variant).

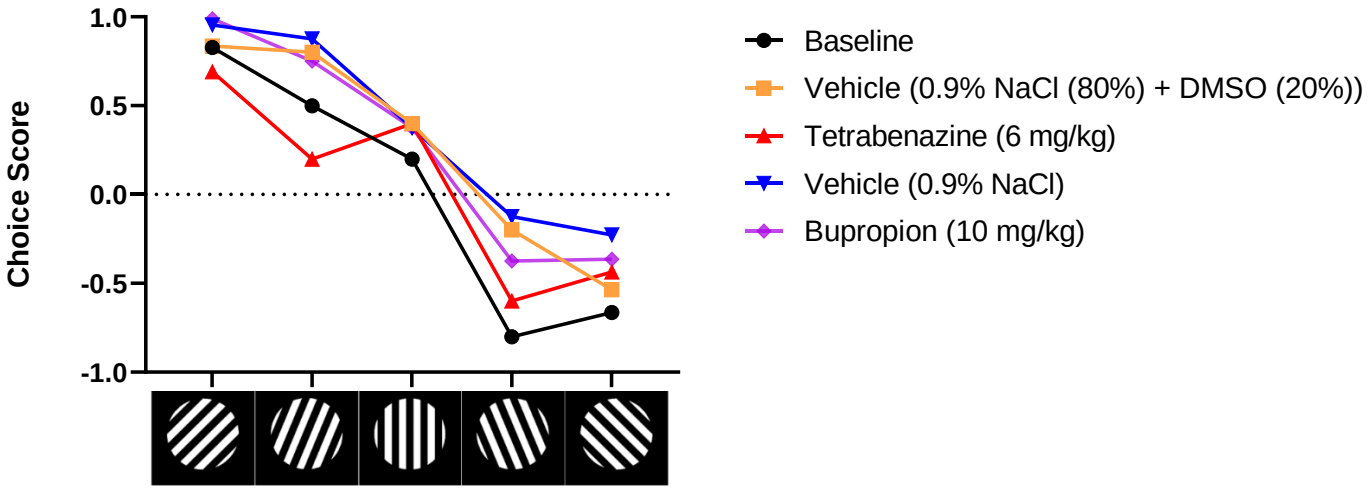

Figure A12. CJB Responding for n = 1 animal. The animal completed baseline and all four testing probe sessions for CJB Task 2 (Krakenberg Variant).

CJB Task 3 (2-Stage Novel Variant)

Testing Probes

One animal (male) successfully completed the baseline session and one probe session (Figure A13). Two animals (males) successfully completed the baseline session and all probe sessions. However, because they were single-housed, their data are not shown. Additionally, five female animals successfully completed the baseline session and all four probe sessions (Figure A14 and A15). Due to the low n, data were graphed but not statistically analyzed and no definitive conclusions can be made. Given the profile of the drug treatments, it appears that TBZ had no effect on CJB responding compared to vehicle. It also appears that BUP slightly increased the CJB choice score, particularly at the near-negative cue.

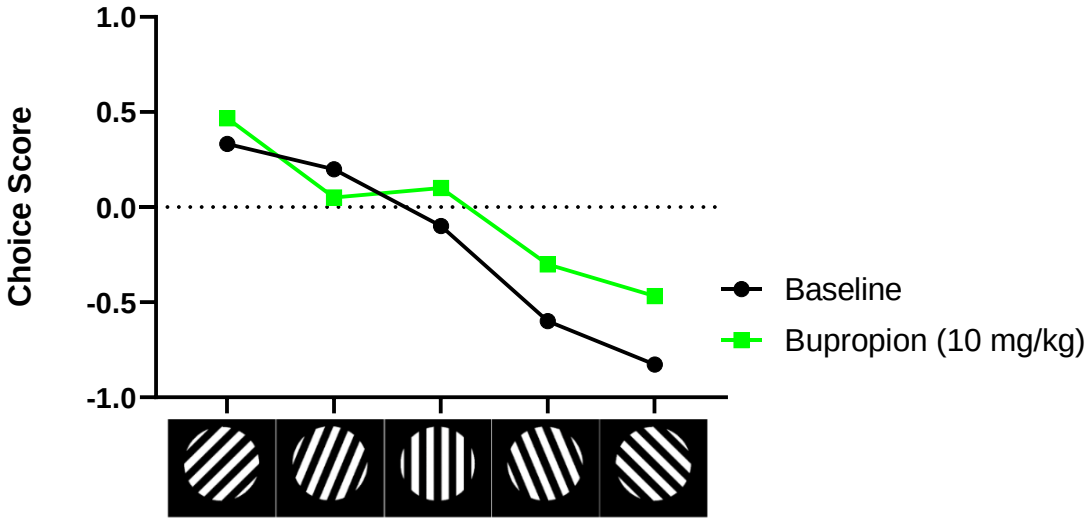

Figure A13. CJB Responding for n = 1 animal. The animal completed baseline and one testing probe session where it received BUP (10 mg/kg) in CJB Task 3 (2-Stage Novel Variant)

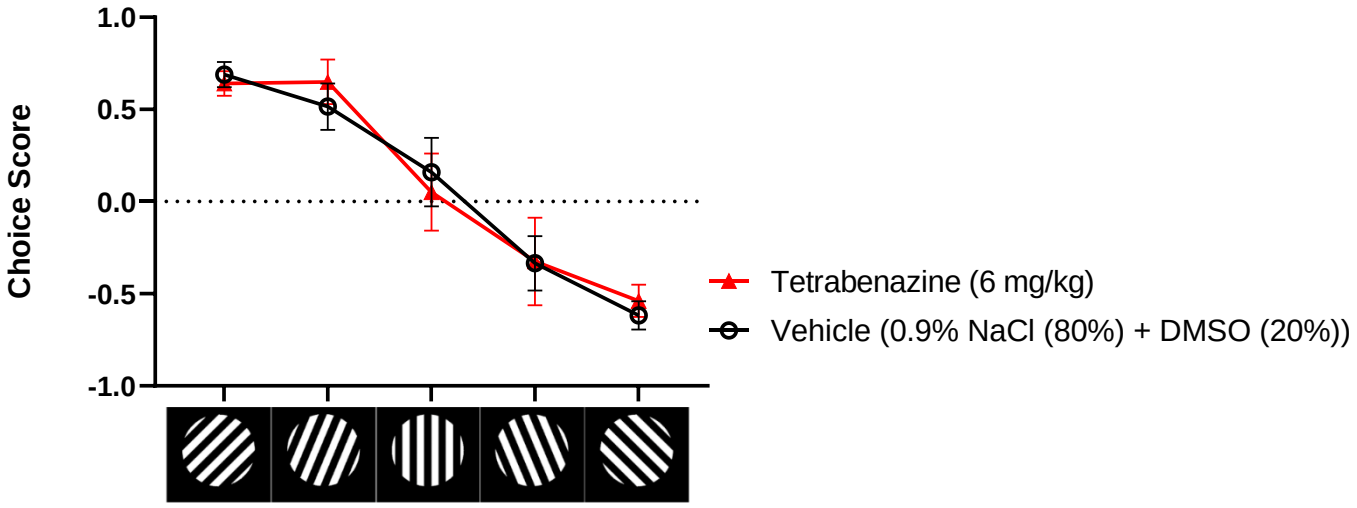

Figure A14. Effect of TBZ on CJB responding in females (n = 5). TBZ appeared to have no effect on CJB choice score compared to vehicle in CJB Task 3 (2-Stage Novel Variant).

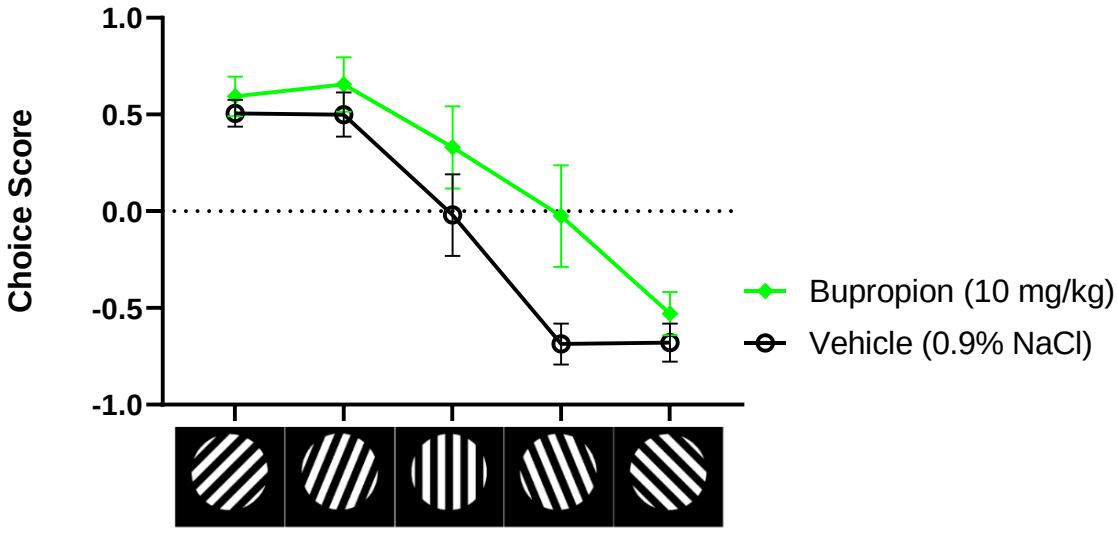

Figure A15. Effect of BUP (10 mg/kg) on CJB responding in females (n = 5). BUP (10 mg/kg) appeared to increase CJB choice score compared to vehicle at the near-negative cue.

Curriculum Vitae

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Post-secondary Education and Degrees:
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Honours and Awards:
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Dean's Honour Roll
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Related Work Experience
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Conferences
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Society for Neuroscience, 2023
Physiology and Pharmacology Research Day, 2023
International Touchscreen Symposium, 2023
Southern Ontario Neuroscience Association Conference, 2023
Physiology and Pharmacology Research Day, 2022
Western Undergraduate Research Conference, 2022
Inspiring Diversity in STEM Conference, 2022